REVIEW

HSC-Derived Fatty Acid Oxidation in Steady-State and Stressed Hematopoiesis

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Metabolism impacts all cellular functions and plays a fundamental role in physiology. Metabolic regulation of hematopoiesis is dynamically regulated under steady-state and stress conditions. It is clear that hematopoietic stem cells (HSCs) impose different energy demands and flexibility during maintenance compared with stressed conditions. However, the cellular and molecular mechanisms underlying metabolic regulation in HSCs remain poorly understood. In this review, we focus on defining the role of fatty acid oxidation (FAO) in HSCs. We first review the existing literature describing FAO in HSCs under steady-state hematopoiesis. Next, we describe the models used to examine HSCs under stress conditions, and, finally, we describe how infection causes a shift toward FAO in HSCs and the impact of using this pathway on emergency hematopoiesis.

HIGHLIGHTS

- Metabolic flexibility is required for hematopoietic stem cell (HSC) function.
- HSCs utilize glycolysis as their main energy source under steady-state hematopoiesis.
- HSCs use β-oxidation as a significant source of metabolic energy during periods of high energy demands.
- New technologies are required to investigate the influence of bone marrow adiposity in supplying free fatty acids for hematopoiesis.

The broad definition of metabolism is the sum of biochemical processes that generate or consume energy in a living organism. This is a vast amount: more than 8,700 reactions and 16,000 metabolites [1]. Pathways utilizing abundant nutrients, such as carbohydrates, fatty acids (FAs), and amino acids, are the basis of core metabolism and are vital for energy homeostasis. Cells utilize these nutrients for energy in the form of adenosine triphosphate (ATP), primarily generated by aerobic respiration using glucose as the metabolite [2]. Glucose and lactate contribute to approximately half of the carbon required for the trichloroacetic acid (TCA) cycle. It has been hypothesized that the remainder is provided by a combination of amino acids and fat [3]. FAs are stored as triglycerides in adipose tissue. Triglycerides are hydrolyzed via lipolysis and secreted into the circulation as free fatty acids (FFAs) and glycerol [4]. FFAs act as a substrate for β-oxidation and can be subsequently used for ATP production [5]. The uptake of FFAs is now generally recognized as an essential process, underpinning cell metabolism. In the context of hematopoiesis, fatty acid oxidation (FAO) is used as an energy source by both HSCs and more committed progenitor cells. In the wider context of stem cells, FAO appears important in maintaining the stem cell state. However, the mechanism by which FAO affects and alters tissue-specific stem cell behavior in healthy and disease states appears to differ. In this review, we aim to describe the importance of FAO in hematopoiesis, with a focus on stem cells and its impact during activation.

FAO

FAO occurs in both mitochondria and peroxisomes and is the process by which FFAs are broken down to generate acetyl coenzyme A (acetyl-CoA), which can feed the TCA cycle to produce energy in the form of ATP. Mitochondria are the main site of FAO of medium- and long-chain FAs, whereas peroxisomes catalyze the FAO of a distinct set of FAs, including very-long-chain FAs. The process of FAO can be broken down into 4 steps. First, FFAs enter the cells and are activated by being coupled with coenzyme A (CoA) within the cytosol. This allows for long-chain fatty-acyl-CoA to transfer the acyl group via carnitine palmitoyl transferase 1 (CPT1), producing acylcarnitine. It is then transported into the inner mitochondrial membrane by carnitine translocase. The final step is inside the mitochondria; long-chain acylcarnitine is oxidized by CPT2 and converted back to long-chain acyl-CoA, which enters the TCA cycle for further production of ATP [6]. FAO is capable of providing a large amount of energy to fuel tumor growth by increasing the production of ATP under metabolic stress [7]. In vitro, CPT1 has been shown to be upregulated in lung tumor models, promoting tumor survival in metabolically stressed conditions [8]. Previous studies have also shown that cancer cells overexpress

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CTP1, which drives ATP production via FAO [9,10]. Taken together, these data show that FAO is an important source of energy under stressed conditions.

