

# Epigenetic and metabolic programming of innate immunity in sepsis

Innate Immunity  
2019, Vol. 25(5) 267–279  
© The Author(s) 2019  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1753425919842320  
journals.sagepub.com/home/ini



Vidula Vachharajani<sup>1,2</sup>  and Charles E McCall<sup>2</sup>

## Abstract

Sepsis, the 10th leading cause of death, is the most expensive condition in the United States. The immune response in sepsis transitions from hyperinflammatory to a hypoinflammatory and immunosuppressive phase; individual variations regarding timing and overlap between hyper- and hypoinflammation exist in a number of patients. While one third of the sepsis-related deaths occur during hyperinflammation, majority of the sepsis-mortality occurs during the hypoinflammatory phase. Currently, no phase-specific molecular-based therapies exist to treat sepsis. Coordinated epigenetic and metabolic perturbations orchestrate this shift from hyper- to hypoinflammation in innate immune cells during sepsis. These epigenetic and metabolic changes during sepsis progression and therapeutic opportunities they pose are described in this review.

## Keywords

Epigenetic programming, hyperinflammation, hypoinflammation, immunosuppression, metabolism, sepsis, septic shock

Date Received: 29 November 2018; revised 22 February 2019; accepted: 13 March 2019

## Introduction

Sepsis and septic shock are the leading causes of death in non-coronary intensive care units. Estimates indicate that nearly 5 million patients are diagnosed with this condition globally and over 200,000 patients die with sepsis in the United States alone each year; no specific therapies presently exist to treat these conditions.<sup>1,2</sup> There is a dire need to find molecular based therapies to treat sepsis and septic shock. Sepsis transitions from early/hyperinflammatory to a late/hypoinflammatory phenotype; a number of co-morbidities determine the timing and persistence of any of these phases in septic patients. To further add to the variability of immune response, a small proportion of critically ill patients undergo a mixed hyper- and hypoinflammatory state, referred to also as persistent inflammation, immunosuppression, and catabolism syndrome (PICS).<sup>3</sup> PICS was first described in trauma patients.<sup>4</sup> Hyperinflammation during sepsis occurs concomitant with innate immune phagocyte cell (neutrophils and monocytes) activation of antimicrobial processes, which include oxidative metabolic sources of reactive oxygen and nitrogen species. The oxidative phenotype of sepsis is cytotoxic to innate and adaptive immune cells and specialized cells of vital organs as cell death

pathways are activated. In response to that, the high energy consuming state of effector immunity/auto-toxicity rapidly transitions to a much lower energy demanding cytoprotective state in immune cells. This cytoprotective response, characterized by increased anti-inflammatory and decreased pro-inflammatory cytokine expression, is reflected in the phenomenon of endotoxin tolerance.<sup>5</sup> The nutrient substrates used to support pro-inflammation and pro-immune mechanisms are predominantly Glc and the Aa glutamine, which together support glycolysis and mitochondrial oxidative phosphorylation as ATP sources.<sup>6</sup> In contrast, lipids through lipolysis of both imported fatty acids and fatty acids stored as triglycerides support the energy conserving phenotype of mitochondrial  $\beta$

<sup>1</sup>Department of Anesthesiology, Wake Forest School of Medicine, Winston-Salem, NC, USA

<sup>2</sup>Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA

### Corresponding author:

Vidula Vachharajani, Critical Care Medicine/Respiratory Institute, Inflammation and Immunity/Lerner Research Institute, Cleveland Lerner College of Medicine, 9500 Euclid Avenue, Cleveland, OH, USA.  
Email: VACHHAV@ccf.org



oxidation. Both, hyper- and hypoinflammatory sepsis-induced pathways profoundly depart from basal homeostasis, which must be restored if the death or prolonged sickness threats of the infection and the autotoxic byproducts of inflammation are to be avoided. Mechanistically, this means that anabolic antimicrobial control and catabolic tolerance control polarities must rebalance. Mechanistic insights into the transition of polarities from hyper- to hypoinflammation and pro-inflammatory to immune-repression are critical for understanding both cell and organism fate during sepsis and for designing molecular based treatments.

Mechanistically, the two categories of phenotypic shifts involved in reprogramming an immune response during the life threatening stress of sepsis are epigenetics and metabolism. Both epigenetics and metabolism depend on mitochondrial anabolic and catabolic bioenergy reprogramming. In this review, we describe how the epigenetic and metabolic pathways reprogram the innate immune response of cells during the sequential acute inflammatory response of life threatening sepsis and septic shock.

### **Hyperinflammation and hypoinflammatory programming of innate immunity in sepsis**

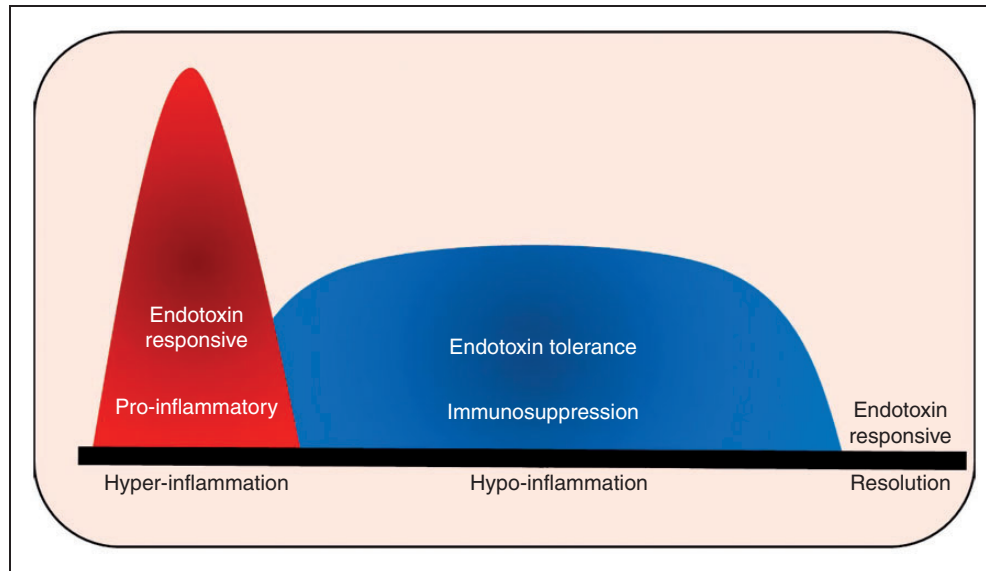
Sepsis-3 defines sepsis as a “life-threatening organ dysfunction caused by a dys-regulated host response to infection” and septic shock as “subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.”<sup>7</sup> Ranked as the 10th leading cause of death sepsis is the most expensive condition in the United States with over \$20 billion annual cost of care.<sup>8</sup> The host “resists” invading pathogen by mounting a systemic inflammatory response in innate immune cells such as macrophages and monocytes that produce pro-inflammatory cytokines, chemokines, and coagulation cascade within minutes. Further fuel to the fire of pro-inflammatory response is added by the activated pro-coagulant factors such as thrombin, Factor X, tissue factor via PAR1 signaling.<sup>9–11</sup>

However, hyperinflammation cannot be sustained, as it also attacks the host tissue and organs indiscriminately. The hyperactive immune cells transition to a deactivation/tolerance state also known as a “hypoinflammatory” and immunosuppressive phenotype. This phase is characterized by increased anti-inflammatory cytokine expression and decreased pro-inflammatory mediators biomarked by endotoxin tolerance (Figure 1).

