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Review Article

Changing the energy of an immune response

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Abstract: The breakdown of nutrients into the critical energy source ATP is the general purpose of cellular metabolism and is essential for sustaining life. Similarly, the immune system is composed of different cell subsets that are indispensable for defending the host against pathogens and disease. The interplay between metabolic pathways and immune cells leads to a plethora of different signaling pathways as well as cellular activities. The activation of T cells via glycolysis-mediated upregulation of surface markers, for example, is necessary for an appropriate effector response against an infection. However, tight regulation of immune cell metabolism is required for protecting the host and resuming homeostasis. An imbalance of immunological metabolic function and/or metabolic byproducts (reactive oxygen species) can oftentimes lead to diseases. In the case of cancer, overactive glucose metabolism can lead to hyperproliferation of cells and subsequent decreases in cytotoxic T cell activity, which attack and destroy the tumor. For this reason and many more, targeting metabolism in immune cells may be a novel therapeutic strategy for treatment of disease. The metabolic pathways of immune cells and the possibilities of immunometabolic therapies will be discussed.

Keywords: Metabolism, immune response, aerobic glycolysis, oxidative phosphorylation

Aerobic respiration

Cellular metabolism is necessary for generating energy and sustaining life. Through a series of steps involved in glycolysis (glucose), fatty acid oxidation, and amino acid (protein) oxidation, cells can break down ingested products into critical energy sources. This energy, better known as adenosine triphosphate or ATP, is synthesized as a result of the degradation of nutrients. Oxygen (O_2) plays a key role in enabling reactions required for the formation of ATP. In human cells, oxidative phosphorylation is the main process leading to the generation of ATP [1]. The degradation of nutrients through the glucose oxidation, fatty acid oxidation, or amino acid oxidation pathways converge to all produce acetyl-CoA, a key molecule that provides a carbon source for fueling the tricarboxylic acid (TCA) cycle. The oxidation of acetyl-CoA to carbon dioxide (aerobic respiration) then allows for the subsequent reduction of nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FAD) via the TCA cycle. The intermediate products, NADH and $FADH_2$, serve as electron transport chain coenzymes for oxidative phosphorylation. For efficient respiration, electrons must be transferred from NADH and $FADH_2$ to oxygen via the mitochondrial complexes along the electron transport chain within the inner mitochondrial membrane. Electrons must be strictly allocated down the electron transport chain while protons must be pumped across the mitochondrial membrane. This movement of electrons not only facilitates the production of H_2O, but also drives a proton gradient that causes the phosphorylation of adenosine diphosphate to adenosine triphosphate. ATP can then be utilized for a number of events including DNA/RNA/protein synthesis, cell signaling, cytoskeletal rearrangement, cell proliferation, and metabolic pathways. Despite the necessity for energy production, the electron transport chain is also responsible for the formation of mitochondrial reactive oxygen species (ROS) through continuous 'leakage' of electrons, causing partial reduction of O_2 molecules [2]. Such events lead to the generation of super-

oxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-), which can both stimulate physiological actions as well as damage important molecules in the body [3], depending on the quantity. At a low abundance, ROS help control normal cellular functions, as demonstrated by the immune system's reliance on this type of signaling for regulation, activation, T cell proliferation [4,5], NF-κB activation [6], and signal transduction [7-9]. Conversely, high levels of ROS lead to oxidative stress, which has been linked to a variety of diseases, aging, and cell death [10-13]. ROS production is, thus, a necessary evil for functional aerobic metabolism.

Aerobic glycolysis

Although aerobic respiration is the main source of ATP in most mammalian cells, an alternative form of metabolism, glycolysis, is crucial in both immunity and disease states. Glycolysis enables the conversion of one glucose molecule to 2 pyruvate molecules, with subsequent production of lactate, NAD+, and ATP. The utilization of glycolysis is a less efficient form of metabolism. During mitochondrial respiration, a cell is able to produce 38 ATP molecules; however, during glycolysis, only 2 molecules of ATP are generated. The obvious 19-fold increase in ATP via oxidative phosphorylation seems more advantageous to the cell, especially since both metabolic pathways can use glucose as the starting material. However, glycolysis is especially important in times of hypoxia (anaerobic) and can occur even in the presence of oxygen (aerobic), such as with tumors and immune cells. Both anaerobic and aerobic glycolysis are crucial for the maintenance of tumor cells [14-16]. Tumor cells switch their energy production from oxidative phosphorylation to glycolysis upon transformation to malignancy [17, 18]. This enables the tumor to rapidly grow in hypoxic environments and evade host immune cell defense mechanisms [19-21]. In oxygenated environments, tumor cells display augmented glucose transport and glycolysis [22, 23]. This counterintuitive metabolic programming has been attributed to over-adaptation to hypoxic environments, a greater need for macromolecules during unrestrained proliferation (glycolysis drives better protein/nucleotide synthesis), and elevated expression of the glycolytic enzyme hexokinase [24, 25]. This high aerobic glycolysis seen in cancer cells is called the Warburg effect [14, 26] and has led to the development of anti-glycolytic drugs for cancer treatment [27, 29]. During proliferation, T cells and cancer cells show similar metabolic programming. Thus T cells, even in the presence of sufficient oxygen, also choose to ferment glucose, as further discussed below.

Immune cell metabolism

The immune system is made up of two different arms: the innate and adaptive immune system. The innate system is a first-line of defense against pathogens and foreign substances. Unlike the adaptive arm, the innate response is non-specific and is mediated by antigen-presenting cells (APCs) as well as granulocytes. Examples of innate cells include dendritic cells, macrophages, and neutrophils. After successful priming by the innate cells, the adaptive immune response, made up primarily of T and B cells, provides antigen-specific protection against the insult, either through the release of cytotoxic granules, cytokines, or antibodies. Immune cells, like most other cells in the body, utilize nutrients via cellular metabolism. At rest, immune cell metabolism is able to regulate cell volume, ion integrity and growth [30]. However, in addition to housekeeping proliferation and sustenance, ATP within the immune cells must be ready to carry out various functional activities such as phagocytosis, activation, antigen presentation/processing, migration, phosphorylation, differentiation, and effector responses [30, 31]. Most of these actions are thermodynamically taxing, requiring notable and rapid changes in metabolism [30, 32]. Furthermore, immune cells must facilitate cytoskeletal changes, increased ion signaling, enhanced phospholipid turnover, and greater macromolecule synthesis in a very short time during rapid energy consumption [33]. Importantly, resting immune cells, especially those of the adaptive arm, contain little glycogen stores, resulting in the dependence of imported glucose to uphold metabolic needs [34-36].

Because of the diverse functionality of immune cells, several important differences exist between their metabolism and that of other cells within the body. Alveolar cells, for example, are reliant primarily on oxidative phosphorylation for the generation of sufficient ATP [37]. Those cells which are in constant contact with

oxygenated blood are especially formulated for mitochondrial respiration. On the other hand, immune cells travel through the body to monitor the peripheral tissues. Once a foreign antigen is detected, APCs migrate to the draining secondary lymphoid organs where they can process and present the antigen to lymphocytes. APCs are present in strategic areas of the body as resident phagocytes. These cells, therefore, are not necessarily exposed to normoxic conditions at all times. For instance, epidermal dendritic cells reside within the deep tissue layers of the skin [38], where oxygen tension is lower than the dermis [39]. Such conditions create a slightly hypoxic environment in which key immune cells must be able to utilize alternate forms of metabolism in order to survive and function properly [40]. Similarly, lymphocytes, upon activation within the secondary lymphoid organs, travel to the site of inflammation by traversing the endothelial cell wall into the target area [41]. Once again, the migration of lymphocytes away from the source of oxygen causes slight hypoxia and a resultant loss of dependence on oxidative phosphorylation. Most sites of inflammation are also areas of lowered oxygen, with innate phagocytes clogging the blood vessels [42-44]. Localization of immune cells thus requires adaptation to different oxygen levels and promotes more glycolytic pathways [45, 46].

Although influential, the environment is not the only element dictating the metabolic choices within immune cells. The activation of both innate and adaptive immune cells is absolutely critical for protecting the body from pathogens and insults. Consequently, the cells cannot afford to be inefficient in their nutrient metabolism. With this being said, it would seem likely that immune cells should generate ATP via oxidative phosphorylation, fostering the most energy from the nutrients provided. However, this is not entirely the case. Although some mitochondrial respiration does occur in immune cells, the level at which it is used depends significantly on the cell's specificity and state of reactivity. Activated (and some inactive) immune cells prefer to utilize glycolysis, as it is 100-times faster than oxidative phosphorylation for macromolecule synthesis and proliferation [47].

As mentioned above, naïve APCs monitor the body for foreign substances. In this resting state, myeloid cells and granulocytes favor glycolysis [48, 49]. Homeostatic protein turnover, degradation, and synthesis all occur via growth factor utilization [50, 51]. Once antigen is phagocytosed, APCs immediately upregulate costimulatory molecules and process and present antigen on their cell surface. Such events require greater ATP; however, APCs retain their dependence upon glycolysis [48, 49]. Dendritic cells, for example, are known to undergo metabolic changes towards greater glycolysis upon toll-like receptor (TLR) stimulation [52]. Moreover, classically activated macrophages (known as M1), which promote proinflammatory cytokines, are known to be regulated by glycolysis; however, alternatively activated macrophages (M2), which are anti-inflammatory, rely more so on mitochondrial respiration [53, 54]. Glycolysis is, therefore, important for inflammatory responses and can be detrimental in the face of chronic inflammatory diseases. Granulocytes, such as neutrophils, also favor glycolysis [48, 55]. Neutrophils are the first mediators at the site of foreign entry. Their immediate degranulation and pyrogenic secretion lead to subsequent cell death [56]. In accordance with the glycolytic reliability of APCs, quick responses are needed by neutrophils, but survival via greater ATP production is not necessary. Fast reactivity and turnover rates of both APCs and granulocytes, therefore, make sense with their choice to generate ATP through the more rapid glycolytic pathway.

Lymphocytes, on the other hand, rely heavily on oxidative phosphorylation during resting states but switch their metabolic needs to glycolysis during activation (**Figure 1**). Subsequently, some lymphocytes return to oxidative phosphorylation after clearance of an antigen to generate memory [57, 59]. Therefore, there is a cyclical pattern of metabolic pathways, fluctuating between anabolism and catabolism, as reviewed by Pearce *et al.* [60]. At rest, T cells need a constant supply of nutrients as well as TCR stimulation [61]. The idea of TCR 'tickling' has long been thought to prevent deletion of mature T cells [62]. The active metabolism behind the quiescent state is a relatively novel idea, with several transcription factors implicated in regulating this process [63]. Circulating naïve lymphocytes undergo oxidative phosphorylation to generate a surplus of ATP reserves via catabolic metabolism, the breakdown of nutrients [36]. In a sense, quiescent

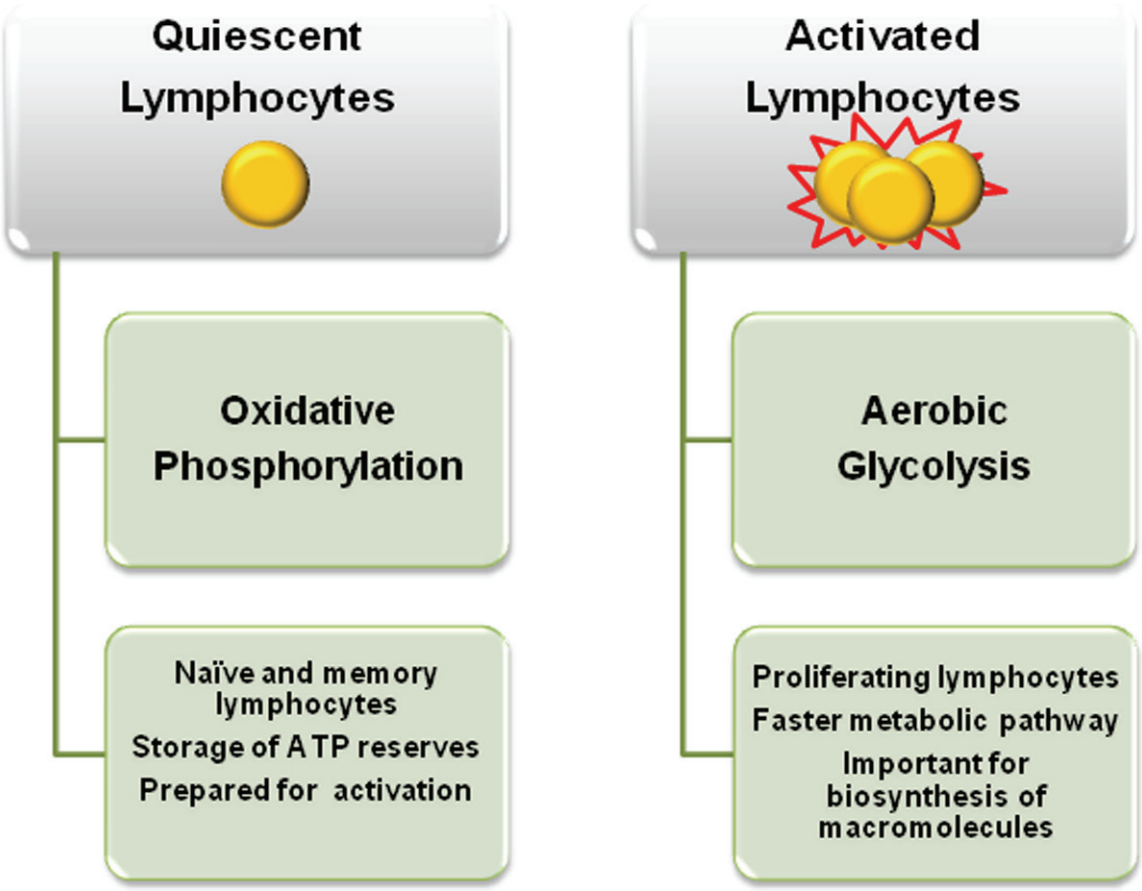

Figure 1. Lymphocyte metabolism fluctuation during resting and activated states. Quiescent, or resting, lymphocytes primarily utilize oxidative phosphorylation to build up reserves of ATP in preparation for activation. Activated, or proliferating, lymphocytes predominately use aerobic glycolysis due to its rapid speed and critical role in forming biosynthetic precursors.