For optimal use of all substrates available and to optimize energy requirements, cells can reprogram protein localization, protein turnover, and gene expression [11]. The first stage in nutrient homeostasis is the uptake of nutrients by cells, which often involves many specific membrane receptors. This is followed by a downstream network of proteins for intracellular nutrient traffic or signal transduction [12]. Long-chain FAs are common nutrients found in the diet and a major source of energy for many cells [13,14]. FAs can also regulate the expression of genes via transcription factors such as peroxisome proliferator-activated receptors (PPARs) [15,16], which regulate numerous metabolic genes [17] and FoxO1 [18]. This is important for adapting to low nutrient availability [19]. The liver and adipose tissue are the only tissues that possess the ability for de novo FA synthesis; all other cells depend on the uptake of FAs for their metabolic needs [20]. However, in the disease state, various studies have shown that targeting the uptake or oxidation of FAs can be used for treatment purposes. Together, this emphasizes not only the physiologic importance of the uptake of FAs into cells but also the clinical importance of targeting the uptake of FAs in disease settings.

STEADY-STATE HSC METABOLISM

In the past several years, the metabolic requirements for HSC function have been well investigated [21–25]. However, many studies have focused on the importance of glycolysis versus that of oxidative phosphorylation (OXPHOS) and the maintenance of HSCs under normal conditions. The HSC niche, where HSCs reside, is known to be hypoxic; this helps maintain stem cell quiescence. Under normal conditions, quiescent HSCs have previously been shown to utilize glycolysis as opposed to mitochondrial OXPHOS to meet their energy demands, a metabolic adaptation influenced by their hypoxic niche [21]. This preference for glycolysis reflects the low metabolic demands of quiescent HSCs because OXPHOS is the most efficient pathway for energy production [26]. A metabolic analysis showed that HSCs have a different metabolic profile compared with more committed cells [27]. HSCs have been shown to have increased pyruvate kinase activity and accrue high levels of fructose-1,6-bisphosphate, suggesting that HSCs metabolize via active glycolysis. They have also been shown to have high levels of pyruvate but low levels of phosphoenolpyruvate, which is the product and substrate of the glycolysis pathway [28]. These data show that under normal conditions, it is most likely that glycolysis is the metabolic pathway of choice for HSCs.

Mitochondria are central to the metabolism of carbohydrates, lipids, and amino acids in most cells, including HSCs. Recent studies have shown that a key characteristic of HSCs is the maintenance of quiescence by limiting mitochondrial respiration [29]. Although the role of mitochondria in stem cells is very complex, the mitochondria in normal HSCs are somewhat inactive [30]. This reduced reliance on mitochondrial function in HSCs allows for the maintenance of low levels of reactive oxygen species (ROS) because HSCs are vulnerable to oxidative stress [31,32]. Furthermore, it has been shown that the mitochondria of HSCs are relatively inactive [30] and that the ROS level associated with mitochondrial activity is lower in HSCs than in more committed progenitors [21,33]. This suggests that HSCs require a rapid burst of mitochondrial metabolism for differentiation. Although it is known that HSCs switch from glycolysis to OXPHOS for the increased energy demand required for differentiation, the mechanisms regulating this switch remain to be elucidated. However, it is probable that the metabolic change is more complex than a switch from one metabolic pathway to another. This metabolic switch in HSCs may have evolved from an increase in energy demand for rapid differentiation in response to exogenous stress.

Notably, it has been shown that HSCs undergoing self-renewal need to minimize mitochondrial activity to remain quiescent [28,29,34]. Moreover, these HSCs have been shown to have a higher long-term, multilineage reconstituting ability in both primary and secondary transplants than HSCs with increased mitochondria membrane potential, indicating mitochondrial biogenesis [35]. This may suggest that during activation, there is a subset of HSCs that does not have increased mitochondrial content to maintain self-renewal and replenish the HSC pool without exhaustion. These data show that under normal conditions, it is most likely that HSCs with low mitochondrial activity utilize glycolysis as the metabolic pathway of choice. However, during stressed hematopoiesis, HSC metabolism needs to switch rapidly from glycolysis to OXPHOS to facilitate the expansion required to respond to, and overcome, the source of the stress. The most common trigger for stressed hematopoiesis is infection, either viral or bacterial, and many researchers use models of infection to understand the physiologic response of the hematopoietic system.