In 1947 Beeson described endotoxin tolerance,<sup>12</sup> which is now defined as “reduced capacity of the host (*in vivo*) or of cultured macrophage/monocyte (*in vitro*) to respond to LPS activation following a primary intravenous stimulus.”<sup>13</sup> LPS binds to LPS-binding protein (LBP) and subsequently forms a complex with CD14 on cell plasma membrane; CD14 is anchored by phosphatidylinositol and does not have intracellular domain,<sup>13</sup> and a protein complex forms to signal danger. While receptors such as complement receptor type III, chemokine receptors, heat shock proteins have all been implicated in the past, since the discovery of TLR family, the research has mainly focused on TLRs, especially TLR4 as major receptor responsible for LPS signaling.<sup>13–16</sup> Endotoxin tolerance is characterized by post receptor complex down-regulation of links to pro-inflammatory cytokine expression such as TNF- $\alpha$ , IL-1 $\beta$ , chemokine expression and up-regulation of anti-inflammatory IL-10 and TGF- $\beta$ ;<sup>17,18</sup> LPS alters expression of thousands of genes in monocytes, which is gene class specific.<sup>19</sup> Thus, the mechanisms involved in endotoxin tolerance are complex and still expanding.<sup>20,21</sup>

The response to bacterial toxins seems to be highly dose-dependent. While “super low” doses of toxins elicit “priming,” “low” doses seem to induce endotoxin tolerance.<sup>22</sup> To that end, an important reaction to bacterial toxins known as the “Shwartzman reaction” is well described in the literature. Shwartzman first described this two-hit phenomenon in 1928 where the investigators administered toxic substance derived from *Bacillus typhosus* subcutaneously (first hit) in rabbits followed by intravenous injection of the same toxin (second hit). Approximately 2 h after the second hit, the subcutaneous-administration area showed inflammatory changes that progressively worsened and upon skin biopsy revealed hemorrhagic lesions.<sup>23</sup> While initially described as a local reaction, later it was discovered to be associated with systemic effects; prevented by glucocorticoid administration and human antiserum.<sup>24–26</sup> The role of circulating leukocytes and platelets was noted later as well.<sup>27</sup> Interestingly, the higher concentrations of the first (preparatory) subcutaneous dose or the “first hit” of toxin was associated with lack of hemorrhagic lesions after intravenous toxin (second hit); however, the same concentration when used as a “second hit” was able to elicit the generalized Shwartzman reaction.<sup>24</sup> The investigators also noted “increased lactate concentration” in the subcutaneous tissue after the second hit, indicating increased glycolysis.<sup>24,26</sup>

Several studies in recent years have investigated Shwartzman and other “priming” phenomena. Evidence indicates that a “super low” dose of endotoxin (0.1 ng/ml range) leads to priming of innate immune



**Figure 1.** Immune response in sepsis: immune response in sepsis transitions from hyperinflammatory to a hypoinflammatory phase followed by resolution. Hyperinflammatory phase of immune cell activation is characterized by endotoxin responsive state with cytokine storm is cytotoxic to immune and other organ cells. Hypoinflammatory phase is characterized by endotoxin tolerance and immunosuppression. Hyper- and hypoinflammatory phases are associated with profound departure from homeostasis. Restoration of homeostasis is achieved during the resolution phase of sepsis.

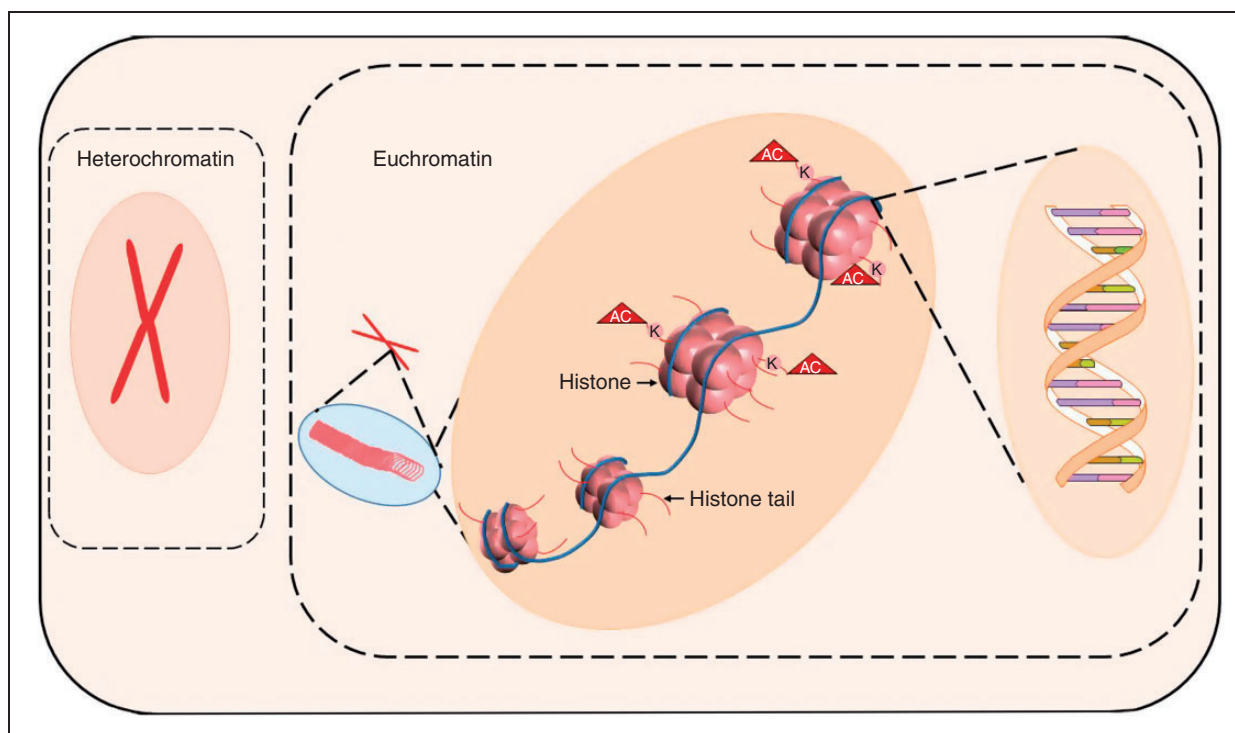
cells via IL-1 receptor associated kinase 1 (IRAK1) via selective induction of CCAAT/enhancer-binding protein d (C/EBPd), without activating NF- $\kappa$ B. IRAK1 then removes the suppressive nuclear receptors from the pro-inflammatory genes. This pathway seems to be completely separate from that of the higher doses (doses of endotoxin  $\geq 1$  ng/ml) lead to endotoxin tolerance.<sup>22,28</sup> Similarly, a crucial role for IL-15 was also noted in pathogenesis of Shwartzman phenomenon.<sup>29</sup> However, during the sepsis and septic shock, perhaps with high doses of endotoxin/other bacterial toxins combined with high sensitivity of human circulating leukocytes, the innate immune cells exhibit endotoxin tolerance.

Cell models of monocytes/macrophages with *in* and/or *ex vivo* LPS stimulation in peripheral blood monocytes have provided mechanistic insights into endotoxin tolerance; however, this should always be confirmed in animal and human studies before assessing them as treatment targets. We delineated the hyper- and hypoinflammatory responses *in vitro* and then *in vivo* in a mouse model of sepsis.<sup>30,31</sup> We used cecal ligation and puncture to induce sepsis, a model used since 1979.<sup>32</sup> We studied leukocyte adhesion in post-capillary venules as a “biomarker” for *in vivo* inflammation in the intestinal microcirculation. Leukocyte adhesion is a rate limiting step in inflammatory response,<sup>33</sup> but often overlooked in studies of introduction tolerance in cell models. We delineated three distinct phases. Within the first 12 h post-sepsis,

a hyperinflammatory phase with leukocyte adhesion significantly increases in response to additional LPS stimulation in septic mice microvasculature, which is followed by a, hypoinflammatory phase in which leukocyte adhesion is tolerant to additional LPS stimulus. As a third phase, mice surviving for at least 72 h post-sepsis restore responsiveness to LPS as defined by adherence competence.<sup>31</sup> We observed that increased leukocyte adhesion assessed *in vivo* were associated with increased ICAM-1 and E-selectin adhesion molecule expression on the endothelial cells and P-selectin glycoprotein ligand, the ligand for the E- and P-selectin adhesion molecule expression on the circulating leukocytes.<sup>31</sup> These findings clearly support linear transition between sepsis hyper- and hypoinflammation in mouse sepsis, a paradigm also supported by cell and human sepsis models in monocytes.<sup>30</sup> It highlights the need to fully understand how the transition in phenotype programming is regulated.