immune cells remain 'at attention' in order to quickly mobilize following antigen stimulation [64]. Preservation of quiescence is mediated by turnover of cell cycle proteins, a very active event which requires a lot of ATP [50, 51], and by upregulation of cyclin-dependent kinase inhibitors [65]. Quiescent cells not only utilize glucose, amino acids, and lipids for ATP generation, but can also extract nutrients from those proteins which are degraded, via autophagy or self-eating [30, 66, 67]. Engagement of the TCR as well as growth factors and homeostatic cytokines, like IL-4, IL-5, IL-7, and IL-3 [68-70], also all play roles in keeping naïve cells alive. Cytokine-receptor signaling, specifically, can activate protein kinases that are necessary for the uptake of adequate ATP to preserve homeostatic processes [36], whereas a lack of TCR interaction will downregulate glucose transport, ATP, and mitochondrial potential [61]. Insufficiencies in glucose uptake by T cells will lead to BAX induction and apoptosis [71]; however, this stringency serves to control the naïve T cell population, ensuring the turnover of existing cells as new cells are produced to avoid over accretion [61].

Like the innate cells, lymphocytes need to become rapidly activated upon antigen stimulation. Such changes in metabolism are well-documented in the case of mitogen-stimulated lymphocytes [60, 4, 72]. Within the first 24h post-mitogen stimulation, lymphocytes considerably enlarge their size [60]. During this time, new macromolecules are being synthesized, including nucleotides and proteins. Following the growth, T cells then divide every 4-6 hours [72] and eventually will differentiate into effector cells. Effector functions such as cytokine production and cytotoxic granule release then

enable the cell to attack the target area. This quick change in cell size and function relies primarily on obtaining nutrients from the environment and driving glycolysis [36]. Instead of initiating the long process of oxidative phosphorylation, T cells convert glucose to pyruvate via the more rapid glycolysis [47], and in the process, generate ATP and lactate, which allows for the conversion of NADH back to NAD+ to retain glycolysis. The dependence of T cells on glucose is so great, that even in the presence of excess glutamine, which is another carbon source that can be metabolized by lymphocytes, proliferation is stunted [68]. T cells are not able to enhance mitochondrial respiration to a level that meets their energy needs. Moreover, T cells require high levels of NADH as macromolecular precursors; therefore, greater lactate production equals more NAD generation [32]. Notably, following mitogen-stimulation, an excess of lactate can be measured from T cells [73, 74]. Aerobic glycolysis ensures that enough energy is made to propel macromolecule synthesis (anabolic metabolism), which is ultimately crucial for clearance of a pathogen, as well as to keep the cells alive and functional [36, 75, 76]. Some oxygen consumption does still occur [77], yet is typically only utilized in situations where glucose is limited [78, 79]. Mitogenic-stimulation of peripheral blood mononuclear cells, for example, causes oxygen consumption attributed to ATPase activity, protein synthesis, and nucleic acid synthesis [31, 64]. Moreover, activation of lymphocytes promotes a calcium flux, which will also drive the upregulation of mitochondrial enzyme activity for sufficient oxidative phosphorylation [80, 81]. In the case of infection, T effector cells promote clearance of the pathogen and primarily utilize glycolysis for rapid growth. By the time pathogen has been cleared, the mitochondrial capacity of effector cells is reduced in such a way that they can only sustain viability through glycolysis. In the absence of adequate nutrients and IL-2 present during infection, effector cells are unstable and eventually will undergo apoptosis. Certain clones, nonetheless, will retain their ability to switch back to oxidative phosphorylation. Such cells will contain greater mitochondrial mass, either through differences in biogenesis or asymmetric division [57]. In autoreactivity [82] as well as graft-versus-host disease [83], chronically stimulated T cells rely on oxidative phosphorylation in contrast to acutely activated cells (those discussed above) which depend on glycolysis. Oftentimes, such illnesses and metabolic outcomes correlate with mitochondrial dysfunction and/or increased mitochondrial mass present during the disease [84]. Lipid oxidation, specifically, is important for the generation of both regulatory T cells (Tregs) [85] and memory CD8+ T cells [86], countering glycolytic effector CD4+ and CD8+ T cells. Following T cell activation, not only will the clearance of antigen help generate memory, but remaining T effector cells will die via apoptosis due to decreased growth factors and metabolism [15]. Memory T cell metabolism is similar to naïve, in that both populations require oxidative phosphorylation; however, some critical changes occur over the course of an immune response to ensure memory T cell survival. Those that become memory cells will have higher TCR affinities, which allows for advantageous survival in conditions of nutrient limitation and low homeostatic engagement [61]. Furthermore, CD8 T cells, described to have substantial spare respiratory capacity after clearance of infection, will be long-lived memory cells [59]. Spare respiratory capacity is especially critical for producing sufficient ATP under times of stress and for boosting long-term cell survival [87, 88], which would be necessary for memory response. Similarly, CD4 T cell memory is generated via blockade of a gene called *Noxa* that is responsible for driving apoptosis under conditions of limiting glucose [89, 90].

Connecting immune signaling and metabolism

Stimulation of T cells via the TCR requires proper engagement by MHC-peptide on the APC. Additionally, costimulation during T cell activation is critical for downstream signaling and effector function. Conversely, a lack of costimulation can lead to T cell anergy and deletion [91, 92]. Appropriate T cell activation is not only governed by mere mechanistic interaction and a cascade of signaling molecules, but there are important links to metabolism. CD28 is the quintessential costimulatory molecule for T cell activation. Its ability to bind CD80/CD86 on APCs enables downstream signaling and promotes T cell differentiation [93, 94]. Analogous to insulin-receptor signaling, CD28 has been shown to enhance glucose metabolism by trig-

gering an accumulation of glycolytic intermediates [35], stimulating glycolysis, and increasing glucose transporter expression [95]. CTLA-4, on the other hand, offsets the effects of CD28, reducing glycolysis and rendering cells quiescent [96]. One of the most essential downstream signaling cascades bridging the gap between T cell activation and metabolism is the PI3K-Akt-mTOR pathway. CD28, IL-2 and TCR engagement leads to PI3K-dependent Akt activation, which in turn increases the amount of glucose transporters on the plasma membrane as well as elevates activities of glycolytic enzymes i.e. hexokinase and phosphofructokinase [32, 36, 70]. PI3K as well as MAPK and NF-κB can all activate Myc, which is responsible for inducing glucose transporters as well as glycolytic enzymes [97, 98]. Akt, in conjunction with STAT5, also plays a role in glucose uptake in resting T cells [99]. Unlike other cell types, lymphocytes only express the Glut1 glucose transporter [32, 61]. In the absence of adequate TCR and/or cytokine stimulation during both the resting and activated states, Glut1 will be internalized, leading to downregulation of surface expression, reduced transport of glucose across the plasma membrane, and decreased viability of the cell [100]. CD28-mediated Akt signaling is especially important for glucose uptake as it is necessary for expression and trafficking of Glut1 to the cell surface [35, 101]. Comparable to T cells, B cells also increase Glut1 expression following BCR engagement [34]. PI3K similarly plays a critical role in B cell proliferation and immunoglobulin synthesis through regulation of glycolysis and Glut1 [102]. Although Glut1 expression is critical for lymphocyte activation, a balance must exist; otherwise, overexpression of Glut1 can manifest into hyperactive lymphocytes and pathologies [101].

mTOR is another critical regulator of metabolism in immune cells [103]. The ability of mTOR to sense nutrient availability [104, 105] leads to the induction of mRNA translation and protein synthesis [106]. Without proper mTOR signaling, T cell proliferation will be blocked [107] and anabolic storage processes will be decreased [108, 109]. Inhibition of the PI3K-Akt-mTOR pathway can also lead to T cell anergy [110, 111], whereas mTOR-deficient T cells do not differentiate into effector T cells, but instead regulatory T cells [112]. Additionally, mTOR has been linked to chemokine-dependent signaling, resulting in T cell migration [113-115] and cancer metastasis [116, 117]. These data highlight the importance of proper metabolic signaling in initiating an effective adaptive immune response as well as reveal targets for therapeutic intervention.

Cytokine binding and cytokine receptor expression have also been connected with metabolism. Although immune cells primarily utilize glycolysis, some oxidative phosphorylation still occurs and is necessary for functionality. If mitochondrial respiration is blocked at different complexes of the electron transport chain, both TNF binding to its receptor on cells [118] and IL-2R expression on lymphocytes are reduced [5]. Interestingly, TNFα-deficient mice are actually protected from obesity-induced insulin resistance, highlighting the importance of proinflammatory cytokines in metabolic signaling [119, 120]. Other innate immune cytokines, such as IL-1, IL-6, IL-3 and IL-7, also contribute to metabolism. IL-1 can prevent fatty acid synthesis [121], whereas IL-6 can both increase the levels of lipid and glucose metabolism [122]. IL-3, which is known to support the growth of myeloid and lymphoid cells, is important for sustaining Glut1 on the surface of lymphocytes [70] and has been directly shown to shift metabolism from oxidative phosphorylation to glycolysis [123]. In order for activated Akt to sustain glucose uptake in both resting and activated T cells, IL-7 must be present [68]. Overall, each cytokine binds a specific receptor and coordinates T cell function with metabolic needs.

Similar to macrophages, adipocytes can release adipokines (i.e. IL-1, IL-6, IFNγ, TNFα, MCP1) to also bridge the gap between immunity and metabolism [95, 124, 125]. Adipokines can recruit monocytes and lymphocytes into the adipose and promote proinflammatory and anti-inflammatory functions. Overnutrition can induce adipocyte hypertrophy, creating a hypoxic core and MCP-1 production, which facilitates macrophage entry into the adipose [126]. Lymphocytes associated with adipose tissue are oftentimes modulators of the infiltrating macrophages [127]. For example, Tregs are present in greater abundance in the adipose of lean mice, correlating with an anti-inflammatory macrophage phenotype [128]. Furthermore,

proinflammatory effector T cells have been detected in the fat of obese mice, leading to the recruitment of even more proinflammatory macrophages [129] and contributing to insulin resistance [130, 131].

Hormone secretion, from the adipose as well as other tissues, is also important in regulating lymphocyte function. Leptin, a hormone released from the adipose, along with insulin, which is secreted from the pancreatic beta cells, both play critical roles in connecting metabolism to the immune system. Leptin, which regulates food intake by inhibiting appetite, is low in times of starvation, resulting in decreased metabolism to maintain vital organs. Consequently, low leptin levels lead to immunosuppression [95]. In a well-nourished environment, leptin can modulate both the innate and adaptive arms of the immune system to promote greater cytokine production [132], decreased apoptosis [133] and skewing of T cells towards the T_H1 lineage [134, 135]. The effects of leptin on T_H1 responses have been especially documented in the context of autoimmunity. Leptin has been shown to accelerate type 1 diabetes onset in NOD mice via enhancement of IFNγ-producing T cells [136]. Furthermore, higher leptin levels have been detected in female animals that are susceptible to EAE induction versus resistant males, positively correlating with an increase in T_H1 responses [137]. Heightened immune responses following leptin signaling may be damaging in autoimmunity; however, in obesity, failure of proper immunity resulting in increased infections has been linked to greater leptin insensitivity in severely overweight individuals [138]. In general, the immune system cannot function properly in times of over or under-nutrition [139], relating the many intricacies between metabolism and immunity. Similarly, insulin, which promotes cellular metabolism by stimulating the uptake of glucose and storage as glycogen, can play a role in modulating the T cell response. Beyond acting as a lymphocyte-specific antigen in type 1 diabetes, insulin helps shape T cell growth and function. Upon activation, insulin receptor is expressed on T cells. Insulin signaling then facilitates glucose uptake, amino acid transport, lipid metabolism, and protein synthesis [140]. Stimulating CD4+ and CD8+ T cells in the presence of insulin can induce more T_H2-type cells and cytokines [141], leading to a more anti-inflammatory environment. This data strongly suggests why lack of insulin signaling in both type 1 and type 2 diabetes can lead to both enhanced T_H1 cells and uncontrolled inflammation [142].