STRESSED HEMATOPOIESIS USING INFECTION MIMICS

To better understand how the mammalian system responds to infection, many studies use infection mimics, with the most popular being bacterial lipopolysaccharide (LPS). LPS has been widely used in models of infection because they mimic the inflammatory effects of cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6. Bacterial LPS is the main component of the outer surface membrane of most gram-negative bacteria [36]. It is a strong activator of the innate and natural immune systems in humans and murine models, and, therefore, it is ideal for mimicking infections [37]. LPS comprise an oligosaccharide region, which is attached to the outer membrane of the bacteria via a carbohydrate, lipid A [38]. The main immunostimulatory effect of LPS is due to lipid A. In mammals, activation of the immune system with highly endotoxic forms of LPS has been shown to be caused by lipid A types of LPS [39]. LPS can trigger a number of physiologic immunostimulatory effects in mammals; however, if the dose is too high, it can lead to induction of septic shock [40]. Myeloid lineage cells have been shown to be the primary sensors of LPS in the immune system [39]. Significant progress has been made to understand the signaling cascade caused by LPS in mammalian phagocytes. It is thought that LPS are first recognized by the LPS-binding protein (LBP), cluster of differentiation (CD)14 (either membrane bound or soluble form), and Toll-like receptor 4 (TLR4)-MD-2 complex. This leads to rapid activation of the intracellular signaling network similar to the signaling of IL-1 and IL-18 [39]. Although LPS alone activate the TLR4 immune response, whole bacterial cells secrete many different virulence factors, which activate numerous TLRs and pattern recognition receptors (PRRs) that can modulate the immune response; therefore, LPS alone may not be truly representative of a bacterial infection. Whole bacterial cells also

replicate and shed LPS, resulting in a more kinetic immune response compared with direct activation of the immune system. Therefore, to assess the effect of the host response to infection in a more standardized manner, LPS can be used in vivo and in vitro as a surrogate for simulating a bacterial infection. However, the use of LPS to mimic infection does have its limitations when used in vivo. One such example includes the fact that the targeted effect of LPS is limited to TLR4 signaling, whereas bacterial infection can not only target other TLRs but also be internalized during phagocytosis, which can lead to activation of downstream proinflammatory signals.

Within the context of metabolic changes, our studies showed that LPS can induce rapid changes in HSC and progenitor cell metabolism [41,42]. In our in vivo models, LPS could induce mitochondrial changes within 2 hours of intraperitoneal injection, which facilitated a rapid shift within HSCs from quiescence to OXPHOS [41]. The switch to FAO requires a longer stimulus with LPS because FA transporters (CD36) need to be transcriptionally upregulated and expressed on the surface of HSCs and progenitors [42]. Without the upregulation of CD36, HSCs enter the cell cycle more slowly and are unable to switch to FAO. The use of LPS in these studies allowed us to determine the speed at which HSCs and progenitors can respond to challenges. However, the use of such mimics needs to be put into the context of the study design and should be aligned with infection models described in the next section.

To mimic a viral infection experimentally in vivo, researchers have also used polyinosinic-polycytidylic acid (poly I:C). Poly I:C is a synthetic, double-stranded RNA molecule polyribonucleotide that has been widely used to induce a type I interferon (IFN) response in many species. Poly I:C is known to interact with TLR3 [43]; upon recognition, TLR3 activates downstream signaling, resulting in the production of type I IFN via activation of IRF3 [44]. Moreover, poly I:C can also activate nuclear factor-κB (NF-κB)-mediated inflammatory responses, resulting in production of IL-6 and TNF-α [45]. When virally infected cells die, the viral double-stranded RNA is released into the extracellular environment. This released viral double-stranded RNA acts as a danger signal, alerting inflammatory cells, and this process can contribute to systemic diseases [46,47]. Poly I:C can stimulate TLR3-induced downstream mechanisms in cells of the innate immune system, including dendritic cells, macrophages, and endothelial cells. Although all these cells express TLR3, their response to poly I:C stimulation differs. Human dendritic cells and macrophages produce the IFN-regulated gene IP-10 but do not produce the inflammatory cytokines TNF-α, IL-6, or IL-8 [48], whereas in human endothelial cells, poly I:C induces activation of NF-κB and mitogen-activated protein kinases (MAPKs) [49]. This indicates a cell-specific response to TLR stimulation by poly I:C, which may cause complications when human inflammation is studied in murine models of inflammatory diseases. Nevertheless, the use of these models allows for a better understanding of the processes of human diseases, thus allowing for improved therapeutic approaches for the treatment of infectious diseases.