### Epigenetic reprogramming of innate immunity in sepsis

Epigenetics is the term first coined by Conrad Waddington in 1942,<sup>34</sup> which by current definition refers to a sustained environmental effects on gene expression program without change in the DNA sequence.<sup>35</sup> The epigenetic regulation of genes modifies the responsive euchromatin into reversibly silent heterochromatin that masks the transcription start sites by



**Figure 2.** Epigenetic modifications: heterochromatin constitute of tightly packaged DNA around histone backbone, making DNA inaccessible to transcription factors. In response to cell signaling including stress, euchromatin formation (unwinding) occurs making DNA accessible for transcription factors. Several histone modifications on histone tails including acetylation, methylation, ubiquitination, and sumoylation modulate winding and unwinding of chromatin. Lysine (K) acetylation (AC) is mostly associated with euchromatin formation while methylation with silencing of DNA (not shown).

chromatin condensation.<sup>36</sup> Thus the epigenetic control, in general terms, revolves around *winding and unwinding* of the chromatin at specific gene set loci. Histones and their interactions with multiple transcription factors and cofactors package the DNA into variably accessible chromatin.<sup>37,38</sup> As depicted in Figure 2, histone modifications on H2A, H2B, H3, and H4 tails control winding and unwinding of chromatin.

Histone modifications include acetylation, methylation, ubiquitination, phosphorylation, and sumoylation.<sup>38</sup> Histone acetylation predominantly supports gene transcription, and histone methylations play a dominant role in heterochromatin silencing of gene expression. How the histone tail modifications translate into euchromatin and heterochromatin formation is a complex but critically important network driving a sepsis outcome at the level of gene expression, as these epigenetic memory may provoke chronic disease.<sup>38</sup>

### Epigenetics of innate immunity hyperinflammation

During hyperinflammation, innate immune responses, intended to kill pathogens, enter a state characterized by excessive up-regulation of pro-inflammatory

chemokines and cytokines that initiate and amplify systemic inflammation during sepsis. Included in the sepsis “cytokine storm” are these cytokines and chemokines arising from macrophages, dendritic cells and circulating neutrophils include cytokines such as TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IL-12, IL-18, and IFN- $\gamma$ , as well as chemokines such as CCL2, CCL3, CCL6, and CXCL8,<sup>39,40</sup> expressed in blood and tissue monocytes macrophages and dendritic cells.<sup>41</sup> Pro-inflammatory cytokine expression is modulated by histone acetylation, supported by the observation that histone deacetylase inhibitor treatment suppress pro-inflammatory genes in response to LPS.<sup>38,41,42</sup> Two antagonistic enzymes that control the acetylation status of chromatin are histone acetyltransferases (HATs) and histone deacetylases (HDACs), which transfer of acetyl molecule to and from acetyl-CoA to the lysines in the amino terminal region of histones. Evidence suggests that the highly positively charged N-terminal tails of histones can potentially interact with the negative phosphodiester backbone of the DNA. Major chromatin modifications associated with hyper- and hypoinflammation during sepsis are summarized in Table 1. Histone acetylation that functionally neutralizes a positive charge on specific lysines and thus loosening the chromatin

structure to facilitate euchromatin formation.<sup>43</sup> Additionally, the newly acetylated lysines also act as the “molecular tags” for transcriptional activation. Thus, HATs are associated with euchromatin formation and transcriptional activation.<sup>44</sup> The availability acetyl-CoA is a rate limiting acetylation support of both transcription factors like NF- $\kappa$ B p65 and transacetylases like the P300 family that initiate epigenetic reprogramming of immune effector genes such as TNF- $\alpha$ , IL-1 b, and IFN- $\gamma$ . An important concept associated with the early epigenetic reprogramming of acute inflammation in many cells including innate immune monocytes and macrophages is the “poised enhancer and promoter” concept, in which cell fate has been determined and cell function is rapidly responsive within euchromatin structure.<sup>45</sup> This allows TLR4 coupling to NF- $\kappa$ B and other pro-inflammatory signaling pathways to launch the acute inflammatory response, which in the case of sepsis is excessive or deregulated.

Removal of repressor “methylation” marks on enhancer and promoter gene region also supports the pro-inflammatory phenotype in innate immune cells during activation. The histone 3 lysine 9 (H3K9me) repressive mark is erased during cell activation and restored during post-activation repression.<sup>46</sup> This demethylation requirement promoted DNA demethylase discovery. Jumonji domain-containing protein 3 (JMJD3) demethylase removes the trimethylation repressor mark on histone 3 lysine 27 (H3K27) during epigenetic chromatin modification. Fully differentiated macrophages functions are restricted from gene expression in response to environmental cues by H3K27 trimethylation of polycomb group (PcG) proteins, JmjC-domain H3K27me demethylase epigenetically derepresses the poised enhancer promoter state during acute inflammation.<sup>47</sup> Subsequently the effect of JMJD3 in derepression was shown to be independent of its demethylase activity.<sup>48</sup> Other demethylases, such as lysine-specific histone demethylase 1A (LSD1) also known as lysine (K)-specific demethylase 1A (KDM1A) are derepressors of acute inflammation and modulation of LSD1 shows therapeutic promise in endotoxic shock.<sup>49</sup> Better understanding of the epigenetic pathways responsible for derepressing inflammation and immunity is an important opportunity for drug discovery.

### *Epigenetic reprogramming of innate immunity hypoinflammation*

The hypoinflammatory phenotype of sepsis and its endotoxin tolerance biomarker epigenetically transition high energy consuming state of early sepsis to a much lower energy state, which in some patients in animal models simulates the status of hibernation, suspended

animation and energy.<sup>50</sup> A likely and ill-fated consequence of the low energy and hypoinflammatory state with endotoxin tolerance is sepsis-induced profound immunosuppression. This potentially lethal phenotype dominates sepsis clinically and is responsible for more deaths that microvascular collapse during hypoinflammatory sepsis.<sup>51</sup> The importance of this undesirable sepsis associated phenotype is evidenced by sustained an opportunistic infection in humans and animals with sepsis.<sup>52</sup>

Others and we have reported epigenetic regulation involved in endotoxin tolerance of innate immune monocytes and macrophages during hypoinflammation is associated with a switch in chromatin status from euchromatin to facultative heterochromatin (Figure 2) formation.<sup>36,53</sup> This switch requires repositioning of nucleosome and concomitant formation of protein complex responsible for transcription repression, which, in turn, requires a switch from NF- $\kappa$ B p65 transcription factor to RelB.<sup>54-57</sup> A clinically relevant and often overlooked observation is that the process of formation of euchromatin and switch to heterochromatin occurs within hours of inflammatory stimulus to an innate immune cell and activation of TLR4.

During the silencing and endotoxin tolerance, NF- $\kappa$ B factor RelA/p65 trans activator is replaced by NF- $\kappa$ B factor RelB. RelB represses a set of genes and supports formation of heterochromatin while activating euchromatin on other sets of genes thus supporting activation.<sup>54</sup> The heterochromatin formation is associated with alteration of histones in the proximal promoters of pro-inflammatory genes. This occurs via formation of protein complex consisting of G9a trans-methylase that dimethylates H3K9, creates a platform for HP1 binding, leads to the recruitment of the DNA methyl transferase Dnmt3a/b and increases promoter CpG methylation, thus forming a stable epigenetic repressor complex.<sup>54,58</sup> Decreased acetylation on histone 4 (AcH4) in addition to trimethylation of lysine 4 on histone 3 (H3K3me3) is supported in endotoxin tolerant pulmonary macrophages in sepsis mice by up-regulation of IRAK-M.<sup>53</sup>

We reported that NAD<sup>+</sup>-dependent class III HDAC family of proteins, sirtuins are crucial to the transition from hyper- to hypoinflammatory response in sepsis.<sup>30,31,59</sup> The critical difference in the SIRTs and other HDAC is their dependence on NAD<sup>+</sup> and their regulation of both immunity and metabolism. There are seven mammalian homologs of sirtuin proteins (SIRT1-7). We have shown that SIRT1 and SIRT2 directly deacetylate NF- $\kappa$ B p65 to deactivate it during hypoinflammatory phase of sepsis; SIRT1 and SIRT2 inhibition during this hypoinflammatory phase not only reverse endotoxin tolerance, but also improve mortality in septic mice.<sup>31,60,61</sup> In addition to

modulating the NF- $\kappa$ B p65 acetylation, sirtuins also direct and work together with the repressor complex mentioned above. In a two-way relationship, sepsis modulates redox-sensitive cysteines; increased sirtuin oxidation during hyperinflammation and decreased oxidation during hypoinflammation.<sup>62,63</sup> Thus, the sirtuin family of proteins are epigenetic, posttranscriptional and posttranslational guardians of homeostasis. They sense and regulate the coordination of redox and intermediary metabolism substrate selection for energy control. In addition, they direct the innate and adaptive immune response. Combined and coordinated molecular controls over all of these networks in mice and their homeostasis rheostat property are supported by the results of our treatment studies.<sup>31,60,64</sup> This raises the question whether flexible metabolic fuel selection directs innate immunity during sepsis.