Modulators of metabolism and potential immunometabolic therapeutic implications

Mitochondrial activity has been implicated as a cause of aging, and metabolic dysfunction and ROS production have been linked to neurodegeneration, cancer, and autoimmunity [10-13]. An accumulation of ROS and redox-damaged byproducts eventually leads to cell dysfunction and death [143]. Indeed, mutations of the electron transport chain can diminish ROS production and thus elongate life [144, 145]. Although immune cells contain higher levels of antioxidants than other cells [146] and rely on both glycolysis and respiration, aging immune cells show accrued impairment, causing reduced lymphocyte proliferation. Functional decline of immune cells, or senescence, oftentimes correlates with age, as free radical production overwhelms antioxidant defenses and the risk of infections/tumors is enhanced [147-149]. During a normal mammalian lifetime, metabolic pathways are kept in check via a number of endogenous mediators such as hypoxia-inducible factor-1 and uncoupling proteins, which prevent oxidative phosphorylation by partially dissipating the mitochondrial proton gradient. Pertaining to aerobic respiration, the existence of antioxidants particularly protects against oxidative stress and damage. Additionally, endogenous mechanisms do exist to restrict ROS production so as not to damage neighboring tissues. For example, Kupffer cells, macrophages residing within the liver, do not undergo respiratory bursts, thus protecting the surrounding parenchyma from any ROS-mediated destruction [150, 151]. Conversely, peritoneal macrophages, which are more involved in clearance of infection, can experience an oxidative burst, with less threat of damage to surrounding tissue [152]. Despite these many mechanisms, improper metabolism of immune cells can oftentimes result in disease. The strict dependence of immune cells on glucose for survival and activation, however, may make them good targets for metabolic therapeutics [60]. Such therapeutics could potentially better control autoimmunity, transplantation rejection, neurodegeneration and cancer.

Hypoxia-inducible factor-1

Hypoxia-inducible factor-1 (HIF-1) is especially important for modulating metabolism in times of low oxygen conditions. HIF-1 is able to inhibit the progression of pyruvate into the TCA cycle by redirecting it to lactate production via activation of pyruvate dehydrogenase kinase [153-155]. Furthermore, HIF-1 can induce glycolytic enzymes while reducing mitochondrial oxygen consumption [155]. Such a switch exists to preserve the viability of cells in times of hypoxia. Interestingly, a similar change occurs in activated lymphocytes, as discussed above. The metabolic similarity between hypoxic cells and lymphocytes begs the question of whether or not HIF-1 plays a role in modulating T cell activation. Hypoxic areas within the body create a need for immune cells to survive and function properly in all environments, hence the importance of HIF-1 activation [40]. Under hypoxic conditions and anaerobic glycolysis, specifically during wound healing, T cells will shift from T_H1 to T_H2 type responses, directing a more anti-inflammatory function in the absence of oxygen [156]. In mice prone to type 2 diabetes, decreased levels of HIF-1 indeed lead to impaired wound healing [157].

Besides hypoxic conditions, HIF-1 is able to induce expression of genes that improve immune cell viability during aerobic glycolysis [75]. The expression of HIF-1 is initially provoked by insulin, IGF1 (insulin-like growth factor 1), and angiotensin, all of which play roles in growth and survival [158, 159]. HIF-1 is increased in activated T cells and promotes expression of Glut1, aiding in T cell survival [40, 160]. In addition to its importance in maintaining T cell viability, HIF-1 also helps regulate T cell subset differentiation. T_H17 cell differentiation requires enhanced glycolysis and expression of the transcription factor RORγT, both of which are increased via HIF-1 activation [161, 162]. Furthermore, HIF-1 is known to directly repress Foxp3, the transcription factor critical for Treg induction [161]. Tregs, unlike other T cell subsets, are primarily powered through lipid oxidation [85]. Likewise, lipid metabolism, which would mainly utilize oxidative phosphorylation, can inhibit glycolytic-dependent T_H17 differentiation [163].

HIF-1 also plays important roles in controlling innate cell functions [153]. ATP, glycolytic enzymes and Glut1 expression are all regulated by HIF-1 in macrophages and neutrophils. Under hypoxic conditions, APC phagocytosis and antigen presentation as well as granulocyte responses are weakened [164, 165]. Upon HIF-1-deficiency, innate cell motility, invasiveness, pathogen killing and T cell-stimulating abilities decrease even more so [49, 166]. Without effective HIF-1 expression, APCs and granulocytes suffer dysfunctional host defenses. On the contrary, chronic inflammation and HIF-1 may together instigate tissue fibrosis, autoimmunity and tumor progression by affecting both innate and adaptive immune cells.

In chronic kidney disease and obesity, HIF-1 can switch from its proangiogenic function to promote fibrosis [167, 168]. In relation to HIF-1's ability to modulate T_H17 differentiation, mice deficient for HIF-1 are resistant to the inducible experimental autoimmune encephalomyelitis (EAE) [161, 162], a rodent model of multiple sclerosis in which disease is largely mediated by T_H17 cells. Furthermore, HIF-1 has been shown to play major roles in prostate cancer tumorigenesis [169], breast cancer prognosis [170], and many other cancer outcomes [171], through induction of genes responsible for cell proliferation, angiogenesis, survival, migration, and glucose metabolism [172]. Immune cells play crucial roles in mediating appropriate wound healing, tolerating self-antigens, and cytotoxic killing of tumor cells. Therefore, blocking HIF-1 may allow for appropriate immunity and alleviation of disease. The list of HIF-1 inhibitors is expanding [171]. For example, digoxin, which inhibits HIF-1 gene and protein expression, can block tumor growth [173] as well as RORγt-dependent T_H17 differentiation [174], yet does not affect other T cell lineages. It is tempting then to speculate that while blockade of HIF-1 may shunt proliferation and T_H17 cells, cytotoxic CD8 T cells may still be active to allow for killing of tumors. Conversely, since HIF-1 is known to repress Treg differentiation, inhibition of this molecule may augment suppressive T cells. In the context of autoimmunity, this side effect may be beneficial in protecting against self-antigen recognition. However, in the case of cancer, combinatorial therapies of digoxin along with chemotherapy and radiation may be necessary for complete regression.

Uncoupling proteins

For oxidative phosphorylation to occur properly, collaboration between electron transfer and proton pumping is a necessity. A disturbance in the 'coupling' of electrons to protons would lead to an increase of futile proton current, decreased ATP production, and diminished ROS levels. In the context of immune cell mitochondrial dysfunction, such as in Alzheimer's and diabetes, an interruption of oxidative phosphorylation may be beneficial for reduction of ROS byproducts. Endogenously, certain proteins exist to manifest this disruption. Uncoupling proteins (UCP) are known proton uniporters that, in the context of a proper activator, can uncouple mitochondrial respiration in a controlled way [175, 176]. Such processes are used for thermogenesis from brown adipose tissue [177] and for reducing the production of free radicals from mitochondria [178]. UCP2 has been suggested to decrease pyruvate entry into the TCA cycle [179, 180], overall limiting ROS production and age-related damage [181-184]. Overexpression of UCP3 in a high-fat diet fed mouse was able to rescue insulin signaling [119] and knockout of UCP2 drives persistent NF-κB activation as well as heightened ROS production in immune cells, resulting in resistance to certain infections [185, 186]. These data reveal UCP2 as a plausible immunometabolic therapeutic target. T cells produce high levels of mitochondrial UCP2 following activation; this has been attributed to the need for rapid proliferation via glycolysis as well as the necessity of low level ROS production for adequate gene expression and signaling activity [187]. In an oxidative stress environment, where interplay between innate immune cells and T cells generates high ROS, uncoupling the electron transport chain may be useful in resetting homeostasis. Moreover, UCP2 decreases glucose-stimulated insulin release [188], highlighting its potential for preventing the release of self-antigen and thus controlling autoreactive T cell responses. To date, uncouplers have not been utilized in the treatment of autoreactive T cells. However, chemical uncouplers do demonstrate abilities in reducing oxidative stress. Rottlerin, a mitochondrial uncoupler, can reduce apoptosis of alveolar macrophages in a model of systemic autoimmune disease [189]. A study utilizing a mitochondrial fission inhibitor, which led to greater uncoupling, normalized oxidative stress levels in hyperglycemia [190]. Another drug, 2,4-dinitrophenol, leads to uncoupling through dissipation of the proton gradient, resulting in decreased hepatic insulin resistance in a non-alcoholic fatty liver disease model [191]. Furthermore, 2,4-dinitrophenol has been shown to enhance the adhesion phenotype (increased collagen and VEGF) for post-peritoneal surgical wound healing [192], a process in which macrophages play an important role [193]. Uncouplers targeting specific cells, therefore, may be a potential therapeutic for immune diseases where oxidative stress is high, whereas uncoupling inhibitors may be utilized for cancer, especially those which are resistant to chemotherapy [194, 195].

Nutrient limitation

The immune system is highly dependent on the glucose levels available. Everyday physiological nutrient limitation throughout the periphery protects from the over accumulation of naïve T cells, allowing for the turnover of older cells and development of new [61]. Likewise, the adaptive immune response also relies on the availability of sufficient amino acids. Innate immune cells can control the supply of amino acids and thus, regulate T cell responsiveness. Upon CD40 ligation or LPS stimulation, APCs can increase their cysteine production and share with interacting T cells, which cannot make their own [196, 197]. Cysteine is critical for T cell survival due to its necessity in glutathione production [198, 199]. Similarly, indoleamine 2,3-dioxygenase (IDO) expression by macrophages can reduce extracellular tryptophan levels, which are also needed by, but not directly produced by T cells. A lack of tryptophan will eventually cause T cell anergy or apoptosis [200, 201], again suppressing an immune response.

In a similar manner, environmental nutrient limitation may also affect an immune response. Caloric restriction causes greater lipid metabolism, lessening the dependence on glucose and thus decreasing an immune response [202]. Lipid metabolism is the chief mechanism of energy production in immune-privileged sites, such as the eye, brain, and placenta. Fatty acid utilization leads to lower costimulatory molecules, resistance to apoptosis, and less damage by free radicals [202-204], correlating with

blunted immune responses. In contrast, immune-sensitive areas that depend on glucose metabolism are more susceptible to infection and death following an effective immune response [69, 71, 205]. Notably, saturated and unsaturated fatty acids also differ in their abilities to stimulate an immune response. Saturated fatty acids, which are considered more detrimental to health, induce greater activation of TLR2 and TLR4 on myeloid cells, whereas unsaturated fatty acids can inhibit TLR signaling and NF-κB activation [206-208]. Particularly, polyunsaturated acids can alter the T cell membrane, negatively impacting signaling and activation of lymphocytes [209, 210]. Moreover, less caloric intake also correlates with better DNA repair, reduced antioxidant decline, diminished cancer rates, and an increase in lifespan [211-214]. In autoimmunity and metabolic syndrome, such confines may be highly effective in quelling inflammation [215, 216]. Specifically, obesity has been associated with dysfunctional phagocytosis and respiratory burst in macrophages [217, 218]. Similar to leptin insensitivity, in obesity, continuous ingestion of saturated fatty acids eventually will lead to a reduction in innate and adaptive immune responses, making individuals more susceptible to cancer and infections [219] and reducing wound healing capacity [220, 221]. Caloric restriction of obese mice, which were at-risk for breast cancer, significantly reduced tumor growth and mimicked mTOR inhibition for regulating cell proliferation [222]. Nutrient limitation via endogenous and exogenous factors may therefore benefit a number of immune diseases with metabolic components.

Anti-glycolitics

Because of the high dependence of immune cells on glucose metabolism, anti-glycolytics have been implemented to limit immunity and treat disease. One of the most studied drugs is rapamycin. Rapamycin is able to inhibit glucose metabolism via blockade of mTOR downstream of PI3K-Akt. Such inhibition leads to decreased T and B cell activation and function as well as decreases cellular proliferation. Rapamycin has especially been used to treat advanced cancer [223, 224] and transplant/graft-versus-host disease patients to mitigate rejection [225-227]. At the immune cell level, rapamycin can affect T cell differentiation and memory. Rapamycin treatment enhanced the quality and quantity of CD8 T cell memory responses by switching metabolism from glycolysis to oxidative phosphorylation [86]. Rapamycin can also mimic dietary restriction, increasing life span [228] as shown additionally in memory T cells [58, 86]. Thus, caloric reduction and fat metabolism discussed above may be used as alternatives to rapamycin and may also improve T cell memory [60, 229]. In CD4 T cells, rapamycin can instigate CD4 Treg development [230-232] as well as modulate chemokine receptors for mobilizing effector cells out of the periphery and back to the lymphoid organs [233]. The immunosuppressive effects of rapamycin, however, have been associated with higher infection rates [234, 235] as well as hyperglycemia, pertaining to its inhibition of the PI3K-Akt-mTOR signaling pathway required for Glut1 expression and translocation to the plasma membrane [236, 237], making it more suitable as a metabolic treatment for chronic inflammation and organ transplantation.