MODELS OF INFECTION

Salmonella typhimurium is the most common cause of invasive, nontyphoidal Salmonella infection. Aggressive strains of nontyphoidal *Salmonella* are the main cause of infection of the bloodstream in African children and adults, with a fatality rate of 20%–25% [50]. S. typhimurium infection causes acute inflammation of the intestine in both human and mice hosts. S. typhimurium can survive in this inflammatory environment and, therefore, has a selective growth advantage over the gut microbiota [51]. Following adherence to the intestine, S. typhimurium invades and crosses the intestinal epithelium [52]. TLRs are the first pathogen recognition receptors to recognize the presence of *Salmonella* [53]. *Salmonella* has been shown to activate several TLRs, including TLR1, TLR2, and TLR6 in vitro via its bacterial lipoproteins; TLR4 via its LPS; TLR5 via its flagellin; and TLR9 via CpG-rich repetitive elements in its DNA [51]. When the ligands bind to the TLRs, MyD88 and TRIF signaling adaptors are activated, initiating a signaling cascade in which the transcriptional factors NF-κB and IRF3 are also activated. This induces the production of several inflammatory cytokines, including IL-8, IL-10, IL-1β, IL-18, and type I IFN [53]. Mice infected with S. typhimurium have been shown to upregulate Stem cells antigen-1 expression. Systemic infection due to S. typhimurium also induced HSC activation as well as γH2AX$^+$ and p53-binding protein 1$^+$foci formation, indicating proliferative stress caused by TLR4-TRIF signaling [54]. Further understanding of how S. typhimurium leads to acute inflammation may lead to better treatment of this highly fatal infection.

In our in vivo studies, we showed that the treatment of mice infected with S. typhimurium with oral gavage leads to acute inflammation within 48–72 hours, as measured by IL-6 serum levels. This is in contrast to the LPS model described above, in which high levels of IL-6 are induced within 16 hours [42]. It is, therefore, clear that timing is critical and needs to be taken into account while using these model systems. In terms of HSC metabolism, we showed that mitochondrial changes and a shift toward FAO occur within 72 hours of S. typhimurium infection [41,42]. Whether these metabolic changes could be detected earlier in this infection model is unknown. In more recent unpublished studies, we have started to use intraperitoneal injection of S. typhimurium. This is because we are concerned that S. typhimurium causes a "leaky gut," thus causing other pathogens to cross from the intestine to the blood and induce an inflammatory response.

Mycobacterium avium is used in models to study infections that can lead to chronic systemic mycobacterial diseases that contrast with acute S. typhimurium infection. *M. avium* complex (MAC), which comprises various mycobacterial species, including *Mycobacterium intracellulare* and *M. avium*, is the most common nontuberculous mycobacteria. Recent studies have shown that high mortality (more than 25%) is associated with MAC pulmonary disease [55]. Mouse models of *M. avium* infection elicit an IFN-γ response but not an IFN-α response [56], which is specifically dependent on IFN-γ-induced activation of macrophages [57]. Studies on mice infected with *M. avium* have shown expansion of Lin-c-kit$^+$Sca-1$^+$ (LSK) cells and that intravenous injection of *M. avium* causes expansion of Lin-c-kit$^+$Sca-1$^+$, CD34$^+$, Flk2$^-$ (ST-HSC) cells 4 weeks after the infection [56,58]. This model is, therefore, a useful tool for the study of the effect of chronic bacterial infection on the maintenance and proliferation of HSCs.

Models of chronic viral infections have also been used in the study of bone marrow (BM) failure and HSC function. A model of lymphocytic choriomeningitis virus has been shown to reduce C-X-C Motif Chemokine Ligand 12-abundant reticular cells and initiate proinflammatory transcriptional remodeling in these cells, which can lead to decreased competitive fitness of HSCs [59]. Viral infections require lymphocytic cells, such as natural killer (NK) and T cells, for clearance;

however, monocytes and granulocytes play an important role in the immune response against bacterial infections. Therefore, this could affect the need for different hematopoietic output upon a viral or bacterial infection. The use of whole viruses and bacteria in mice to model infectious diseases in humans or larger animals is important because characterization of the immune response to these organisms can lead to the development of therapeutic interventions. However, in our experience, the use of live bacteria in mouse models can lead to high variation in the results obtained. These variations can be limited by the use of sex- and age-matched mice, although the results obtained from such experiments can be misleading because they do not represent the target human population with diseases, which is often very young or very old.