### **Metabolic reprogramming of innate immunity in sepsis**

Growing evidence supports that the metabolic shifts are crucial for temporally changing the hyper- and hypoinflammatory innate immune cell phenotypes in inflammatory phenotype during sepsis. Studies in cell models of sepsis and isolated leukocytes from sepsis patient samples suggest that these shifts occur during sepsis-inflammation. Furthermore, the metabolic shifts may precede the immune cell phenotypic phase shifts.<sup>31,65</sup>

#### ***Metabolic reprogramming of hyperinflammation***

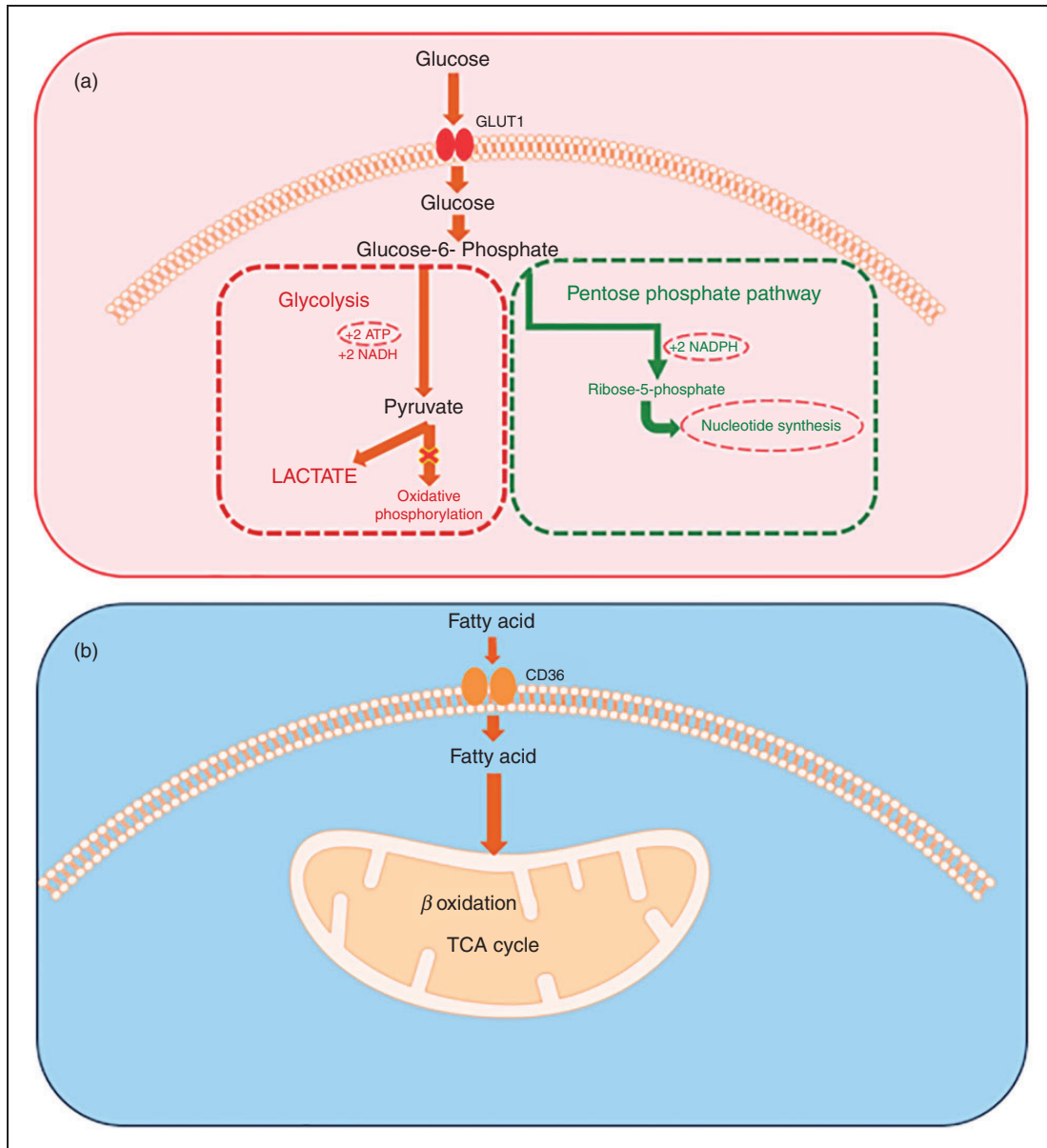
During hyperinflammatory phase of sepsis, the innate effector cells are tasked with pathogen clearance, a process that consumes large quantities of energy in the form of ATP. This energy is needed for differentiation as well as immune effector microbial accounting processes. The innate immune cells have three specific requirements: (1) high energy, (2) activating effector immunity, and (3) rapid cell regeneration. A major source of ATP in monocytes/macrophages/dendritic cells during extreme stress is glycolysis. In innate immune cells during hyperinflammation, a switch to aerobic glycolysis or “Warburg effect” common to cancer cells achieves this, as depicted in Figure 3a. Otto Warburg described this phenomenon when cancer cells preferentially utilize glycolysis to provide substrate for nucleotide synthesis for regeneration and ATP requirement to sustain proliferation.<sup>66</sup>

### **High energy**

Innate immune cells, the first responders of the inflammatory stimulus mobilize to phagocytose and kill the invading pathogen. They are mobilized in response to the inflammatory stimulus by consuming energy available to cell from Glc carbon combustion.<sup>67–69</sup> Disrupting glycolysis has profound adverse effects on phagocytosis, but disrupting mitochondrial-Glc oxidation does not.<sup>68,70</sup> Glc enters the immune cells via up-regulated GLUT1 expression in response to an inflammatory stimulus.<sup>71–73</sup> Once inside the cell, Glc converts to pyruvate during multistep glycolysis.<sup>65</sup> Pyruvate is either converted to lactate and is secreted rapidly, or enters mitochondria after decarboxylation by pyruvate dehydrogenase complex (PDC) to acetyl-CoA. Intramitochondrial acetyl-CoA can enter the tricarboxylic acid cycle and electron transport chain in the mitochondria, while cytosolic acetyl-CoA fuels fatty acid synthesis. The extramitochondrial glycolysis nets only two molecules of ATP, while the total yield of ATP molecules from the extra- and intramitochondrial Glc oxidation is 36 molecules of ATP. However, while highly efficient in regard to ATP generation, oxidative phosphorylation is a slow process while glycolysis that can be up-regulated extremely rapidly and meet the demand for ATP generation quickly. Thus, the pathogen-fighting innate immune cells use glycolysis preferentially over the oxidative phosphorylation to meet the high energy life threatening microbial invasions by increasing Glc flux and glycolysis to lactate. In fact, the oxidative phosphorylation is inhibited in immune cells during acute stress from infection.<sup>66,74–77</sup>

### **Activating effector immunity**

Innate immune cells use “weapons” to kill or contain invading microbes. Reactive oxygen species (ROS) promote pathogen killing.<sup>78–80</sup> Aerobic glycolysis seen in the innate immune cells during hyperinflammation along with the inhibition of oxidative phosphorylation leads to accumulation of Glc-6-phosphate; Glc-6-phosphate then feeds into the pentose phosphate pathway (PPP) which in addition to the formation of Rib-5-phosphate, supports generation of NADPH molecules and in turn generation of NADPH oxidase, a ROS, or the “killing capacity” much needed by these cells.<sup>65,81,82</sup> Glc-6-phosphate dehydrogenase (G-6PD), a key regulatory enzyme of PPP, is essential for neutrophil extracellular trap (NET) formation to further assist with phagocytosis of pathogens. Furthermore, G-6PD deficiency is associated with increased susceptibility and mortality in sepsis, likely due to decreased phagocytosis with impairment of PPP and NADPH activity.<sup>81,83–86</sup>



**Figure 3.** Metabolic changes of during sepsis. (a) Aerobic glycolysis by immune cells during hyperinflammatory phase of sepsis. During this phase, there is inhibition of oxidative phosphorylation and selective increase in PPP. Aerobic glycolysis provides ATP generation fulfilling the “high energy” demand of cells. In addition, there is generation of NADPH and Rib-5-phosphate to fulfil the “activation of effector immunity” and “cell regeneration” to support pathogenic killing, as detailed in the text. (b) The fatty acid uptake during the hypoinflammatory and cytoprotective response of sepsis. This is associated with increased CD36 expression on immune cell surface. Hypoinflammatory phase is associated with increased  $\beta$ -oxidation and tricarboxylic acid (TCA) cycle to produce energy for cell survival.