3-bromopyruvate is another anti-glycolytic drug preferentially used for the treatment of cancer. During glycolysis, a series of enzymes are necessary for the breakdown of glucose into pyruvate. 3-bromopyruvate is able to inhibit the activity of the first enzyme in the glycolytic pathway, hexokinase [238]. Hexokinase is often overexpressed in tumors and contributes to the high glycolytic activity seen in cancerous cells [24, 25]. Treatment of tumor cells with 3-bromopyruvate drains intracellular ATP levels, resulting in cell death [239]. Although systemic treatment may be detrimental to actively proliferating and cytokine-secreting T cells, intra-tumoral treatment may allow for the induction of anti-tumor CD8 memory T cells, since a reversal of metabolism from glycolysis to oxidative phosphorylation is necessary for the maintenance of adaptive memory [59, 89]. Additionally, 3-bromopyruvate is quite specific for cancer cells, with little to no toxicity of healthy tissue [240, 241]. Therefore, eradication of the cancerous cell growth with 3-bromopyruvate may diminish the suppressive microenvironment surrounding the tumor, allowing for greater infiltration of cytotoxic killer cells and subsequent tumor immunity – an area of research that still requires investigation.

Anti-mitochondrial drugs

Blocking different mitochondrial complexes along the electron transport chain can manifest in phagocytic defects [242] and NF-κB inactivation [243]. These types of treatments are standard in lab settings; however, drugs that can be used in the clinic need to be better characterized. Metformin, for example, is an anti-oxidative phosphorylation drug and is predominantly used as an anti-diabetic drug. It enhances glucose disposal in muscle and reduces hepatic gluconeogenesis. Additionally, metformin was shown to enhance the levels of UCP2 [244]. Unlike rapamycin, which directly blocks mTOR, metformin can activate AMP-activated protein kinase (AMPK), which in turn blocks mTOR function, inhibiting cell proliferation [245, 246]. AMPK activation induces glucose uptake from the blood, increasing glycolysis and lessening the dependence of cells on oxygen [247]. Metformin can specifically impede complex I of the electron transport chain and inhibit oxygen consumption in cancer cells [248, 249]. Particularly, metformin usage for cancer therapy has been widely studied in those with type 2 diabetes [250, 251], a known risk factor for tumor formation [252]. Like the anti-glycolytics mentioned previously, reductions in cancer cell growth may permit the cytotoxic killing of tumor cells by T cells, albeit when treatment is administered directly to the tumor. The impact of metformin has also been characterized in a mouse model of cancer vaccination. Upon metformin treatment of ova-specific T cells, a significant enhancement of memory CD8 T cells was detected along with better tumor regression. These results depict the ability of metformin to modulate the immune system outside of its anti-proliferative effects. Additionally, in the context of proinflammatory cytokines, metformin is able to block the activation of NF-κB, resulting in diminished cytokine-induced endothelial cell adhesion molecule expression [253, 254]. Such an effect may lead to decreases in chronic macrophage infiltration into the adipocytes of type 2 diabetics as well as lower the damaging consequences of autoimmunity. Of course, better understanding of metformin's selective effects on the immune system is necessary for these types of indications.

As another approach to metabolic control for immune regulation, antioxidants have been utilized in a plethora of disease models, including autoimmunity, infections, neurodegeneration, and cancer [255-259]. Although antioxidants do not specifically block oxidative phosphorylation, they are important for decreasing ROS produced from the electron transport chain during mitochondrial respiration, thus reducing damaging side effects. During aerobic respiration, the glutathione transported from the cytosol into the mitochondrial membrane is the only antioxidant available for metabolizing H_2O_2 [260]. Therefore, the augmentation of antioxidants present in immune cells may alleviate certain cellular dysfunctions. Specifically, antioxidants are able to show improvement of immune-mediated disorders, such as lymphocyte and macrophage function in the face of aging, septic shock, asthma, and type 1 diabetes [259, 261-264]. The mechanisms by which antioxidants improve the immune system vary greatly. An antioxidant present in green tea called epigallocatechin-3-gallate can reduce T cell signaling via downregulation of cytokine receptors [265] as well as ameliorate EAE through an enhancement of regulatory T cells [266]. Other studies demonstrated the antioxidant ability of resveratrol to decrease collagen-induced arthritis by suppressing T_H17 responses [267] and to enhance B cell lymphoma recognition by CD4+ T cells through upregulation of HLA class II molecules [268]. Consistently, other antioxidants have shown promise in restricting NF-κB activation [269-271], leading to many anti-inflammatory effects. On the metabolic side, a recent study utilizing α-tocopherol, the antioxidant component of Vitamin E, demonstrated a reduction in glycolysis in lymphoma cells through blockade of lactate dehydrogenase activity [272].

Our group focuses on the usage of manganese porphyrins as catalytic antioxidants for the scavenging of ROS (superoxide, peryoxynitrite) as well as the mimicking of superoxide dismutase [259, 273]. Unlike other antioxidants, the metalloporphyrins are catalytic and can repetitively eliminate ROS, resulting in many immunological effects. These antioxidants have shown promise in reducing type 1 diabetes incidence through autoreactive T_H1 cell modulation [259, 274, 275] and in protecting islets during isolation for transplantation [276, 277]. Furthermore, NF-κB activation [271] and CD8 T cell effector function [278] are reduced

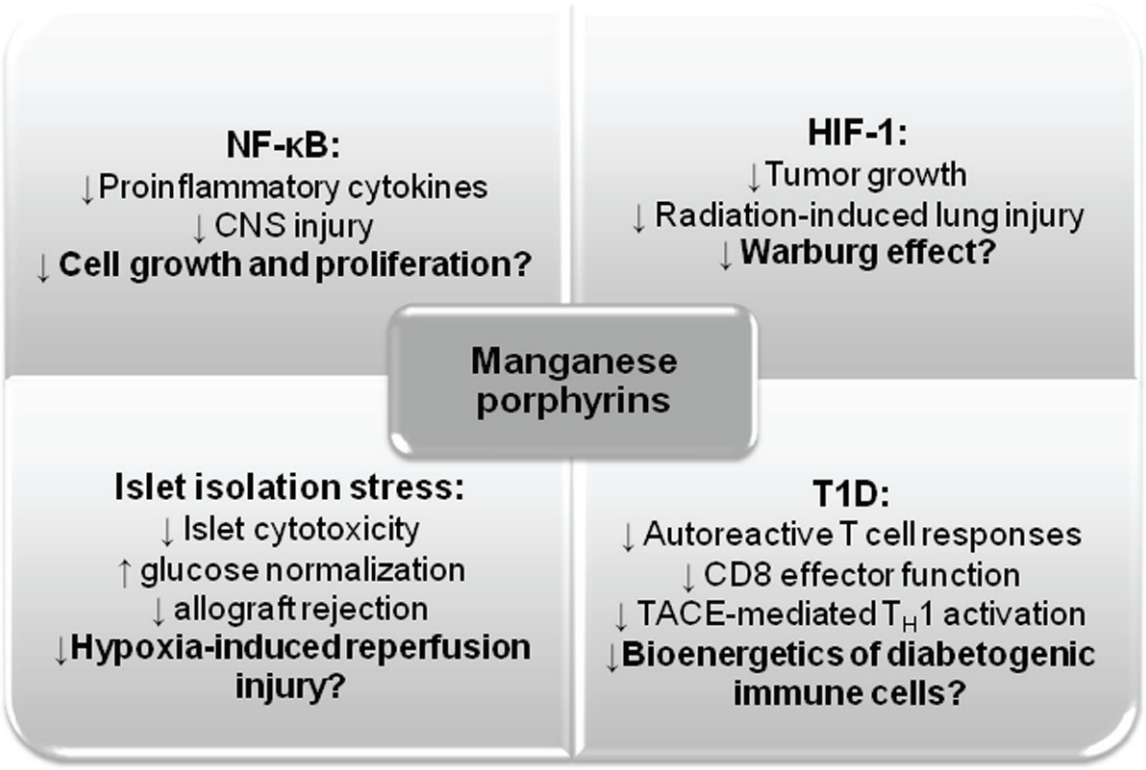

Figure 2. Metabolic modulation potential of manganese porphyrins. Manganese porphyrins, or catalytic antioxidants, have been shown to decrease NF-κB and HIF-1 activation, reduce T cell-mediated type 1 diabetes progression/onset, and maintain islet cell function during isolation and transplantation. Proposed metabolic effects of metalloporphyrins include decreasing: cell growth, hypoxia-induced reperfusion injury, the Warburg effect, and bioenergetics of diabetogenic immune cells.

upon metalloporphyrin treatment. Inhibition of NF-κB after metalloporphyrin administration has also shown promise in decreasing acute central nervous system injury, effectively enhancing neurologic function following ischemic stroke [279]. Additionally, metalloporphyrins can protect lungs from radiation-induced injury via HIF-1 inactivation [280-282] and kidneys from ischemia/reperfusion injury through induction of ATP synthase [283]. Interestingly, metalloporphyrins display oxidoreductase abilities, where they can act as a scavenger in the cytoplasm, as in the context of reduced TACE oxidation [274], yet work as an oxidizer in the nucleus, inhibiting the reduction of the p50 subunit of NF-κB and effectively blocking DNA binding [271]. In the context of cancer, metalloporphyrins are also able to block HIF-1 activation, decrease hypoxia, reduce tumor-protective cytokine release and ultimately suppress tumor growth [284]. HIF-1, as mentioned above, is critical for facilitating glycolysis in times of low oxygen; moreover, tumor cells rely heavily on glycolysis to survive, making them an obvious target of metalloporphyrin-induced regulation. The wide scope of metalloporphyrin effectiveness allows for their usage in a range of immunologic diseases, all centered around restoring redox balance; yet the effect of metalloporphyrins on fundamental immune cell metabolism has yet to be described (**Figure 2**). In conjunction with the cancer studies, we have observed promotion of hyporesponsive T cells after antioxidant treatment [274, 285]. Preliminary studies suggest decreases in aerobic glycolysis following metalloporphyrin administration. This metabolic reduction may then decrease T cell differentiation and return cells to stasis or quiescence, all while retaining viability, as metalloporphyrins are not toxic [273, 276, 278]. If T cells are in fact displaying lowered aerobic glycolysis, the potential for treating chronic inflammatory conditions, such as autoimmunity, is widespread. With frontline therapeutics, such as anti-metabolites, rapamycin, and antibodies against costimula-

tory molecules, either failing in the clinic or leading to unwanted side effects, agents that modulate immune function, have mild side effects, and show no toxicity are highly sought after. Metalloporphyrins meet all of these demands and therefore, may be beneficial for reducing inflammatory disorders/potentiating cancer regression and should elicit greater attention in the search for alternative metabolic therapies.

Summary

The immune system plays a vital role in maintaining a fine balance in the battle against infections and cancer, but requires rigorous control in order to walk the fine line of regulator and menace leading to self-reactivity and autoimmunity. Both oxidative phosphorylation and glycolysis are critical for fulfilling the metabolic needs of immune cells. In innate immune cells, glycolysis is the predominant form of metabolism, whereas adaptive immune cells fluctuate between oxidative phosphorylation and glycolysis, depending on their activation status. Nonetheless, the heavy reliance of immune cells on glucose utilization makes them good targets for immunometabolic therapies. A number of endogenous molecules can be pursued, including HIF-1 and UCP2. Alternatively, caloric restriction, anti-glycolytics, and antioxidants all exhibit potential in resetting homeostasis in chronic inflammation while possibly enhancing immunity in cancer models. Overall, metabolic regulation should be an active line of research for the control of immune-mediated disorders.

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References

[1] Da Poian AT, El-Bacha T and Luz MR. Nutrient Utilization in Humans: Metabolism Pathways. Nature Education 2010; 3: 11.

[2] Ames BN, Shigenaga MK and Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993; 90: 7915-7922.

[3] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44-84.

[4] Roth S and Droge W. Regulation of T-cell activation and T-cell growth factor (TCGF) production by hydrogen peroxide. Cell Immunol 1987; 108: 417-424.

[5] Karlsson H and Nassberger L. In vitro metabolic inhibition of the human lymphocyte: influence on the expression of interleukin-2 receptors. Immunol Cell Biol 1992; 70: 309-313.

[6] Schreck R and Baeuerle PA. A role for oxygen radicals as second messengers. Trends Cell Biol 1991; 1: 39-42.

[7] Lo YY, Wong JM and Cruz TF. Reactive oxygen species mediate cytokine activation of c-Jun NH2-terminal kinases. J Biol Chem 1996; 271: 15703-15707.

[8] Monteiro HP and Stern A. Redox modulation of tyrosine phosphorylation-dependent signal transduction pathways. Free Radic Biol Med 1996; 21: 323-333.

[9] Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82: 47-95.

[10] Baynes JW and Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999; 48: 1-9.

[11] Schonfeld P and Wojtczak L. Fatty acids as modulators of the cellular production of reactive oxygen species. Free Radic Biol Med 2008; 45: 231-241.

[12] Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC and Rabinovitch PS. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 2005; 308: 1909-1911.

[13] Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005; 39: 359-407.

[14] Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, Zhuang H, Cinalli RM, Alavi A, Rudin CM and Thompson CB. Akt stimulates aerobic glycolysis in cancer cells. Cancer Res 2004; 64: 3892-3899.

