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Epigenetic coordination of acute systemic inflammation: potential therapeutic targets

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Abstract

Epigenetic reprogramming of thousands of genes directs the course of acute systemic inflammation, which is highly lethal when dysregulated during sepsis. No molecular-based treatments for sepsis are available. A new concept supports that sepsis is an immunometabolic disease and that loss of control of nuclear epigenetic regulator Sirtuin 1 (SIRT-1), a NAD⁺ sensor directs immune and metabolic pathways during sepsis. SIRT-1, acting as homeostasis checkpoint, controls hyper and hypo inflammatory responses of sepsis at the microvascular interface, which disseminates inflammatory injury to cause multiple organ failure. Modifying SIRT-1 activity, which can prevent or treat established sepsis in mice, may provide a new way treat sepsis by epigenetically restoring immunometabolic homeostasis.

Introduction

This discussion highlights an emerging concept that the highly lethal acute systemic inflammatory reaction associated with sepsis is epigenetically controlled as it deviates from and is able to return to homeostasis in survivors, but not in non-survivors. It emphasizes new data supporting that sepsis is an immunometabolic process, which switches immune and metabolic phenotypes in response to shifts in cell bioenergetics and nutritional needs¹. It highlights the microvascular tissue interface (MVI) as a poorly defined but critically important site of immunometabolic phenotypic shifts associated with sepsis. It promotes the concept that a sirtuin 1 (SIRT-1) dependent homeostat provides a checkpoint for controlling immunometabolic phenotypes and holds promise as a target to treat sepsis by counterbalancing dysregulated epigenetics. Sirtuins are mammalian homologues of silent information regulator (sir2) protein first described in yeast, as described below. Homeostasis restoration of inflammation and metabolism represents a new concept in sepsis treatment with high impact potential, since sepsis mortality rates remain over 30% with death of millions worldwide^{2,3} and is without successful therapeutics targeted to specific pathophysiology.⁴

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Inflammation exemplifies the Darwinian principle of a highly conserved biological process designed “defend and mend” the fittest, but if too far deviated from homeostasis causes disease or death. Successful inflammation involves changes in expression of thousands of genes, which deviate from basal homeostasis and must be counterbalanced in order to restore normal physiology. Of increasing interest is that epigenetic reprogramming of the germ line genome directs the deviation from homeostasis during the inflammatory process. The term epigenetics was first coined in 1942 by Waddington^{5,6}. Currently, the most accepted definition is the heritable changes in gene function that cannot be explained by differences in DNA sequences⁷.

Fundamentals of epigenetic controls are depicted in Figure 1. In general terms, epigenetic regulation of gene function occurs by organizing the gene loci on chromatin into transcriptionally active or silent states⁷. Transcriptionally *active* “euchromatin” is accessible to transcription factors and polymerases while the transcriptionally *silent* “heterochromatin” is inaccessible to those. Histones are responsible for packaging the DNA in a chromatin structure. Their modifications (histone codes) at H1, H2A, H2B, H3 and H4 tails unwinds or winds chromatin structure to make it accessible (euchromatin) or inaccessible (heterochromatin) respectively for transcription. Chemical modifications of histone tails include acetylation, methylation, ubiquitination and phosphorylation. An acetylation of histone lysines usually supports transcription and replacement of lysine acetyl with methyl groups usually promotes silent heterochromatin formation. An orderly modification of the specific histone residues along with DNA methylation and other epigenetic alterations of the nearby DNA dictate the transcriptional status of the gene locus by exposing it to transcriptionally active euchromatin or silent heterochromatin. It is easy to appreciate how changes in chromatin landscape can be guided by environmental processes that prompt sensing and signaling to inform the genetic and epigenomic code of inflammation.

Epigenetic reprogramming temporally controls the acute systemic inflammatory response

Figure 2 depicts two important genomic features of acute systemic inflammation. **Panel A** represents changes in expression of the total genome over time. In mild sepsis, both the genes that increase expression of mRNA and those that repress mRNA return to homeostasis in a timely fashion. In contrast, severe sepsis is associated with prolonged mRNA changes—either increased (gene activation) or decreased (gene repression). Strikingly, there are no detectable differences in the gene expression changes in mild vs. severe acute systemic inflammation with high mortality, suggesting defective regulation of homeostasis without qualitative gene shifting. This similarity supports the need to determine how homeostasis is regulated. **Panel B** depicts the epigenetic and phenotypic shifts associated with acute inflammation. This counter regulatory principle supports a pivotal checkpoint that shifts to homeostasis or becomes dysregulated to allow too much inflammation early and too much hypo-inflammation/immunosuppression later. Controversy exists over whether to refer to the later phenotype of acute systemic inflammation as a compensatory anti-inflammatory response (CARS), since it is often uncontrolled and the host remains chronically ill or dies. Newer terms such as mixed anti-inflammatory syndrome (MARS)⁸ and persistent

inflammation, immunosuppression, and catabolic syndrome (PICS)⁹ are emerging, underscoring the basic need to better understand how acute inflammation rebalances homeostasis at the molecular level.