### Rapid cell regeneration

Apoptosis with profound lymphopenia occurs early and persists during sepsis with 10–20% of cells dying;<sup>87,88</sup> while exact mechanism/mechanisms remain unclear, many overlapping cells death mechanisms likely occur concomitantly. Accordingly, there is a need for continued regeneration of immune cells to continue effective phagocytosis, pathogen killing and inflammation

resolution. Rapid regeneration and differentiation of immune cells requires broad increases in biomass. The PPP generates Rib-5-phosphate, a substrate for nucleotide synthesis, to support the much-needed cell generation during early sepsis. Thus, once again, the rapid regeneration of immune cells, much like that of cancer cells utilizes “Warburg effect” to fulfil the biomass requirement.<sup>65</sup> Glycolysis also fuels a lipid synthesis and promotes protein synthesis from Aas,

and RNA and DNA synthesis by one carbon glycolysis support of serine glycine and synthesis. Thus, during the hyperinflammatory phase of sepsis, aerobic glycolysis at least partially fulfills all the three requirements of the immune cells to resist uncontrolled systemic infection.

The hyperinflammatory response is a double-edged sword; while resisting infecting organism, the cells and organs must protect themselves from cytotoxicity arising from the “effector response.” To do this, innate and adaptive immune cells and selective organ cells such as kidney epithelium, endothelium, intestine villi, and hepatocytes invoke evolution’s two survival principles of resistance and tolerance. At the metabolic level, the infection-resistance and auto-toxicity are in conflict during sepsis: immune resistance during hyperinflammation requires anabolism and its support of increased biomass, but tolerance during hypoinflammation requires a catabolic low energy source provided by oxidizing fatty acids, and may even need to enter a state of anergy or suspended animation.

### Metabolic reprogramming of hypoinflammation

Profound depletion of energy during hyperinflammation cause immune cells to enter “cell hibernation” and abandon resistance to infection during hypoinflammatory response of late sepsis.<sup>50</sup> This transition from hyper- to hypoinflammatory response occurs in both, the innate and adaptive immune cells.<sup>89,90</sup> The hypoinflammatory phase resembles suspended animation or hibernation characterized by mitochondrial dysfunction and resultant perturbations in cellular metabolism. Evidence supports that a hypometabolic state replaces a more transient hypermetabolic anabolic state of cell, animal, and human models of sepsis. Sirtuins play a crucial role in this switch from hyper- to hypometabolic state concomitant with the switch from hyper- to hypoinflammatory response. Mitochondrial SIRT3, which is controlled by SIRT1 promotes increased  $\beta$ -oxidation and Aa anaplerosis as mitochondria-driven catabolism.<sup>91</sup> This major shift is controlled by both AMPK disruption of mTOR-dependent protein synthesis and combined sirtuins 1, 3, and 6 response in support of the catabolic energetics, which together drive endotoxin tolerance and promote innate immune suppression.<sup>71,91,92</sup> Recent data also suggest that SIRT1 may act through dendritic cells to increase immune repressor cell function and decrease CD4+ T cell pro-immune responses.<sup>93</sup> If confirmed, this supports the notion that innate and adaptive immune competence are coordinated during sepsis by changes in metabolic substrates that reciprocally fuel

anabolism and catabolism. Interestingly, the anabolic, excessively oxidative state of hyperinflammation is countered by hypoinflammatory response with the support of antioxidants such as glutathione, superoxide dismutase, and thioredoxin. High levels of ROS directly inactivate cysteines in the zinc tetrathiolate motif of sirtuins; mechanistically, this is highly relevant for fine-tuning of inflammatory response of sepsis. Specifically, a direct and reversible cysteine thiol oxidation on SIRTs 1, 2, and 6 derepresses pro-inflammatory genes enabling hyperinflammation and anabolism.<sup>62,63,94</sup>

Nutrients and oxygen consumption decrease in muscle and innate immune cells during sepsis in animals and humans.<sup>95,96</sup> If the biomass expansion and cell regeneration were to continue in the face of decreased oxygen and ATP supply, the resulting energy deprivation would massively activate cell apoptosis.<sup>97</sup> Perhaps the hibernation and suspended animation phenotypes of sepsis hypoinflammation are survival necessities that counter death pathways by a “switch off” from high energy to low energy.<sup>95</sup> Support for this concept and for metabolic mechanisms underlying sepsis outcomes during tolerance are mounting.

We and others found that innate immune monocytes and macrophages, obtained from the hypoinflammatory phase from different models of sepsis, change their energy source substrate selection from Glc-fueled glycolysis to fatty acid oxidation,<sup>71,91</sup> as depicted in Figure 3b. Netea et al. reported broad defects in both glycolysis, fatty acids metabolism, and mitochondrial bioenergetics in human and rodent blood monocytes with highly lethal septic shock.<sup>39</sup> The investigators also found that this energy deficit-state with immunoparalysis of blood monocytes could be partially reversed by interferon  $\gamma$  treatment of human sepsis patients or their monocytes.<sup>92</sup>

Our studies of lipid metabolism during the hypoinflammatory phase differed in that CD36 plasma membrane fatty acid importer and CPT-1 mitochondrial membrane importer of acyl carnitine derived fatty acids increased.<sup>71</sup> This difference may be related to dynamic shifts in nutrient sources and metabolism in monocytes and macrophages during sepsis. Reports also support that macrophages undergo alternative M2 phenotype activation under support of STAT6 and PGC-1, thus directly linking oxidative phosphorylation with anti-inflammatory response;<sup>98</sup> notably, sirtuin 1, lies proximal to PGC-1 during hypoinflammatory phase of sepsis.<sup>71</sup>

The role of metabolism in directing the course of sepsis is growing as well. We found that the catabolic phenotypes are controlled by increased expression pyruvate dehydrogenase kinases, which maintains an inflexible tolerance-phenotype by precluding a reversal



of catabolic energetics to anabolic energetics balance. As a result, cell regeneration processes might arrest.<sup>99</sup>

Although indirect support of the innate immunity immunometabolism concept, a large non-biased metabolomics study of plasma from sepsis patients and non-human primates suggests a relationship between fatty acid oxidation pathway and sepsis-survival.<sup>100,101</sup> The studies indicate that dys-regulation of fatty acid oxidation pathway and accumulation of short and long chain acyl carnitine fatty acids early in the course of the disease predict sepsis-survival. Specifically, survivors showed increased levels of six carnitine metabolites while 16 carnitine esters increased in non-survivors of sepsis. In this study as well, fatty acid CPT1 transporter levels decreased in sepsis non-survivors.<sup>100,101</sup>

### Epigenetic and metabolic targeting during sepsis

Epigenetic and metabolic reprogramming in sepsis pose several opportunities for therapeutic targeting. Several such targets have been investigated over the past decade.

#### Epigenetic targets

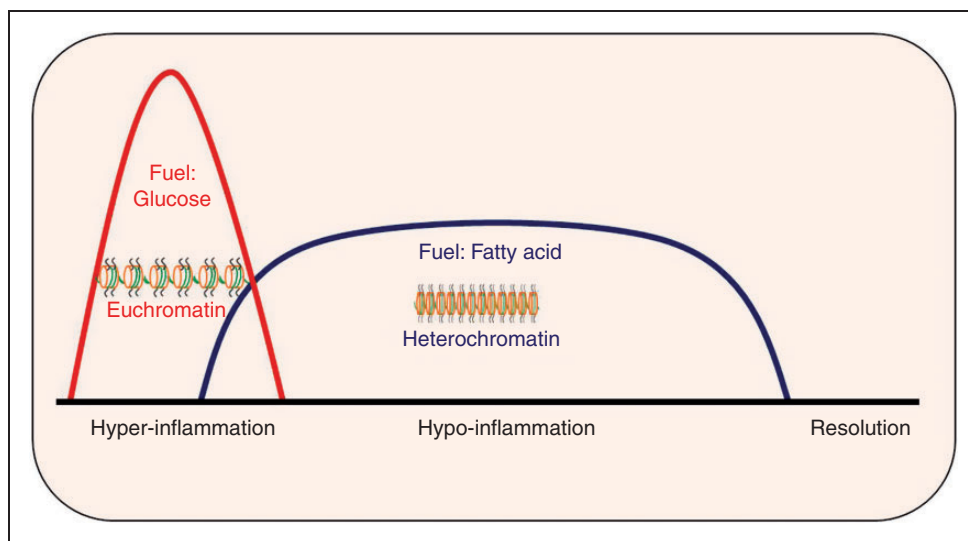
Histone deacetylase inhibition used prior to sepsis induction in mice with sepsis is associated with increased survival via attenuation of hyperinflammatory response.<sup>102,103</sup> We have shown that sirtuin over-expression during the hyperinflammatory phase of sepsis also increases survival in rodent sepsis. Others and we have shown that resveratrol pre-treatment in sepsis is associated with increased survival in mice

with sepsis via attenuation of hyperinflammatory response.<sup>61,104–106</sup> Interestingly, an old drug procainamide is also shown to be effective in attenuating hyperinflammation and short-term survival in endotoxemic rats via inhibition of DNA methylation.<sup>107</sup>