[15] Hockel M and Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001; 93: 266-276.

[16] Koukourakis MI, Giatromanolaki A, Harris AL and Sivridis E. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. Cancer Res 2006; 66: 632-637.

[17] Chen M, Zhang J and Manley JL. Turning on a fuel switch of cancer: hnRNP proteins regulate alternative splicing of pyruvate kinase mRNA. Cancer Res 2010; 70: 8977-8980.

[18] Jose C, Bellance N and Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Biochim Biophys Acta 2011; 1807: 552-561.

[19] Gatenby RA and Gawlinski ET. The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models. Cancer Res 2003; 63: 3847-3854.

[20] Gatenby RA and Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004; 4: 891-899.

[21] Gillies RJ, Robey I and Gatenby RA. Causes and consequences of increased glucose metabolism of cancers. J Nucl Med 2008; 49 Suppl 2: 24S-42S.

[22] Jones RG and Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev 2009; 23: 537-548.

[23] Koppenol WH, Bounds PL and Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer 2011; 11: 325-337.

[24] Bustamante E and Pedersen PL. High aerobic glycolysis of rat hepatoma cells in culture: role of mitochondrial hexokinase. Proc Natl Acad Sci U S A 1977; 74: 3735-3739.

[25] Pedersen PL, Mathupala S, Rempel A, Geschwind JF and Ko YH. Mitochondrial bound type II hexokinase: a key player in the growth and survival of many cancers and an ideal prospect for therapeutic intervention. Biochim Biophys Acta 2002; 1555: 14-20.

[26] Warburg O. On respiratory impairment in cancer cells. Science 1956; 124: 269-270.

[27] Lee SC, Marzec M, Liu X, Wehrli S, Kantekure K, Ragunath PN, Nelson DS, Delikatny EJ, Glickson JD and Wasik MA. Decreased lactate concentration and glycolytic enzyme expression reflect inhibition of mTOR signal transduction pathway in B-cell lymphoma. NMR Biomed 2013; 26: 106-14.

[28] Liu Y, Cao Y, Zhang W, Bergmeier S, Qian Y, Akbar H, Colvin R, Ding J, Tong L, Wu S, Hines J and Chen X. A Small-Molecule Inhibitor of Glucose Transporter 1 Downregulates Glycolysis, Induces Cell-Cycle Arrest, and Inhibits Cancer Cell Growth In Vitro and In Vivo. Mol Cancer Ther 2012; 11: 1672-82.

[29] Suh DH, Kim MK, No JH, Chung HH and Song YS. Metabolic approaches to overcoming chemoresistance in ovarian cancer. Ann N Y Acad Sci 2011; 1229: 53-60.

[30] Krauss S, Brand MD and Buttgereit F. Signaling takes a breath--new quantitative perspectives on bioenergetics and signal transduction. Immunity 2001; 15: 497-502.

[31] Buttgereit F, Burmester GR and Brand MD. Bioenergetics of immune functions: fundamental and therapeutic aspects. Immunol Today 2000; 21: 192-199.

[32] Frauwirth KA and Thompson CB. Regulation of T lymphocyte metabolism. J Immunol 2004; 172: 4661-4665.

[33] Crabtree GR and Clipstone NA. Signal transmission between the plasma membrane and nucleus of T lymphocytes. Annu Rev Biochem 1994; 63: 1045-1083.

[34] Doughty CA, Bleiman BF, Wagner DJ, Dufort FJ, Mataraza JM, Roberts MF and Chiles TC. Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. Blood 2006; 107: 4458-4465.

[35] Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, Elstrom RL, June CH and Thompson CB. The CD28 signaling pathway regulates glucose metabolism. Immunity 2002; 16: 769-777.

[36] Fox CJ, Hammerman PS and Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 2005; 5: 844-852.

[37] Ouiddir A, Planes C, Fernandes I, VanHesse A and Clerici C. Hypoxia upregulates activity and expression of the glucose transporter GLUT1 in alveolar epithelial cells. Am J Respir Cell Mol Biol 1999; 21: 710-718.

[38] Schuler G and Steinman RM. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J Exp Med 1985; 161: 526-546.

[39] Winter GD. Oxygen and epidermal wound healing. Adv Exp Med Biol 1977; 94: 673-678.

[40] Sitkovsky M and Lukashev D. Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. Nat Rev Immunol 2005; 5: 712-721.

[41] von Andrian UH and Mackay CR. T-cell function and migration. Two sides of the same coin. N Engl J Med 2000; 343: 1020-1034.

[42] Arnold F, West D and Kumar S. Wound healing: the effect of macrophage and tumour derived angiogenesis factors on skin graft vascularization. Br J Exp Pathol 1987; 68: 569-574.

[43] Helmlinger G, Yuan F, Dellian M and Jain RK. Interstitial pH and pO2 gradients in solid tu-

mors in vivo: high-resolution measurements reveal a lack of correlation. Nat Med 1997; 3: 177-182.
[44] Simmen HP, Battaglia H, Giovanoli P and Blaser J. Analysis of pH, pO2 and pCO2 in drainage fluid allows for rapid detection of infectious complications during the follow-up period after abdominal surgery. Infection 1994; 22: 386-389.
[45] Masopust D, Vezys V, Marzo AL and Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. Science 2001; 291: 2413-2417.
[46] Roman E, Miller E, Harmsen A, Wiley J, Von Andrian UH, Huston G and Swain SL. CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. J Exp Med 2002; 196: 957-968.
[47] Pfeiffer T, Schuster S and Bonhoeffer S. Cooperation and competition in the evolution of ATP-producing pathways. Science 2001; 292: 504-507.
[48] Borregaard N and Herlin T. Energy metabolism of human neutrophils during phagocytosis. J Clin Invest 1982; 70: 550-557.
[49] Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N and Johnson RS. HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 2003; 112: 645-657.
[50] Land SC and Hochachka PW. Protein turnover during metabolic arrest in turtle hepatocytes: role and energy dependence of proteolysis. Am J Physiol 1994; 266: C1028-1036.
[51] Princiotta MF, Finzi D, Qian SB, Gibbs J, Schuchmann S, Buttgereit F, Bennink JR and Yewdell JW. Quantitating protein synthesis, degradation, and endogenous antigen processing. Immunity 2003; 18: 343-354.
[52] Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, Cross JR, Jung E, Thompson CB, Jones RG and Pearce EJ. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood 2010; 115: 4742-4749.
[53] Lacy-Hulbert A and Moore KJ. Designer macrophages: oxidative metabolism fuels inflammation repair. Cell Metab 2006; 4: 7-8.
[54] Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ and Chawla A. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab 2006; 4: 13-24.
[55] Healy DA, Watson RW and Newsholme P. Glucose, but not glutamine, protects against spontaneous and anti-Fas antibody-induced apoptosis in human neutrophils. Clin Sci (Lond) 2002; 103: 179-189.
[56] Savill JS, Wyllie AH, Henson JE, Walport MJ, Henson PM and Haslett C. Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. J Clin Invest 1989; 83: 865-875.
[57] Chang JT, Palanivel VR, Kinjyo I, Schambach F, Intlekofer AM, Banerjee A, Longworth SA, Vinup KE, Mrass P, Oliaro J, Killeen N, Orange JS, Russell SM, Weninger W and Reiner SL. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. Science 2007; 315: 1687-1691.
[58] Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG and Choi Y. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 2009; 460: 103-107.
[59] van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, Pearce EJ and Pearce EL. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 2012; 36: 68-78.
[60] Pearce EL. Metabolism in T cell activation and differentiation. Curr Opin Immunol 2010; 22: 314-320.
[61] Rathmell JC, Vander Heiden MG, Harris MH, Frauwirth KA and Thompson CB. In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. Mol Cell 2000; 6: 683-692.
[62] Brocker T. Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class II-expressing dendritic cells. J Exp Med 1997; 186: 1223-1232.
[63] Yusuf I and Fruman DA. Regulation of quiescence in lymphocytes. Trends Immunol 2003; 24: 380-386.
[64] Schmid D, Burmester GR, Tripmacher R, Kuhnke A and Buttgereit F. Bioenergetics of human peripheral blood mononuclear cell metabolism in quiescent, activated, and glucocorticoid-treated states. Biosci Rep 2000; 20: 289-302.
[65] Valcourt JR, Lemons JM, Haley EM, Kojima M, Demuren OO and Coller HA. Staying alive: metabolic adaptations to quiescence. Cell Cycle 2012; 11: 1680-1696.
[66] Lum JJ, DeBerardinis RJ and Thompson CB. Autophagy in metazoans: cell survival in the land of plenty. Nat Rev Mol Cell Biol 2005; 6: 439-448.
[67] Yang Z and Klionsky DJ. Eaten alive: a history of macroautophagy. Nat Cell Biol 2010; 12: 814-822.
[68] Maciver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL and Rathmell JC. Glucose metabolism in lymphocytes is a regulated process with

significant effects on immune cell function and survival. J Leukoc Biol 2008; 84: 949-957.
[69] Plas DR and Thompson CB. Cell metabolism in the regulation of programmed cell death. Trends Endocrinol Metab 2002; 13: 75-78.
[70] Wieman HL, Wofford JA and Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. Mol Biol Cell 2007; 18: 1437-1446.
[71] Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM and Thompson CB. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. Mol Cell Biol 2003; 23: 7315-7328.
[72] van Stipdonk MJ, Hardenberg G, Bijker MS, Lemmens EE, Droin NM, Green DR and Schoenberger SP. Dynamic programming of CD8+ T lymphocyte responses. Nat Immunol 2003; 4: 361-365.
[73] Culvenor JG and Weidemann MJ. Phytohaemagglutinin stimulation of rat thymus lymphocytes glycolysis. Biochim Biophys Acta 1976; 437: 354-363.
[74] Hedeskov CJ. Early effects of phytohaemagglutinin on glucose metabolism of normal human lymphocytes. Biochem J 1968; 110: 373-380.
[75] Lu H, Forbes RA and Verma A. Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. J Biol Chem 2002; 277: 23111-23115.
[76] Vander Heiden MG, Cantley LC and Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324: 1029-1033.
[77] Marelli-Berg FM, Fu H and Mauro C. Molecular mechanisms of metabolic reprogramming in proliferating cells: implications for T-cell-mediated immunity. Immunology 2012; 136: 363-369.
[78] Brand K, Von Hintzenstern J, Langer K and Fekl W. Pathways of glutamine and glutamate metabolism in resting and proliferating rat thymocytes: comparison between free and peptide-bound glutamine. J Cell Physiol 1987; 132: 559-564.
[79] Buttgereit F, Brand MD and Muller M. ConA induced changes in energy metabolism of rat thymocytes. Biosci Rep 1992; 12: 381-386.
[80] Buttgereit F and Brand MD. A hierarchy of ATP-consuming processes in mammalian cells. Biochem J 1995; 312: 163-167.
[81] Duchen MR. Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. J Physiol 1999; 516: 1-17.
[82] Wahl DR, Petersen B, Warner R, Richardson BC, Glick GD and Opipari AW. Characterization of the metabolic phenotype of chronically activated lymphocytes. Lupus 2010; 19: 1492-1501.
[83] Gatza E, Wahl DR, Opipari AW, Sundberg TB, Reddy P, Liu C, Glick GD and Ferrara JL. Manipulating the bioenergetics of alloreactive T cells causes their selective apoptosis and arrests graft-versus-host disease. Sci Transl Med 2011; 3: 67ra68.
[84] Gergely P Jr, Grossman C, Niland B, Puskas F, Neupane H, Allam F, Banki K, Phillips PE and Perl A. Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. Arthritis Rheum 2002; 46: 175-190.
[85] Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, Sullivan SA, Nichols AG and Rathmell JC. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J Immunol 2011; 186: 3299-3303.
[86] Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP and Ahmed R. mTOR regulates memory CD8 T-cell differentiation. Nature 2009; 460: 108-112.
[87] Choi SW, Gerencser AA and Nicholls DG. Bioenergetic analysis of isolated cerebrocortical nerve terminals on a microgram scale: spare respiratory capacity and stochastic mitochondrial failure. J Neurochem 2009; 109: 1179-1191.
[88] Nicholls DG. Spare respiratory capacity, oxidative stress and excitotoxicity. Biochem Soc Trans 2009; 37: 1385-1388.
[89] Alves NL, Derks IA, Berk E, Spijker R, van Lier RA and Eldering E. The Noxa/Mcl-1 axis regulates susceptibility to apoptosis under glucose limitation in dividing T cells. Immunity 2006; 24: 703-716.
[90] Yamashita M, Kuwahara M, Suzuki A, Hirahara K, Shinnaksu R, Hosokawa H, Hasegawa A, Motohashi S, Iwama A and Nakayama T. Bmi1 regulates memory CD4 T cell survival via repression of the Noxa gene. J Exp Med 2008; 205: 1109-1120.
[91] Harding FA, McArthur JG, Gross JA, Raulet DH and Allison JP. CD28-mediated signalling costimulates murine T cells and prevents induction of anergy in T-cell clones. Nature 1992; 356: 607-609.
[92] Wells AD. New insights into the molecular basis of T cell anergy: anergy factors, avoidance sensors, and epigenetic imprinting. J Immunol 2009; 182: 7331-7341.
[93] Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N and Glimcher LH. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2