HSC RESPONSE TO INFECTION

Infectious diseases exert a major selective pressure on mammalian evolution and are still a leading cause of global mortality. Of the top 10 causes of global death in 2019, 2 are communicable or infectious diseases: diarrheal diseases and lower-respiratory infections [60]. The innate immune response to the challenge of infection must be rapid and robust to generate significant numbers of leukocytes in a short period of time. The most abundant leukocytes found in mammals are neutrophils, and they contribute to infection resolution by activation of other leukocytes, capturing microbes within extracellular traps, phagocytosis, and enzyme-mediated lysis [61]. During infection, the turnover of neutrophils and other immune cells is immense, there is an increased demand, and the hematopoietic system responds by rapidly switching from steady-state hematopoiesis to emergency granulopoiesis [62]. This transition involves interactions between both hematopoietic and nonhematopoietic cells of the BM microenvironment, including exchange of cytokines and growth factors [63–65]. Bone marrow stromal cells (BMSCs) specifically have previously been shown to support the increased demand for hematopoiesis during inflammatory conditions, preventing HSC exhaustion [66].

The BM is an important site for granulopoiesis, erythropoiesis, and lymphopoiesis, which are all key to the response to infection. Microbial challenges, including acute infection, are linked to significant changes in the HSC compartment. This, in turn, has an impact on the differentiation and migration of HSCs [67]. The BM has a central role in maintaining this immune homeostasis; during infection, the hematopoietic system drives the immune response necessary for host survival. HSC differentiation can be influenced by a variety of stimuli, including contact with pathogens, to combat the cellular needs of the immune response, resulting in physiologic changes in the BM [68]. Infection can, therefore, dysregulate progenitor cells and interfere with normal homeostasis in the BM, including availability of blood cells [69]. Under a pathogenic challenge, emergency granulopoiesis and rapid mobilization of neutrophils out of the BM are key to overcome pathogen invasion [70]. Erythropoiesis is similarly increased in response to acute inflammation upon pathogen detection. Under systemic challenges, such as malaria and toxoplasmosis, there is a preferential increase in granulocytes in the BM. This is at the expense of the lymphocyte, erythrocyte, and megakaryocyte populations [71].

Increases in the number of progenitors or HSCs in the BM are a trademark of infection [72]. Immune challenges with Plasmodium chabaudi or Pneumocystis carinii increase the number of HSCs in the BM and peripheral blood, which subsequently causes an increase in multipotent progenitors cells [73]. It has not been distinguished whether these changes in the BM are a consequence of the infection or part of the response to minimize the infection. It is also unclear whether the role of HSCs in infection includes immune sensing; however, it has been reported that the proliferation of HSCs could be a component of the primary immune response alongside the replenishment of depleted progenitors [74]. Studies have indicated that HSCs can directly participate in the primary response to acute and chronic infections. Mice infected with *Escherichia coli* showed expansion of the LSK population, and this led to an increase in the level of granulocyte colony-stimulating factor (G-CSF) before leukopenia [75,76]. This suggests that LSK cells respond to the infection directly rather than as a secondary response to cytopenia. Using a model of *M. avium* infection, it was found that HSC activation in mice was initiated by IFN-γ signaling. This was also shown to be the case in vitro, wherein HSC activation was directly stimulated by an IFN-mediated immune response, irrespective of the infection or other cell types present [56]. Nevertheless, despite these drastic changes in the number of HSCs and makeup of BM microenvironment populations caused by infection, once cleared, the BM returns to normal homeostasis.

The long-term effects of repeated infections on the function of HSCs and hematopoiesis can be detrimental. It has recently been shown that repeated inflammatory challenges with poly I:C result in the depletion of functional HSCs and that these HSCs do not have the ability to self-renew. This depletion had no initial consequences on mice; however, they developed clinically relevant features of aging, comparable with those of elderly humans [77]. Similarly, when mice were given a low dose of LPS daily for 1 month, HSCs were unable to maintain quiescence, and following transplantation of these HSCs, their progeny was skewed toward a myeloid lineage, concurrent with aging [78]. Overall, the hematopoietic system has an effective efficient response to immune challenges; however, sustained or repeated infections can have an adverse effect and accelerate aging.