Increased sirtuin expression not only is critical to switch from hyper- to hypoinflammation, but also for sustained hypoinflammatory response in rodent sepsis. Accordingly, we showed that sirtuin inhibition during the hypoinflammatory phase of sepsis not only reverses hypoinflammatory response but also improves survival in rodent sepsis. Sirtuin regulation during hypoinflammatory response of sepsis seems to depend on biological context. To that end, we showed that SIRT1 inhibition in lean and SIRT2 inhibition in obese mice during the hypoinflammatory phase are associated with significant increase in survival.<sup>31,59</sup>

#### Metabolic targeting

As discussed earlier, there is decreased Glc utilization by immune cells during the hypoinflammatory phase of sepsis. We have shown recently that PDC plays a critical role in modulating this response; moreover, inhibition of pyruvate dehydrogenase kinase using dichloroacetate (DCA) reactivates PDC, increases mitochondrial oxidative bioenergetics in immune cells and hepatocytes, promotes immune, and organ homeostasis. In addition, we also showed that DCA accelerates bacterial clearance and improved survival in mice with sepsis.<sup>99</sup> DCA is already in clinical trials for other disease processes, the clinical use of DCA in sepsis patients remains to be studied.



**Figure 4.** Summary of epigenetic and metabolic changes of sepsis: epigenetic and metabolic changes coordinate to change hyper- to hypoinflammatory phase in immune cells.

**Table 1.** Chromatin modifications in sepsis induced inflammation.

Chromatin modification	Action
Acetylation	Lysine acetylation during hyperinflammation acetylation of histone facilitates euchromatin formation. <sup>43</sup> Lysine acetylation on NF- $\kappa$ B p65: decreased interaction with I $\kappa$ B $\alpha$ ; increased affinity to DNA binding. <sup>108</sup>
Deacetylation	Lysine deacetylation of histone during hypoinflammation: heterochromatin formation. <sup>43,53</sup> Lysine deacetylation of NF- $\kappa$ B p65: increased interaction with I $\kappa$ B $\alpha$ and export from nucleus; decreased affinity to DNA binding. <sup>53,59</sup>
Methylation	Lysine methylation of histone during hypoinflammation: methylation acts as a gene-repressor. <sup>53,108</sup> Thus, during post-activation phase of hypoinflammation, lysine methylation is restored. <sup>47</sup>
Demethylation	Lysine demethylation of histone during hyperinflammation: demethylation actively removes the repressor-methylation mark via demethylase enzymes to activate the gene poised enhancer promoter during pro-inflammatory response of hyperinflammation. <sup>47</sup>

### Challenges to therapeutic targeting

While various exciting metabolic and epigenetic targets are studied in pre-clinical models of sepsis, several roadblocks continue to exist before the true translational potential of these targets can be studied in sepsis patients. One of the main roadblocks is recognition of the exact phase the patient belongs to at any given time. Sepsis is a dynamic disease; the immune response in sepsis patients transitions from hyper- to hypoinflammation. This transition of phase remains elusive in sepsis patients. There is a dire need for a single biomarker or a panel of biomarkers that can be rapidly tested and available for use in patients before employing these phase-specific targets; one example being sirtuin up-regulation during hyperinflammation while sirtuin blockade during the hypoinflammatory phase of sepsis. These biomarkers need to take the epigenetic and metabolic changes in the innate immune response into account.

### Conclusions

The immune response in sepsis transitions from a hyperinflammatory to a hypoinflammatory phenotype. Epigenetic and metabolic changes cooperatively drive this polarity in immune and non-immune organ cells and tissue, providing opportunities for therapeutic targeting, as summarized in Figure 4. Developing biomarkers to identify the hyper- and hypoinflammatory kinetics is urgently needed to guide opportunities for molecular targeting in sepsis.

### Author's note

Vidula Vachharajani is now affiliated with Critical Care Medicine/Respiratory Institute, Inflammation and Immunity/Lerner Research Institute, Cleveland, OH.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Institutes of Health (Grant Numbers R01GM099807, R01AI065791, R01AI079144, and 1R35GM126922).

### ORCID iD

Vidula Vachharajani  <http://orcid.org/0000-0002-0892-7084>

### References

1. Dombrovskiy VY, Martin AA, Sunderram J, et al. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. *Crit Care Med* 2007; 35: 1244–1250.
2. Williams SC. After Xigris, researchers look to new targets to combat sepsis. *Nat Med* 2012; 18: 1001.
3. Horiguchi H, Loftus TJ, Hawkins RB, et al. Innate immunity in the persistent inflammation, immunosuppression, and catabolism syndrome and its implications for therapy. *Front Immunol* 2018; 9: 595.
4. Gentile LF, Cuenca AG, Efron PA, et al. Persistent inflammation and immunosuppression: a common syndrome and new horizon for surgical intensive care. *J Trauma Acute Care Surg* 2012; 72: 1491–1501.
5. Cook JA. Molecular basis of endotoxin tolerance. *Ann NY Acad Sci* 1998; 851: 426–428.
6. Ardawi MS, Jamal YS, Ashy AA, et al. Glucose and glutamine metabolism in the small intestine of septic rats. *J Lab Clin Med* 1990; 115: 660–668.
7. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315: 801–810.