developmental pathways: application to autoimmune disease therapy. Cell 1995; 80: 707-718.
[94] Sperling AI and Bluestone JA. The complexities of T-cell co-stimulation: CD28 and beyond. Immunol Rev 1996; 153: 155-182.
[95] Matarese G and La Cava A. The intricate interface between immune system and metabolism. Trends Immunol 2004; 25: 193-200.
[96] Rathmell JC, Elstrom RL, Cinalli RM and Thompson CB. Activated Akt promotes increased resting T cell size, CD28-independent T cell growth, and development of autoimmunity and lymphoma. Eur J Immunol 2003; 33: 2223-2232.
[97] Grumont R, Lock P, Mollinari M, Shannon FM, Moore A and Gerondakis S. The mitogen-induced increase in T cell size involves PKC and NFAT activation of Rel/NF-kappaB-dependent c-myc expression. Immunity 2004; 21: 19-30.
[98] Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J and Green DR. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity 2011; 35: 871-882.
[99] Wofford JA, Wieman HL, Jacobs SR, Zhao Y and Rathmell JC. IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. Blood 2008; 111: 2101-2111.
[100] Vander Heiden MG, Plas DR, Rathmell JC, Fox CJ, Harris MH and Thompson CB. Growth factors can influence cell growth and survival through effects on glucose metabolism. Mol Cell Biol 2001; 21: 5899-5912.
[101] Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ and Rathmell JC. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. J Immunol 2008; 180: 4476-4486.
[102] Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW and Cantley LC. Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha. Science 1999; 283: 393-397.
[103] Delgoffe GM and Powell JD. mTOR: taking cues from the immune microenvironment. Immunology 2009; 127: 459-465.
[104] Dann SG and Thomas G. The amino acid sensitive TOR pathway from yeast to mammals. FEBS Lett 2006; 580: 2821-2829.
[105] Howell JJ and Manning BD. mTOR couples cellular nutrient sensing to organismal metabolic homeostasis. Trends Endocrinol Metab 2011; 22: 94-102.
[106] Brunn GJ, Hudson CC, Sekulic A, Williams JM, Hosoi H, Houghton PJ, Lawrence JC Jr and Abraham RT. Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. Science 1997; 277: 99-101.
[107] Kay JE, Kromwel L, Doe SE and Denyer M. Inhibition of T and B lymphocyte proliferation by rapamycin. Immunology 1991; 72: 544-549.
[108] Brown NF, Stefanovic-Racic M, Sipula IJ and Perdomo G. The mammalian target of rapamycin regulates lipid metabolism in primary cultures of rat hepatocytes. Metabolism 2007; 56: 1500-1507.
[109] Sipula IJ, Brown NF and Perdomo G. Rapamycin-mediated inhibition of mammalian target of rapamycin in skeletal muscle cells reduces glucose utilization and increases fatty acid oxidation. Metabolism 2006; 55: 1637-1644.
[110] Powell JD, Lerner CG and Schwartz RH. Inhibition of cell cycle progression by rapamycin induces T cell clonal anergy even in the presence of costimulation. J Immunol 1999; 162: 2775-2784.
[111] Zheng Y, Collins SL, Lutz MA, Allen AN, Kole TP, Zarek PE and Powell JD. A role for mammalian target of rapamycin in regulating T cell activation versus anergy. J Immunol 2007; 178: 2163-2170.
[112] Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC and Powell JD. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity 2009; 30: 832-844.
[113] Finlay D and Cantrell D. Phosphoinositide 3-kinase and the mammalian target of rapamycin pathways control T cell migration. Ann N Y Acad Sci 2010; 1183: 149-157.
[114] Murooka TT, Rahbar R, Platanias LC and Fish EN. CCL5-mediated T-cell chemotaxis involves the initiation of mRNA translation through mTOR/4E-BP1. Blood 2008; 111: 4892-4901.
[115] Munk R, Ghosh P, Ghosh MC, Saito T, Xu M, Carter A, Indig F, Taub DD and Longo DL. Involvement of mTOR in CXCL12 mediated T cell signaling and migration. PLoS One 2011; 6: e24667.
[116] Hashimoto I, Koizumi K, Tatematsu M, Minami T, Cho S, Takeno N, Nakashima A, Sakurai H, Saito S, Tsukada K and Saiki I. Blocking on the CXCR4/mTOR signalling pathway induces the anti-metastatic properties and autophagic cell death in peritoneal disseminated gastric cancer cells. Eur J Cancer 2008; 44: 1022-1029.
[117] Wang J, Lu Y, Koch AE, Zhang J and Taichman RS. CXCR6 induces prostate cancer progression by the AKT/mammalian target of rapamycin signaling pathway. Cancer Res 2008; 68: 10367-10376.
[118] Sanchez-Alcazar JA, Hernandez I, De la Torre MP, Garcia I, Santiago E, Munoz-Yague MT and Solis-Herruzo JA. Down-regulation of tumor ne-

crosis factor receptors by blockade of mitochondrial respiration. J Biol Chem 1995; 270: 23944-23950.
[119] Choi CS, Fillmore JJ, Kim JK, Liu ZX, Kim S, Collier EF, Kulkarni A, Distefano A, Hwang YJ, Kahn M, Chen Y, Yu C, Moore IK, Reznick RM, Higashimori T and Shulman GI. Overexpression of uncoupling protein 3 in skeletal muscle protects against fat-induced insulin resistance. J Clin Invest 2007; 117: 1995-2003.
[120] Warne JP. Tumour necrosis factor alpha: a key regulator of adipose tissue mass. J Endocrinol 2003; 177: 351-355.
[121] Matsuki T, Horai R, Sudo K and Iwakura Y. IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. J Exp Med 2003; 198: 877-888.
[122] Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, Ohlsson C and Jansson JO. Interleukin-6-deficient mice develop mature-onset obesity. Nat Med 2002; 8: 75-79.
[123] Bauer DE, Harris MH, Plas DR, Lum JJ, Hammerman PS, Rathmell JC, Riley JL and Thompson CB. Cytokine stimulation of aerobic glycolysis in hematopoietic cells exceeds proliferative demand. FASEB J 2004; 18: 1303-1305.
[124] Cousin B, Munoz O, Andre M, Fontanilles AM, Dani C, Cousin JL, Laharrague P, Casteilla L and Penicaud L. A role for preadipocytes as macrophage-like cells. FASEB J 1999; 13: 305-312.
[125] Wellen KE and Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 2003; 112: 1785-1788.
[126] Takahashi K, Mizuarai S, Araki H, Mashiko S, Ishihara A, Kanatani A, Itadani H and Kotani H. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. J Biol Chem 2003; 278: 46654-46660.
[127] Ouchi N, Parker JL, Lugus JJ and Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011; 11: 85-97.
[128] Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S and Mathis D. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 2009; 15: 930-939.
[129] Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T and Nagai R. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009; 15: 914-920.
[130] Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH and Libby P. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Circ Res 2008; 103: 467-476.
[131] Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker DJ, Engleman E, Winer D and Dosch HM. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med 2009; 15: 921-929.
[132] Martin-Romero C, Santos-Alvarez J, Goberna R and Sanchez-Margalet V. Human leptin enhances activation and proliferation of human circulating T lymphocytes. Cell Immunol 2000; 199: 15-24.
[133] Fujita Y, Murakami M, Ogawa Y, Masuzaki H, Tanaka M, Ozaki S, Nakao K and Mimori T. Leptin inhibits stress-induced apoptosis of T lymphocytes. Clin Exp Immunol 2002; 128: 21-26.
[134] Fantuzzi G and Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukoc Biol 2000; 68: 437-446.
[135] La Cava A, Matarese G, Ebling FM and Hahn BH. Leptin-based immune intervention: current status and future directions. Curr Opin Investig Drugs 2003; 4: 1327-1332.
[136] Matarese G, Sanna V, Lechler RI, Sarvetnick N, Fontana S, Zappacosta S and La Cava A. Leptin accelerates autoimmune diabetes in female NOD mice. Diabetes 2002; 51: 1356-1361.
[137] Matarese G, Sanna V, Di Giacomo A, Lord GM, Howard JK, Bloom SR, Lechler RI, Fontana S and Zappacosta S. Leptin potentiates experimental autoimmune encephalomyelitis in SJL female mice and confers susceptibility to males. Eur J Immunol 2001; 31: 1324-1332.
[138] Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD and Diehl AM. Leptin regulates proinflammatory immune responses. FASEB J 1998; 12: 57-65.
[139] Scrimshaw NS and SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr 1997; 66: 464S-477S.
[140] Helderman JH. Role of insulin in the intermediary metabolism of the activated thymic-derived lymphocyte. J Clin Invest 1981; 67: 1636-1642.
[141] Viardot A, Grey ST, Mackay F and Chisholm D. Potential antiinflammatory role of insulin via the preferential polarization of effector T cells toward a T helper 2 phenotype. Endocrinology 2007; 148: 346-353.
[142] Stentz FB and Kitabchi AE. Activated T lymphocytes in Type 2 diabetes: implications from in vitro studies. Curr Drug Targets 2003; 4: 493-503.

[143] McCarron M, Osborne Y, Story CJ, Dempsey JL, Turner DR and Morley AA. Effect of age on lymphocyte proliferation. Mech Ageing Dev 1987; 41: 211-218.

[144] Feng J, Bussiere F and Hekimi S. Mitochondrial electron transport is a key determinant of life span in Caenorhabditis elegans. Dev Cell 2001; 1: 633-644.

[145] Rea S and Johnson TE. A metabolic model for life span determination in Caenorhabditis elegans. Dev Cell 2003; 5: 197-203.

[146] Knight JA. Review: Free radicals, antioxidants, and the immune system. Ann Clin Lab Sci 2000; 30: 145-158.

[147] Ames BN and Shigenaga MK. Oxidants are a major contributor to aging. Ann N Y Acad Sci 1992; 663: 85-96.

[148] De la Fuente M. Effects of antioxidants on immune system ageing. Eur J Clin Nutr 2002; 56 Suppl 3: S5-8.

[149] Stadtman ER. Protein oxidation and aging. Science 1992; 257: 1220-1224.

[150] Ding A and Nathan C. Analysis of the nonfunctional respiratory burst in murine Kupffer cells. J Exp Med 1988; 167: 1154-1170.

[151] Lepay DA, Nathan CF, Steinman RM, Murray HW and Cohn ZA. Murine Kupffer cells. Mononuclear phagocytes deficient in the generation of reactive oxygen intermediates. J Exp Med 1985; 161: 1079-1096.

[152] Reichner JS, Mulligan JA and Bodenheimer HC Jr. Electron transport chain activity in normal and activated rat macrophages. J Surg Res 1995; 59: 636-643.

[153] Dehne N and Brune B. HIF-1 in the inflammatory microenvironment. Exp Cell Res 2009; 315: 1791-1797.

[154] Kim JW, Tchernyshyov I, Semenza GL and Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006; 3: 177-185.

[155] Papandreou I, Cairns RA, Fontana L, Lim AL and Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 2006; 3: 187-197.

[156] Lederer JA, Rodrick ML and Mannick JA. The effects of injury on the adaptive immune response. Shock 1999; 11: 153-159.

[157] Mace KA, Yu DH, Paydar KZ, Boudreau N and Young DM. Sustained expression of Hif-1alpha in the diabetic environment promotes angiogenesis and cutaneous wound repair. Wound Repair Regen 2007; 15: 636-645.

[158] Richard DE, Berra E and Pouyssegur J. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. J Biol Chem 2000; 275: 26765-26771.

[159] Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW and Semenza GL. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000; 60: 1541-1545.

[160] Kominsky DJ, Campbell EL and Colgan SP. Metabolic shifts in immunity and inflammation. J Immunol 2010; 184: 4062-4068.

[161] Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR, Luo W, Zeller K, Shimoda L, Topalian SL, Semenza GL, Dang CV, Pardoll DM and Pan F. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell 2011; 146: 772-784.

[162] Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR and Chi H. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med 2011; 208: 1367-1376.

[163] Klotz L, Burgdorf S, Dani I, Saijo K, Flossdorf J, Hucke S, Alferink J, Nowak N, Beyer M, Mayer G, Langhans B, Klockgether T, Waisman A, Eberl G, Schultze J, Famulok M, Kolanus W, Glass C, Kurts C and Knolle PA. The nuclear receptor PPAR gamma selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS autoimmunity. J Exp Med 2009; 206: 2079-2089.

[164] Panther E, Corinti S, Idzko M, Herouy Y, Napp M, la Sala A, Girolomoni G and Norgauer J. Adenosine affects expression of membrane molecules, cytokine and chemokine release, and the T-cell stimulatory capacity of human dendritic cells. Blood 2003; 101: 3985-3990.

[165] Thiel M, Chouker A, Ohta A, Jackson E, Caldwell C, Smith P, Lukashev D, Bittmann I and Sitkovsky MV. Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. PLoS Biol 2005; 3: e174.

[166] Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG, Volke M, Glasner J, Warnecke C, Wiesener MS, Eckardt KU, Steinkasserer A, Hensel M and Willam C. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. J Immunol 2008; 180: 4697-4705.