ROLE OF INFECTION ON HSC METABOLISM

The HSC energy requirements during differentiation and expansion following infection are vast and require rapid and efficient production of energy. The change from steady-state hematopoiesis to emergency hematopoiesis places a substantial metabolic demand on the hematopoietic system. A better understanding of the metabolic changes in HSCs during normal physiologic stress can help in understanding how these processes can become dysregulated during aging or diseases associated with aging, such as cancer. As HSCs differentiate into progenitor cells, they exit the niche, and many of these cycling cells are situated near vascular endothelial cells. The oxygen levels increase to a more normoxic level, and the progenitor cells start to use OXPHOS to generate ATP [79]. However, under stressed hematopoiesis, the primary source of ATP was thought to be glycolysis. During stress, the BM becomes acidic because of increased lactate production, a byproduct of glycolysis [80]. The BM is also full of triglyceride-containing adipocytes, which release FFAs and glycerol in response to stress conditions [81].

Given the supporting data on increased mitochondrial activity in more committed progenitor cells, it would be expected that these cells also have increased mitochondrial content during infection. However, we found that 2 hours following LPS treatment, there was

no increase in the mitochondrial content in the committed progenitor population [41]. It has previously been reported that activated immune cells can favor glycolysis because they generate ATP more rapidly compared with OXPHOS [82]. Moreover, the metabolic profile of committed progenitors differs from that of HSCs [28]. Therefore, this could be the reason why more committed progenitors do not have increased mitochondrial mass following infection. Moreover, we found that mitochondrial transfer occurs from BMSCs to HSCs before the onset of mitochondrial biogenesis [41]. It has previously been shown that the transition from quiescent to active HSCs is inherently associated with increased mitochondrial biogenesis [83–86]. During this transition, mitochondrial health is tightly regulated by mitochondrial unfolded protein response (UPR^{mt}). Sirtuin 7, a vital component of UPR^{mt}, can suppress mitochondrial biogenesis to conserve the HSC pool under stress [87]. These data highlight the ability of HSCs to activate numerous mechanisms to regulate metabolic activity, which is a fundamental determinant of HSC maintenance and cell fate. It also suggests that HSCs critically need to increase their mitochondrial mass before undergoing differentiation.

When HSCs undergo differentiation in response to stress, it has been shown that there is a metabolic switch to mitochondrial OXPHOS, which is associated with elevated ROS levels [41]. Because active cells require a different energy source compared with quiescent cells, changes in cellular metabolism from dormant to cycling are important [88]. Increasing the ROS levels, as occurs with infection, promotes the differentiation of HSCs for repopulation, whereas low levels of ROS help maintain quiescence [89]. This suggests that reprogramming of HSC metabolism can occur depending on the demand. For example, normal HSCs rely on the maintenance of quiescence via anaerobic glycolysis but can rapidly switch to mitochondrial OXPHOS to meet the energy demands of repopulation under stressed conditions [27,90,91].

The question that remains is what fuels the TCA cycle in response to infection. A single-cell gene expression analysis showed that FAO plays a crucial role in HSC expansion [92]. FAO has also been shown to be associated with HSC maintenance via the promyelocytic leukemia (PML)-PPAR-δ pathway via regulation of asymmetric division [93]. Inhibition of FAO causes HSCs to undergo symmetric divisions, leading to the exhaustion of HSCs and depletion of the stem cell pool [93]. FAO has also been shown to be pertinent in sustaining ATP production in breast epithelial cells when contact with the extracellular matrix is lost [94]. This, therefore, suggests that increased ATP production by FAO protects HSCs following differentiation when contact with the BM niche is lost. Moreover, a recent study showed that when mice undergo short-term starvation, there is a decrease in the number of HSCs [95]. These data further suggest that HSC differentiation is reliant on FAO because the loss of the number of HSCs can be attributed to increased FAO during starvation, causing the HSCs to undergo differentiation and initiating the loss of the HSC pool.

In our study, we identified that HSCs take up FFAs in response to acute infection, and this uptake is critical to the immune response [42] (Figure 1). In this study, we showed that the uptake of FFAs by HSCs in response to acute bacterial infection facilitates increased reliance of the HSCs on β-oxidation. The uptake of FFAs occurs after the onset of transcriptional changes in the FA transporter CD36. Furthermore, we identified that without CD36 on the surface, HSCs enter the cell cycle more slowly and are unable to switch to FAO [95]. The uptake of FFAs is now commonly recognized as an essential process, sustaining cell metabolism in both malignant and nonmalignant cells. In a nonmalignant setting, quiescent HSCs have a high rate of FAO, and the inhibition of FAO leads to HSC dysfunction [93]. Furthermore, in malignant cells, FFAs are used as a major energy source in ovarian [96] and prostate cancers [97]. This process is similar to observations in acute myeloid leukemia (AML), wherein FFAs are taken up by AML blasts to enhance proliferation through a