8. Lagu T, Rothberg MB, Shieh MS, et al. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. *Crit Care Med* 2012; 40: 754–761.
9. Dellinger RP. Inflammation and coagulation: implications for the septic patient. *Clin Infect Dis* 2003; 36: 1259–1265.
10. Opal SM. Interactions between coagulation and inflammation. *Scand J Infect Dis* 2003; 35: 545–554.
11. Pawlinski R and Mackman N. Tissue factor, coagulation proteases, and protease-activated receptors in endotoxemia and sepsis. *Crit Care Med* 2004; 32: S293–S297.
12. Beeson PB. Tolerance to bacterial pyrogens. *J Exp Med* 1947; 86: 29–41.
13. Fan H and Cook JA. Molecular mechanisms of endotoxin tolerance. *J Endotoxin Res* 2004; 10: 71–84.
14. Ingalls RR, Monks BG, Savedra R Jr, et al. CD11/CD18 and CD14 share a common lipid A signaling pathway. *J Immunol* 1998; 161: 5413–5420.
15. Ingalls RR and Golenbock DT. CD11c/CD18, a transmembrane signaling receptor for lipopolysaccharide. *J Exp Med* 1995; 181: 1473–1479.
16. Dobrovolskaia MA and Vogel SN. Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes Infect* 2002; 4: 903–914.
17. Draisma A, Bemelmans R, van der Hoeven JG, et al. Microcirculation and vascular reactivity during endotoxemia and endotoxin tolerance in humans. *Shock* 2009; 31: 581–585.
18. Draisma A, Pickkers P, Bouw MP, et al. Development of endotoxin tolerance in humans in vivo. *Crit Care Med* 2009; 37: 1261–1267.
19. Foster SL, Hargreaves DC and Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* 2007; 447: 972–978.
20. Liu D, Cao S, Zhou Y, et al. Recent advances in endotoxin tolerance. *J Cell Biochem* 2019; 120: 56–70.
21. Lopez-Collazo E and del Fresno C. Pathophysiology of endotoxin tolerance: mechanisms and clinical consequences. *Critical Care* 2013; 17: 242.
22. Morris M and Li L. Molecular mechanisms and pathological consequences of endotoxin tolerance and priming. *Arch Immunol Ther Exp* 2012; 60: 13–18.
23. Shwartzman G. Studies on *Bacillus typhosus* toxic substances. I. Phenomenon of local skin reactivity to *B. typhosus* culture filtrate. *J Exp Med* 1928; 48: 247–268.
24. Thomas L and Good RA. Studies on the generalized Shwartzman reaction. I. General observations concerning the phenomenon. *J Exp Med* 1952; 96: 605–624.
25. Thomas L and Good RA. The effect of cortisone on the Shwartzman reaction: the production of lesions resembling the dermal and generalized Shwartzman reactions by a single injection of bacterial toxin in cortisone-treated rabbits. *J Exp Med* 1952; 95: 409–428.
26. Ziegler EJ, Douglas H and Braude AI. Human antiserum for prevention of the local shwartzman reaction and death from bacterial lipopolysaccharides. *J Clin Invest* 1973; 52: 3236–3238.
27. Stetson CA Jr. Studies on the mechanism of the Shwartzman phenomenon: certain factors involved in the production of the local hemorrhagic necrosis. *J Exp Med* 1951; 93: 489–504.
28. Maitra U, Gan L, Chang S, et al. Low-dose endotoxin induces inflammation by selectively removing nuclear receptors and activating CCAAT/enhancer-binding protein delta. *J Immunol* 2011; 186: 4467–4473.
29. Fehniger TA, Yu H, Cooper MA, et al. Cutting edge: IL-15 costimulates the generalized Shwartzman reaction and innate immune IFN-gamma production in vivo. *J Immunol* 2000; 164: 1643–1647.
30. Liu TF, Yoza BK, El Gazzar M, et al. NAD<sup>+</sup>-dependent SIRT1 deacetylase participates in epigenetic re-programming during endotoxin tolerance. *J Biol Chem* 2011; 286: 9856–9864.
31. Vachharajani VT, Fu Liu T, Brown CM, et al. SIRT1 inhibition during the hypoinflammatory phenotype of sepsis enhances immunity and improves outcome. *J Leukoc Biol* 2014; 96: 785–796.
32. Chaudry IH, Hirasawa H and Baue AE. Impairment of reticuloendothelial system function with sepsis and its improvement with ATP-MgCl<sub>2</sub> plus glucose administration. *Adv Shock Res* 1979; 2: 153–162.
33. Jung U, Norman KE, Scharffetter-Kochanek K, et al. Transit time of leukocytes rolling through venules controls cytokine-induced inflammatory cell recruitment in vivo. *J Clin Invest* 1998; 102: 1526–1533.
34. Slack JM. Conrad Hal Waddington: the last Renaissance biologist? *Nat Rev Genet* 2002; 3: 889–895.
35. Delcuve GP, Rastegar M and Davie JR. Epigenetic control. *J Cell Physiol* 2009; 219: 243–250.
36. Yoza BK and McCall CE. Facultative heterochromatin formation at the IL-1 beta promoter in LPS tolerance and sepsis. *Cytokine* 2011; 53: 145–152.
37. Fischle W, Wang Y and Allis CD. Histone and chromatin cross-talk. *Curr Opin Cell Biol* 2003; 15: 172–183.
38. Carson WF, Cavassani KA, Dou Y, et al. Epigenetic regulation of immune cell functions during post-septic immunosuppression. *Epigenetics* 2011; 6: 273–283.
39. Netea MG, van der Meer JW, van Deuren M, et al. Proinflammatory cytokines and sepsis syndrome: not enough, or too much of a good thing? *Trends Immunol* 2003; 24: 254–258.
40. Coelho AL, Hogaboam CM and Kunkel SL. Chemokines provide the sustained inflammatory bridge between innate and acquired immunity. *Cytokine Growth Factor Rev* 2005; 16: 553–560.
41. Brogdon JL, Xu Y, Szabo SJ, et al. Histone deacetylase activities are required for innate immune cell control of Th1 but not Th2 effector cell function. *Blood* 2007; 109: 1123–1130.
42. Tsaprouni LG, Ito K, Adcock IM, et al. Suppression of lipopolysaccharide- and tumour necrosis factor-alpha-induced interleukin (IL)-8 expression by glucocorticoids involves changes in IL-8 promoter acetylation. *Clin Exp Immunol* 2007; 150: 151–157.

43. Gregory PD, Wagner K and Horz W. Histone acetylation and chromatin remodeling. *Exp Cell Res* 2001; 265: 195–202.
44. Ciarlo E, Savva A and Roger T. Epigenetics in sepsis: targeting histone deacetylases. *Int J Antimicrob Agents* 2013; 42(Suppl): S8-S12.
45. Heinz S, Romanoski CE, Benner C, et al. The selection and function of cell type-specific enhancers. *Nat Rev Mol Cell Biol* 2015; 16: 144–154.
46. Saccani S and Natoli G. Dynamic changes in histone H3 Lys 9 methylation occurring at tightly regulated inducible inflammatory genes. *Genes Dev* 2002; 16: 2219–2224.
47. De Santa F, Totaro MG, Prosperini E, et al. The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* 2007; 130: 1083–1094.
48. De Santa F, Narang V, Yap ZH, et al. Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *Embo J* 2009; 28: 3341–3352.
49. Wang J, Saijo K, Skola D, et al. Histone demethylase LSD1 regulates hematopoietic stem cells homeostasis and protects from death by endotoxic shock. *Proc Natl Acad Sci USA* 2018; 115: E244–E252.
50. Singer M. Cellular dysfunction in sepsis. *Clin Chest Med* 2008; 29: 655–660.
51. Otto GP, Sossdorf M, Claus RA, et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. *Crit Care* 2011; 15: R183.
52. Hotchkiss RS, Coopersmith CM, McDunn JE, et al. The sepsis seesaw: tilting toward immunosuppression. *Nat Med* 2009; 15: 496–497.
53. Lyn-Kew K, Rich E, Zeng X, et al. IRAK-M regulates chromatin remodeling in lung macrophages during experimental sepsis. *PLoS One* 2010; 5: e11145.
54. Vachharajani V, Liu T and McCall CE. Epigenetic coordination of acute systemic inflammation: potential therapeutic targets. *Expert Rev Clin Immunol* 2014; 10: 1141–1150.
55. El Gazzar M, Yoza BK, Hu JY, et al. Epigenetic silencing of tumor necrosis factor alpha during endotoxin tolerance. *J Biol Chem* 2007; 282: 26857–26864.
56. El Gazzar M, Yoza BK, Chen X, et al. Chromatin-specific remodeling by HMGB1 and linker histone H1 silences proinflammatory genes during endotoxin tolerance. *Mol Cell Biol* 2009; 29: 1959–1971.
57. McCall CE, Yoza B, Liu T, et al. Gene-specific epigenetic regulation in serious infections with systemic inflammation. *J Innate Immun* 2010; 2: 395–405.
58. El Gazzar M, Yoza BK, Chen X, et al. G9a and HP1 couple histone and DNA methylation to TNFalpha transcription silencing during endotoxin tolerance. *J Biol Chem* 2008; 283: 32198–32208.
59. Wang X, Buechler NL, Martin A, et al. Sirtuin-2 regulates sepsis inflammation in ob/ob mice. *PLoS One* 2016; 11: e0160431.
60. Vachharajani VT, Liu T, Wang X, et al. Sirtuins link inflammation and metabolism. *J Immunol Res* 2016; 2016: 8167273.
61. Wang X, Buechler NL, Yoza BK, et al. Resveratrol attenuates microvascular inflammation in sepsis via SIRT1-Induced modulation of adhesion molecules in ob/ob mice. *Obesity* 2015; 23: 1209–1217.
62. Wang X, Buechler NL, Long DL, et al. Cysteine thiol oxidation on SIRT2 regulates inflammation in obese mice with sepsis. *Inflammation* 2019; 42: 156–169.
63. Long D, Wu H, Tsang AW, et al. The oxidative state of cysteine thiol 144 regulates the SIRT6 glucose homeostat. *Sci Rep* 2017; 7: 11005.
64. Wang X, Buechler NL, Yoza BK, et al. Adiponectin treatment attenuates inflammatory response during early sepsis in obese mice. *J Inflammation Res* 2016; 9: 167–174.
65. Arts RJ, Gresnigt MS, Joosten LA, et al. Cellular metabolism of myeloid cells in sepsis. *J Leukoc Biol* 2017; 101: 151–164.
66. Warburg O, Gawehn K and Geissler AW. Metabolism of leukocytes. *Z Naturforsch B Chem Biochem Biophys Biol* 1958; 13B: 515–516.
67. Borregaard N and Herlin T. Energy metabolism of human neutrophils during phagocytosis. *J Clin Invest* 1982; 70: 550–557.
68. Boxer LA, Baehner RL and Davis J. The effect of 2-deoxyglucose on guinea pig polymorphonuclear leukocyte phagocytosis. *J Cell Physiol* 1977; 91: 89–102.
69. Selvaraj RJ and Sbarra AJ. Phagocytosis inhibition and reversal. II. Possible role of pyruvate as an alternative source of energy for particle uptake by guinea-pig leukocytes. *Biochim Biophys Acta* 1966; 127: 159–171.
70. Sbarra AJ and Karnovsky ML. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. *J Biol Chem* 1959; 234: 1355–1362.
71. Liu TF, Vachharajani VT, Yoza BK, et al. NAD<sup>+</sup>-dependent sirtuin 1 and 6 proteins coordinate a switch from glucose to fatty acid oxidation during the acute inflammatory response. *J Biol Chem* 2012; 287: 25758–25769.
72. Michalek RD, Gerriets VA, Jacobs SR, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4<sup>+</sup> T cell subsets. *J Immunol* 2011; 186: 3299–3303.
73. Venet F and Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol* 2017; 14: 121.
74. Guthrie LA, McPhail LC, Henson PM, et al. Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *J Exp Med* 1984; 160: 1656–1671.
75. Krawczyk CM, Holowka T, Sun J, et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 2010; 115: 4742–4749.
76. Srivastava A and Mannam P. Warburg revisited: lessons for innate immunity and sepsis. *Front Physiol* 2015; 6: 70.