[167] Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S, Wang ZV, Landskroner-Eiger S, Dineen S, Magalang UJ, Brekken RA and Scherer PE. Hypoxia-inducible factor 1alpha induces fibrosis and insulin re-

sistance in white adipose tissue. Mol Cell Biol 2009; 29: 4467-4483.

[168] Higgins DF, Kimura K, Iwano M and Haase VH. Hypoxia-inducible factor signaling in the development of tissue fibrosis. Cell Cycle 2008; 7: 1128-1132.

[169] Grebhardt S, Veltkamp C, Strobel P and Mayer D. Hypoxia and HIF-1 increase S100A8 and S100A9 expression in prostate cancer. Int J Cancer 2012; 131: 2785-94.

[170] Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S, Bersiga A, Allevi G, Milani M, Aguggini S, Gandolfi V, Dogliotti L, Bottini A, Harris AL and Fox SB. Hypoxia-inducible factor-1alpha expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. Clin Cancer Res 2006; 12: 4562-4568.

[171] Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 2010; 29: 625-634.

[172] Harris AL. Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer 2002; 2: 38-47.

[173] Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, Dang CV, Liu JO and Semenza GL. Digoxin and other cardiac glycosides inhibit HIF-1alpha synthesis and block tumor growth. Proc Natl Acad Sci U S A 2008; 105: 19579-19586.

[174] Huh JR, Leung MW, Huang P, Ryan DA, Krout MR, Malapaka RR, Chow J, Manel N, Ciofani M, Kim SV, Cuesta A, Santori FR, Lafaille JJ, Xu HE, Gin DY, Rastinejad F and Littman DR. Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORgammat activity. Nature 2011; 472: 486-490.

[175] Brand MD and Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2005; 2: 85-93.

[176] Nicholls DG and Rial E. A history of the first uncoupling protein, UCP1. J Bioenerg Biomembr 1999; 31: 399-406.

[177] Klingenberg M and Huang SG. Structure and function of the uncoupling protein from brown adipose tissue. Biochim Biophys Acta 1999; 1415: 271-296.

[178] Skulachev VP. Uncoupling: new approaches to an old problem of bioenergetics. Biochim Biophys Acta 1998; 1363: 100-124.

[179] Bouillaud F. UCP2, not a physiologically relevant uncoupler but a glucose sparing switch impacting ROS production and glucose sensing. Biochim Biophys Acta 2009; 1787: 377-383.

[180] Emre Y and Nubel T. Uncoupling protein UCP2: when mitochondrial activity meets immunity. FEBS Lett 2010; 584: 1437-1442.

[181] Fridell YW, Sanchez-Blanco A, Silvia BA and Helfand SL. Targeted expression of the human uncoupling protein 2 (hUCP2) to adult neurons extends life span in the fly. Cell Metab 2005; 1: 145-152.

[182] Hoerter J, Gonzalez-Barroso MD, Couplan E, Mateo P, Gelly C, Cassard-Doulcier AM, Diolez P and Bouillaud F. Mitochondrial uncoupling protein 1 expressed in the heart of transgenic mice protects against ischemic-reperfusion damage. Circulation 2004; 110: 528-533.

[183] Mattiasson G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S, Warden CH, Castilho RF, Melcher T, Gonzalez-Zulueta M, Nikolich K and Wieloch T. Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. Nat Med 2003; 9: 1062-1068.

[184] Vincent AM, Olzmann JA, Brownlee M, Sivitz WI and Russell JW. Uncoupling proteins prevent glucose-induced neuronal oxidative stress and programmed cell death. Diabetes 2004; 53: 726-734.

[185] Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, Goubern M, Surwit R, Bouillaud F, Richard D, Collins S and Ricquier D. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat Genet 2000; 26: 435-439.

[186] Bai Y, Onuma H, Bai X, Medvedev AV, Misukonis M, Weinberg JB, Cao W, Robidoux J, Floering LM, Daniel KW and Collins S. Persistent nuclear factor-kappa B activation in Ucp2-/- mice leads to enhanced nitric oxide and inflammatory cytokine production. J Biol Chem 2005; 280: 19062-19069.

[187] Rupprecht A, Brauer AU, Smorodchenko A, Goyn J, Hilse KE, Shabalina IG, Infante-Duarte C and Pohl EE. Quantification of Uncoupling Protein 2 Reveals Its Main Expression in Immune Cells and Selective Up-Regulation during T-Cell Proliferation. PLoS One 2012; 7: e41406.

[188] Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB and Lowell BB. Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. Cell 2001; 105: 745-755.

[189] Brown JM, Schwanke CM, Pershouse MA, Pfau JC and Holian A. Effects of rottlerin on silica-exacerbated systemic autoimmune disease in New Zealand mixed mice. Am J Physiol Lung Cell Mol Physiol 2005; 289: L990-998.

[190] Galloway CA, Lee H, Nejjar S, Jhun BS, Yu T, Hsu W and Yoon Y. Transgenic Control of Mitochondrial Fission Induces Mitochondrial Un-

coupling and Relieves Diabetic Oxidative Stress. Diabetes 2012; 61: 2093-104.
[191] Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ and Shulman GI. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem 2004; 279: 32345-32353.
[192] Shavell VI, Fletcher NM, Jiang ZL, Saed GM and Diamond MP. Uncoupling oxidative phosphorylation with 2,4-dinitrophenol promotes development of the adhesion phenotype. Fertil Steril 2012; 97: 729-733.
[193] White JC, Jiang ZL, Diamond MP and Saed GM. Macrophages induce the adhesion phenotype in normal peritoneal fibroblasts. Fertil Steril 2011; 96: 758-763 e753.
[194] Dalla Pozza E, Fiorini C, Dando I, Menegazzi M, Sgarbossa A, Costanzo C, Palmieri M and Donadelli M. Role of mitochondrial uncoupling protein 2 in cancer cell resistance to gemcitabine. Biochim Biophys Acta 2012; 1823: 1856-1863.
[195] Derdak Z, Mark NM, Beldi G, Robson SC, Wands JR and Baffy G. The mitochondrial uncoupling protein-2 promotes chemoresistance in cancer cells. Cancer Res 2008; 68: 2813-2819.
[196] Angelini G, Gardella S, Ardy M, Ciriolo MR, Filomeni G, Di Trapani G, Clarke F, Sitia R and Rubartelli A. Antigen-presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. Proc Natl Acad Sci U S A 2002; 99: 1491-1496.
[197] Edinger AL and Thompson CB. Antigen-presenting cells control T cell proliferation by regulating amino acid availability. Proc Natl Acad Sci U S A 2002; 99: 1107-1109.
[198] Gmunder H, Eck HP, Benninghoff B, Roth S and Droge W. Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine. Cell Immunol 1990; 129: 32-46.
[199] Stipanuk MH, Coloso RM, Garcia RA and Banks MF. Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr 1992; 122: 420-427.
[200] Bauer TM, Jiga LP, Chuang JJ, Randazzo M, Opelz G and Terness P. Studying the immunosuppressive role of indoleamine 2,3-dioxygenase: tryptophan metabolites suppress rat allogeneic T-cell responses in vitro and in vivo. Transpl Int 2005; 18: 95-100.
[201] Munn DH, Sharma MD and Mellor AL. Ligation of B7-1/B7-2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. J Immunol 2004; 172: 4100-4110.
[202] Newell MK, Villalobos-Menuey E, Schweitzer SC, Harper ME and Camley RE. Cellular metabolism as a basis for immune privilege. J Immune Based Ther Vaccines 2006; 4: 1.
[203] Bhushan A, Kupperman JL, Stone JE, Kimberly PJ, Calman NS, Hacker MP, Birge RB, Tritton TR and Newell MK. Drug resistance results in alterations in expression of immune recognition molecules and failure to express Fas (CD95). Immunol Cell Biol 1998; 76: 350-356.
[204] Harper ME, Antoniou A, Villalobos-Menuey E, Russo A, Trauger R, Vendemelio M, George A, Bartholomew R, Carlo D, Shaikh A, Kupperman J, Newell EW, Bespalov IA, Wallace SS, Liu Y, Rogers JR, Gibbs GL, Leahy JL, Camley RE, Melamede R and Newell MK. Characterization of a novel metabolic strategy used by drug-resistant tumor cells. FASEB J 2002; 16: 1550-1557.
[205] Steinman L. Myelin-specific CD8 T cells in the pathogenesis of experimental allergic encephalitis and multiple sclerosis. J Exp Med 2001; 194: F27-30.
[206] Lee JY and Hwang DH. The modulation of inflammatory gene expression by lipids: mediation through Toll-like receptors. Mol Cells 2006; 21: 174-185.
[207] Lee JY, Sohn KH, Rhee SH and Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 2001; 276: 16683-16689.
[208] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H and Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116: 3015-3025.
[209] Grimble RF. Dietary lipids and the inflammatory response. Proc Nutr Soc 1998; 57: 535-542.
[210] Shaikh SR and Edidin M. Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation. Am J Clin Nutr 2006; 84: 1277-1289.
[211] Hursting SD, Lavigne JA, Berrigan D, Perkins SN and Barrett JC. Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. Annu Rev Med 2003; 54: 131-152.
[212] Roe FJ, Lee PN, Conybeare G, Tobin G, Kelly D, Prentice D and Matter B. Risks of premature death and cancer predicted by body weight in early adult life. Hum Exp Toxicol 1991; 10: 285-288.
[213] Weindruch R, McFeeters G and Walford RL. Food intake reduction and immunologic alterations in mice fed dehydroepiandrosterone. Exp Gerontol 1984; 19: 297-304.
[214] Weraarchakul N, Strong R, Wood WG and Richardson A. The effect of aging and dietary restriction on DNA repair. Exp Cell Res 1989; 181: 197-204.

[215] Beach RS, Gershwin ME and Hurley LS. Nutritional factors and autoimmunity. III. Zinc deprivation versus restricted food intake in MRL/1 mice--the distinction between interacting dietary influences. J Immunol 1982; 129: 2686-2692.
[216] Reddy Avula CP, Lawrence RA, Zaman K and Fernandes G. Inhibition of intracellular peroxides and apoptosis of lymphocytes in lupus-prone B/W mice by dietary n-6 and n-3 lipids with calorie restriction. J Clin Immunol 2002; 22: 206-219.
[217] Neels JG and Olefsky JM. Inflamed fat: what starts the fire? J Clin Invest 2006; 116: 33-35.
[218] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL and Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-1808.
[219] Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B and Grangette C. Feeding our immune system: impact on metabolism. Clin Dev Immunol 2008; 2008: 639803.
[220] Scheinfeld NS. Obesity and dermatology. Clin Dermatol 2004; 22: 303-309.
[221] Wilson JA and Clark JJ. Obesity: impediment to postsurgical wound healing. Adv Skin Wound Care 2004; 17: 426-435.
[222] Nogueira LM, Dunlap SM, Ford NA and Hursting SD. Calorie restriction and rapamycin inhibit MMTV-Wnt-1 mammary tumor growth in a mouse model of postmenopausal obesity. Endocr Relat Cancer 2012; 19: 57-68.
[223] Fagone P, Donia M, Mangano K, Quattrocchi C, Mammana S, Coco M, Libra M, McCubrey JA and Nicoletti F. Comparative Study of Rapamycin and Temsirolimus Demonstrates Superimposable Anti-Tumour Potency on Prostate Cancer Cells. Basic Clin Pharmacol Toxicol 2012; 112: 63-9.
[224] Stallone G, Schena A, Infante B, Di Paolo S, Loverre A, Maggio G, Ranieri E, Gesualdo L, Schena FP and Grandaliano G. Sirolimus for Kaposi's sarcoma in renal-transplant recipients. N Engl J Med 2005; 352: 1317-1323.
[225] Abouelnasr A, Cohen S, Kiss T, Roy J and Lachance S. Defining The Role of Sirolimus in the Management of Graft-versus-Host Disease: from Prophylaxis to Treatment. Biol Blood Marrow Transplant 2012; 19: 12-21.
[226] Charbonnier LM and Le Moine A. Rapamycin as immunosuppressant in murine transplantation model. Methods Mol Biol 2012; 821: 435-445.
[227] Dutcher JP. Mammalian target of rapamycin (mTOR) Inhibitors. Curr Oncol Rep 2004; 6: 111-115.
[228] Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E and Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 2009; 460: 392-395.
[229] Wang MC, O'Rourke EJ and Ruvkun G. Fat metabolism links germline stem cells and longevity in C. elegans. Science 2008; 322: 957-960.
[230] Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T and Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. J Immunol 2006; 177: 8338-8347.
[231] Kopf H, de la Rosa GM, Howard OM and Chen X. Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells. Int Immunopharmacol 2007; 7: 1819-1824.
[232] Hester J, Schiopu A, Nadig SN and Wood KJ. Low-dose rapamycin treatment increases the ability of human regulatory T cells to inhibit transplant arteriosclerosis in vivo. Am J Transplant 2012; 12: 2008-2016.
[233] Sinclair LV, Finlay D, Feijoo C, Cornish GH, Gray A, Ager A, Okkenhaug K, Hagenbeek TJ, Spits H and Cantrell DA. Phosphatidylinositol-3-OH kinase and nutrient-sensing mTOR pathways control T lymphocyte trafficking. Nat Immunol 2008; 9: 513-521.
[234] Fielhaber JA, Carroll SF, Dydensborg AB, Shourian M, Triantafillopoulos A, Harel S, Hussain SN, Bouchard M, Qureshi ST and Kristof AS. Inhibition of mammalian target of rapamycin augments lipopolysaccharide-induced lung injury and apoptosis. J Immunol 2012; 188: 4535-4542.
[235] Watt K, Dierkhising R, Heimbach J and Charlton M. Impact of sirolimus and tacrolimus on mortality & graft loss in liver transplant recipients with and without HCV - an analysis of the SRTR database. Liver Transpl 2012; 18: 1029-36.
[236] Cohen EE, Wu K, Hartford C, Kocherginsky M, Eaton KN, Zha Y, Nallari A, Maitland ML, Fox-Kay K, Moshier K, House L, Ramirez J, Undevia SD, Fleming GF, Gajewski TF and Ratain MJ. Phase I Studies of Sirolimus Alone or in Combination with Pharmacokinetic Modulators in Advanced Cancer Patients. Clin Cancer Res 2012; 18: 4785-93.
[237] Deblon N, Bourgoin L, Veyrat-Durebex C, Peyrou M, Vinciguerra M, Caillon A, Maeder C, Fournier M, Montet X, Rohner-Jeanrenaud F and Foti M. Chronic mTOR inhibition by rapamycin induces muscle insulin resistance despite weight loss in rats. Br J Pharmacol 2012; 165: 2325-2340.
[238] Ko YH, Pedersen PL and Geschwind JF. Glucose catabolism in the rabbit VX2 tumor model

for liver cancer: characterization and targeting hexokinase. Cancer Lett 2001; 173: 83-91.