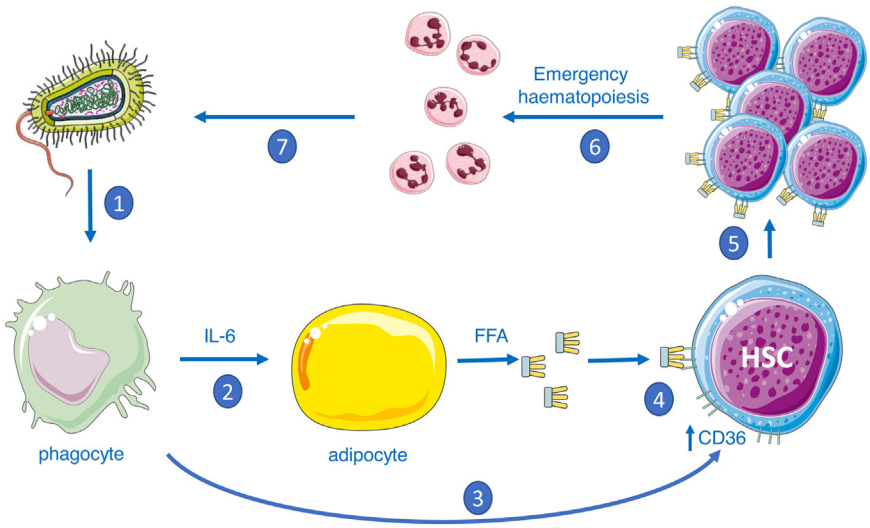

Figure 1 Schematic of the proposed model of hematopoietic stem cell (HSC) response to bacterial infection. **(A)** Phagocyte senses a bacterial infection. **(B)** The interleukin 6 cytokine is released into the peripheral system, causing lipolysis. **(C)** An unknown mechanism induces cluster of differentiation (CD)36 expression on HSCs. **(D)** HSCs take up free fatty acids through a CD36-dependent mechanism. **(E)** β-oxidation induces HSC expansion. **(F)** Emergency hematopoiesis. **(G)** Eradicate infection. *FFA*=free fatty acid; *HSC*=hematopoietic stem cell.

mechanism that increases β-oxidation in the blast [98]. This, therefore, suggests that the uptake FFAs by AML cells is a process that has been hijacked from the normal physiologic response to stress. It has not yet been investigated whether FAO is the preferred metabolic pathway in HSC differentiation. Models using *Drosophila* have demonstrated that FAO is critical for the differentiation of blood cell progenitors and that these progenitors are unable to differentiate in the absence of FAO [99]. Interestingly, it has been reported that muscle stem cells, endothelial precursors, intestinal stem cells, and neural stem cells [100] have metabolic dependence on FAO. In our study, we added HSCs to the list of cells that are dependent on FAO metabolism. We showed that during infection, HSCs take up FFAs and that this, in turn, causes a rapid shift from baseline, quiescent glycolytic metabolism to increased dependency on FAO. Furthermore, mitochondria are the main sites for FAO, where FAs are broken down to generate ATP. This suggests that imported mitochondria in HSCs following infection could be using the acquired FAs to fuel FAO. Moreover, targeting increased uptake of FAs or enhanced FAO to facilitate the expansion of immune cells may be a therapeutic approach, which could help the aging immune system.

SUMMARY

Understanding the metabolism in HSCs has become one of the leading scientific fields that have contributed to the investigation of metabolic regulation in other types of stem cells. In this review, we highlighted the metabolic flexibility of HSCs under stressed conditions. We highlighted the importance of FAO for HSC proliferation and the response to stress. However, many questions remain, including what drives HSCs to exit glycolysis under stress and how do other metabolic pathways, such as the pentose phosphate pathway and amino acid metabolism, impact HSC regulation under stress. With regard to FAO, the main question that remains is the source of FFAs. The physical location inside the niche may suggest that BM adipocytes are an important energy source in response to stress. However, the full extent of the role of adipocytes in the BM is not well established. This is because of difficulties in technology, especially the isolation and study of BM adipocytes from both humans and mice, which is partially because of the location of adipocytes. Thus, additional studies need to be undertaken to further explore the roles of adipocytes in the HSC niche.

Conflict of Interest Disclosure

The authors do not have conflicts of interest to declare in relation to this work.

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