77. Wang L, Li JJ, Guo LY, et al. Molecular link between glucose and glutamine consumption in cancer cells mediated by CtBP and SIRT4. *Oncogenesis* 2018; 7: 26.
78. McPhail LC, DeChatelet LR, Shirley PS, et al. Deficiency of NADPH oxidase activity in chronic granulomatous disease. *J Pediatr* 1977; 90: 213–217.
79. Kovacs I, Horvath M, Lanyi A, et al. Reactive oxygen species-mediated bacterial killing by B lymphocytes. *J Leukoc Biol* 2015; 97: 1133–1137.
80. Vernon PJ and Tang D. Eat-me: autophagy, phagocytosis, and reactive oxygen species signaling. *Antioxid Redox Signaling* 2013; 18: 677–691.
81. Spolarics Z, Siddiqi M, Siegel JH, et al. Increased incidence of sepsis and altered monocyte functions in severely injured type A<sup>-</sup> glucose-6-phosphate dehydrogenase-deficient African American trauma patients. *Crit Care Med* 2001; 29: 728–736.
82. Wang Y, Wang X, Yang W, et al. Effect of simvastatin on the intestinal Rho/ROCK signaling pathway in rats with sepsis. *J Surg Res* 2018; 232: 531–538.
83. Azevedo EP, Rochael NC, Guimaraes-Costa AB, et al. A metabolic shift toward pentose phosphate pathway is necessary for amyloid fibril- and phorbol 12-myristate 13-acetate-induced neutrophil extracellular trap (NET) formation. *J Biol Chem* 2015; 290: 22174–22183.
84. Rostami-Far Z, Ghadiri K, Rostami-Far M, et al. Glucose-6-phosphate dehydrogenase deficiency (G6PD) as a risk factor of male neonatal sepsis. *J Med Life* 2016; 9: 34–38.
85. Wilmanski J, Villanueva E, Deitch EA, et al. Glucose-6-phosphate dehydrogenase deficiency and the inflammatory response to endotoxin and polymicrobial sepsis. *Crit Care Med* 2007; 35: 510–518.
86. Cooper MR, DeChatelet LR, McCall CE, et al. Leucocyte G.-6-P.D. deficiency. *Lancet* 1970; 1: 110.
87. Girardot T, Rimmele T, Venet F, et al. Apoptosis-induced lymphopenia in sepsis and other severe injuries. *Apoptosis* 2017; 22: 295–305.
88. Hotchkiss RS, Tinsley KW, Swanson PE, et al. Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J Immunol* 2002; 168: 2493–2500.
89. Anantha RV, Mazzuca DM, Xu SX, et al. T helper type 2-polarized invariant natural killer T cells reduce disease severity in acute intra-abdominal sepsis. *Clin Exp Immunol* 2014; 178: 292–309.
90. Mehta A, Brewington R, Chatterji M, et al. Infection-induced modulation of m1 and m2 phenotypes in circulating monocytes: role in immune monitoring and early prognosis of sepsis. *Shock* 2004; 22: 423–430.
91. Liu TF, Vachharajani V, Millet P, et al. Sequential actions of SIRT1-RELB-SIRT3 coordinate nuclear-mitochondrial communication during immunometabolic adaptation to acute inflammation and sepsis. *J Biol Chem* 2015; 290: 396–408.
92. Cheng SC, Scicluna BP, Arts RJ, et al. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nat Immunol* 2016; 17: 406–413.
93. Martin AN, Alexander-Miller M, Yoza BK, et al. Sirtuin1 targeting reverses innate and adaptive immune tolerance in septic mice. *J Immunol Res* 2018; 2018: 2402593.
94. Jung SB, Kim CS, Kim YR, et al. Redox factor-1 activates endothelial SIRTUIN1 through reduction of conserved cysteine sulphydryls in its deacetylase domain. *PLoS One* 2013; 8: e65415.
95. Singer M. Mitochondrial function in sepsis: acute phase versus multiple organ failure. *Crit Care Med* 2007; 35: S441-S448.
96. Liu TF, Brown CM, El Gazzar M, et al. Fueling the flame: bioenergy couples metabolism and inflammation. *J Leukoc Biol* 2012; 92: 499–507.
97. Singer M, De Santis V, Vitale D, et al. Multiorgan failure is an adaptive, endocrine-mediated, metabolic response to overwhelming systemic inflammation. *Lancet* 2004; 364: 545–548.
98. Vats D, Mukundan L, Odegaard JI, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* 2006; 4: 13–24.
99. McCall CE, Zabalawi M, Liu T, et al. Pyruvate dehydrogenase complex stimulation promotes immunometabolic homeostasis and sepsis survival. *JCI Insight* 2018; 3.
100. Langley RJ, Tipper JL, Bruse S, et al. Integrative “omic” analysis of experimental bacteremia identifies a metabolic signature that distinguishes human sepsis from systemic inflammatory response syndromes. *Am J Respir Crit Care Med* 2014; 190: 445–455.
101. Langley RJ, Tsalik EL, van Velkinburgh JC, et al. An integrated clinico-metabolomic model improves prediction of death in sepsis. *Sci Transl Med* 2013; 5: 195ra95.
102. Zhang L, Jin S, Wang C, et al. Histone deacetylase inhibitors attenuate acute lung injury during cecal ligation and puncture-induced polymicrobial sepsis. *World J Surg* 2010; 34: 1676–1683.
103. Kim SJ, Park JS, Lee DW, et al. Trichostatin A protects liver against septic injury through inhibiting Toll-like receptor signaling. *Biomol Ther* 2016; 24: 387–394.
104. Xu S, Gao Y, Zhang Q, et al. SIRT1/3 activation by resveratrol attenuates acute kidney injury in a septic rat model. *Oxid Med Cell Longevity* 2016; 2016: 7296092.
105. Gan Y, Tao S, Cao D, et al. Protection of resveratrol on acute kidney injury in septic rats. *Hum Exp Toxicol* 2017; 36: 1015–1022.
106. Holthoff JH, Wang Z, Seely KA, et al. Resveratrol improves renal microcirculation, protects the tubular epithelium, and prolongs survival in a mouse model of sepsis-induced acute kidney injury. *Kidney Int* 2012; 81: 370–378.
107. Shih CC, Liao MH, Hsiao TS, et al. Procainamide inhibits DNA methylation and alleviates multiple organ dysfunction in rats with endotoxic shock. *PLoS One* 2016; 11: e0163690.
108. Glozak MA, Sengupta N, Zhang X, et al. Acetylation and deacetylation of non-histone proteins. *Gene* 2005; 363: 15–23.