[239] Ganapathy-Kanniappan S, Vali M, Kunjithapatham R, Buijs M, Syed LH, Rao PP, Ota S, Kwak BK, Loffroy R and Geschwind JF. 3-bromopyruvate: a new targeted antiglycolytic agent and a promise for cancer therapy. Curr Pharm Biotechnol 2010; 11: 510-517.

[240] Buijs M, Vossen JA, Geschwind JF, Ishimori T, Engles JM, Acha-Ngwodo O, Wahl RL and Vali M. Specificity of the anti-glycolytic activity of 3-bromopyruvate confirmed by FDG uptake in a rat model of breast cancer. Invest New Drugs 2009; 27: 120-123.

[241] Ko YH, Smith BL, Wang Y, Pomper MG, Rini DA, Torbenson MS, Hullihen J and Pedersen PL. Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochem Biophys Res Commun 2004; 324: 269-275.

[242] van Rensburg MJ and Coyne VE. The role of electron transport in the defence response of the South African abalone, Haliotis midae. Fish Shellfish Immunol 2009; 26: 171-176.

[243] Josse C, Legrand-Poels S, Piret B, Sluse F and Piette J. Impairment of the mitochondrial electron chain transport prevents NF-kappa B activation by hydrogen peroxide. Free Radic Biol Med 1998; 25: 104-112.

[244] Anedda A, Rial E and Gonzalez-Barroso MM. Metformin induces oxidative stress in white adipocytes and raises uncoupling protein 2 levels. J Endocrinol 2008; 199: 33-40.

[245] Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA and Cantley LC. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell 2004; 6: 91-99.

[246] Zakikhani M, Dowling R, Fantus IG, Sonenberg N and Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res 2006; 66: 10269-10273.

[247] Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR and Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005; 330: 1304-1305.

[248] Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B and Thompson CB. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res 2007; 67: 6745-6752.

[249] El-Mir MY, Nogueira V, Fontaine E, Averet N, Rigoulet M and Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem 2000; 275: 223-228.

[250] Bodmer M, Meier C, Krahenbuhl S, Jick SS and Meier CR. Long-term metformin use is associated with decreased risk of breast cancer. Diabetes Care 2010; 33: 1304-1308.

[251] Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B and Gandini S. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2010; 3: 1451-1461.

[252] Frasca F, Pandini G, Sciacca L, Pezzino V, Squatrito S, Belfiore A and Vigneri R. The role of insulin receptors and IGF-I receptors in cancer and other diseases. Arch Physiol Biochem 2008; 114: 23-37.

[253] Hattori Y, Suzuki K, Hattori S and Kasai K. Metformin inhibits cytokine-induced nuclear factor kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. Hypertension 2006; 47: 1183-1188.

[254] Kim SA and Choi HC. Metformin inhibits inflammatory response via AMPK-PTEN pathway in vascular smooth muscle cells. Biochem Biophys Res Commun 2012; 455: 866-72.

[255] Esrefoglu M. Oxidative stress and benefits of antioxidant agents in acute and chronic hepatitis. Hepat Mon 2012; 12: 160-167.

[256] Essa MM, Vijayan RK, Castellano-Gonzalez G, Memon MA, Braidy N and Guillemin GJ. Neuroprotective effect of natural products against Alzheimer's disease. Neurochem Res 2012; 37: 1829-1842.

[257] Fuchs-Tarlovsky V. Role of antioxidants in cancer therapy. Nutrition 2012; 29: 15-21.

[258] Zhu H and Li YR. Oxidative stress and redox signaling mechanisms of inflammatory bowel disease: updated experimental and clinical evidence. Exp Biol Med (Maywood) 2012; 237: 474-480.

[259] Delmastro MM and Piganelli JD. Oxidative stress and redox modulation potential in type 1 diabetes. Clin Dev Immunol 2011; 2011: 593863.

[260] Fernandez-Checa JC, Garcia-Ruiz C, Colell A, Morales A, Mari M, Miranda M and Ardite E. Oxidative stress: role of mitochondria and protection by glutathione. Biofactors 1998; 8: 7-11.

[261] Correa R, Blanco B, Del Rio M, Victor V, Guayerbas N, Medina S and De la Fuente M. Effect of a diet supplemented with thioproline on murine macrophage function in a model of premature ageing. Biofactors 1999; 10: 195-200.

[262] De la Fuente M and Victor VM. Anti-oxidants as modulators of immune function. Immunol Cell Biol 2000; 78: 49-54.

[263] Victor VM and De la Fuente M. N-acetylcysteine improves in vitro the function of macrophages from mice with endotoxin-induced oxidative stress. Free Radic Res 2002; 36: 33-45.

[264] Guo CH, Liu PJ, Lin KP and Chen PC. Nutritional supplement therapy improves oxidative stress,

immune response, pulmonary function, and quality of life in allergic asthma patients: an open-label pilot study. Altern Med Rev 2012; 17: 42-56.

[265] Wang J, Pae M, Meydani SN and Wu D. Epigallocatechin-3-gallate inhibits expression of receptors for T cell regulatory cytokines and their downstream signaling in mouse CD4+ T cells. J Nutr 2012; 142: 566-571.

[266] Wang J, Ren Z, Xu Y, Xiao S, Meydani SN and Wu D. Epigallocatechin-3-gallate ameliorates experimental autoimmune encephalomyelitis by altering balance among CD4+ T-cell subsets. Am J Pathol 2012; 180: 221-234.

[267] Xuzhu G, Komai-Koma M, Leung BP, Howe HS, McSharry C, McInnes IB and Xu D. Resveratrol modulates murine collagen-induced arthritis by inhibiting Th17 and B-cell function. Ann Rheum Dis 2012; 71: 129-135.

[268] Radwan FF, Zhang L, Hossain A, Doonan BP, God JM and Haque A. Mechanisms regulating enhanced human leukocyte antigen class II-mediated CD4 + T cell recognition of human B-cell lymphoma by resveratrol. Leuk Lymphoma 2012; 53: 305-314.

[269] Checker R, Sandur SK, Sharma D, Patwardhan RS, Jayakumar S, Kohli V, Sethi G, Aggarwal BB and Sainis KB. Potent anti-inflammatory activity of ursolic acid, a triterpenoid antioxidant, is mediated through suppression of NF-kappaB, AP-1 and NF-AT. PLoS One 2012; 7: e31318.

[270] Li DY, Xue MY, Geng ZR and Chen PY. The suppressive effects of Bursopentine (BP5) on oxidative stress and NF-kB activation in lipopolysaccharide-activated murine peritoneal macrophages. Cell Physiol Biochem 2012; 29: 9-20.

[271] Tse HM, Milton MJ and Piganelli JD. Mechanistic analysis of the immunomodulatory effects of a catalytic antioxidant on antigen-presenting cells: implication for their use in targeting oxidation-reduction reactions in innate immunity. Free Radic Biol Med 2004; 36: 233-247.

[272] Sharma R and Vinayak M. Antioxidant alpha-tocopherol checks lymphoma promotion via regulation of expression of protein kinase C-alpha and c-Myc genes and glycolytic metabolism. Leuk Lymphoma 2012; 53: 1203-1210.

[273] Batinic-Haberle I, Spasojevic I, Tse HM, Tovmasyan A, Rajic Z, St Clair DK, Vujaskovic Z, Dewhirst MW and Piganelli JD. Design of Mn porphyrins for treating oxidative stress injuries and their redox-based regulation of cellular transcriptional activities. Amino Acids 2012; 42: 95-113.

[274] Delmastro MM, Styche AJ, Trucco MM, Workman CJ, Vignali DA and Piganelli JD. Modulation of redox balance leaves murine diabetogenic TH1 T cells "LAG-3-ing" behind. Diabetes 2012; 61: 1760-1768.

[275] Piganelli JD, Flores SC, Cruz C, Koepp J, Batinic-Haberle I, Crapo J, Day B, Kachadourian R, Young R, Bradley B and Haskins K. A metalloporphyrin-based superoxide dismutase mimic inhibits adoptive transfer of autoimmune diabetes by a diabetogenic T-cell clone. Diabetes 2002; 51: 347-355.

[276] Bottino R, Balamurugan AN, Tse H, Thirunavukkarasu C, Ge X, Profozich J, Milton M, Ziegenfuss A, Trucco M and Piganelli JD. Response of human islets to isolation stress and the effect of antioxidant treatment. Diabetes 2004; 53: 2559-2568.

[277] Sklavos MM, Bertera S, Tse HM, Bottino R, He J, Beilke JN, Coulombe MG, Gill RG, Crapo JD, Trucco M and Piganelli JD. Redox modulation protects islets from transplant-related injury. Diabetes 2010; 59: 1731-1738.

[278] Sklavos MM, Tse HM and Piganelli JD. Redox modulation inhibits CD8 T cell effector function. Free Radic Biol Med 2008; 45: 1477-1486.

[279] Sheng H, Spasojevic I, Tse HM, Jung JY, Hong J, Zhang Z, Piganelli JD, Batinic-Haberle I and Warner DS. Neuroprotective efficacy from a lipophilic redox-modulating Mn(III) N-Hexylpyridylporphyrin, MnTnHex-2-PyP: rodent models of ischemic stroke and subarachnoid hemorrhage. J Pharmacol Exp Ther 2011; 338: 906-916.

[280] Gauter-Fleckenstein B, Fleckenstein K, Owzar K, Jiang C, Reboucas JS, Batinic-Haberle I and Vujaskovic Z. Early and late administration of MnTE-2-PyP5+ in mitigation and treatment of radiation-induced lung damage. Free Radic Biol Med 2010; 48: 1034-1043.

[281] Rabbani ZN, Batinic-Haberle I, Anscher MS, Huang J, Day BJ, Alexander E, Dewhirst MW and Vujaskovic Z. Long-term administration of a small molecular weight catalytic metalloporphyrin antioxidant, AEOL 10150, protects lungs from radiation-induced injury. Int J Radiat Oncol Biol Phys 2007; 67: 573-580.

[282] Rabbani ZN, Salahuddin FK, Yarmolenko P, Batinic-Haberle I, Thrasher BA, Gauter-Fleckenstein B, Dewhirst MW, Anscher MS and Vujaskovic Z. Low molecular weight catalytic metalloporphyrin antioxidant AEOL 10150 protects lungs from fractionated radiation. Free Radic Res 2007; 41: 1273-1282.

[283] Saba H, Batinic-Haberle I, Munusamy S, Mitchell T, Lichti C, Megyesi J and MacMillan-Crow LA. Manganese porphyrin reduces renal injury and mitochondrial damage during ischemia/reperfusion. Free Radic Biol Med 2007; 42: 1571-1578.

[284] Rabbani ZN, Spasojevic I, Zhang X, Moeller BJ, Haberle S, Vasquez-Vivar J, Dewhirst MW, Vujaskovic Z and Batinic-Haberle I. Antiangiogenic action of redox-modulating Mn(III) mesotetrakis(N-ethylpyridinium-2-yl)porphyrin, MnTE-2-PyP(5+), via suppression of oxidative stress in a mouse model of breast tumor. Free Radic Biol Med 2009; 47: 992-1004.

[285] Tse HM, Milton MJ, Schreiner S, Profozich JL, Trucco M and Piganelli JD. Disruption of innate-mediated proinflammatory cytokine and reactive oxygen species third signal leads to antigen-specific hyporesponsiveness. J Immunol 2007; 178: 908-917.