In the molecular language of the acute inflammatory process, Toll like receptors (TLR) sense microbial products such as lipopolysaccharide endotoxin (LPS) from gram negative bacteria or other microbial products or non-infectious cell derived alarmins such as high mobility group protein B1 (HMGB1). Molecular changes in complement and coagulation also are sensed by immune cells. Within minutes of inflammatory stimulus, TLR-derived signaling pathways activate a complex cascade that usually requires the nuclear factor kappa B (NF- κ B) RelA/p65 component [with cooperation of other transcription factors such as interferon response transcription factors (IRF) and activator protein 1 (AP1)] to activate transcription of acute pro-inflammatory genes at the poised promoters of euchromatic genes responsible for initiating acute inflammation. Virtually coincident with this pro-inflammatory gene transcription program is increased transcription of counter-regulator immunorepressor genes and repressed transcription of genes that regulate a broad array of cell physiology e. g. protein synthesis¹⁰. In addition to transcription, post-transcriptional, translational and post-translational processes contribute the ultimate protein profiles and cell physiology. Importantly in mild sepsis both the genes that increase expression of mRNA and those that repress mRNA return to homeostasis in a timely fashion. In contrast, severe sepsis is associated with prolongation of mRNA regulation—either activation or repression. As previously stated, there are no detectable differences in the overall gene expression changes in mild versus severe sepsis with high mortality, suggesting defective adaptation without qualitative gene shifting. This genomic symmetry has been used to support the concept that there are no separate phases of sepsis¹⁰. This position, however, does not take into consideration the epigenetic program of sepsis that alters the phenotypes of hyper-inflammation and immunosuppression and returns to homeostasis if the process succeeds.

Homeostasis restoration at the molecular level requires a counter-regulatory epigenetic process that shifts away from the early hyper-inflammatory state to a hypo-inflammatory and adaptive phenotype in addition to removal of source of inflammation/infection such as focus of infection. During both mild and lethal acute systemic inflammation, the same epigenetic shift from initial hyper-inflammation shift to hypo-inflammatory phenotype occurs, but with different magnitude and persistence. In severe acute inflammation, the magnitude of the initial response dictates the level of hyper-inflammation which in turn determines the degree and persistence of the adaptive hypo-inflammatory phenotype and the level of clinical immunosuppression. Thus, pronounced deviations from homeostasis occur during the mild and severe phenotypes, but with different kinetics underlying homeostasis restoration. The hypo-inflammatory or chronic inflammatory phenotype can persist for days or weeks and is accompanied by a profound state of repressed innate and adaptive immunity¹¹. During this time, sepsis patients are more susceptible to new infections as well as retaining the original infection. During this chronic disease phase, increased susceptibility to infections associated with higher mortality may persist for weeks or months. An important question is whether a new homeostasis set point could underlie increased sepsis mortality.

Accumulating data implicate epigenetic reprogramming of innate immune myeloid derived phagocytes (monocyte/macrophages and neutrophils) during acute inflammation^{1,7,12} but has not been clearly defined in different organs/tissue or in adaptive immunity. Importantly, the early epigenetic switch from hyper-inflammation to hypo-inflammation cannot be clinically detected in human sepsis. This has resulted in a claim that there is only one phase of sepsis¹⁰, which overlooks dynamic phenotype shifts that evolve during sepsis. Epigenetic reprogramming ultimately must reestablish homeostasis during resolution. Figure 3 partially details mechanisms that control the epigenetic shifts that temporally evolve during sepsis. As epigenetic programming progresses from basal state to hyper-inflammatory state in response to inflammatory stimulus, transcription factors, DNA regulators and histone modifiers modify chromatin structure from the “poised” (basal) state to active euchromatin state. This is followed by a hypo-inflammatory and immunosuppressed state that switches activated euchromatin to silenced heterochromatin at specific gene sets^{1,7,12}. Other genes important in sepsis remain in the euchromatic state, in which transcription activation is still possible. This switch of selected gene sets from euchromatin to heterochromatin requires nucleosome repositioning or remodeling on proximal promoter DNA^{13,14}, concomitant with formation of a transcription repressosome^{15–17}. Importantly, this process of opening and closing chromatin structure occurs within hours of initial inflammatory stimulus sensing by Toll-like receptor 4 (TLR4)—and likely other pro-inflammatory membrane sensors. NF- κ B DNA binding sites and play a crucial role in chromatin reprogramming that silences acute response innate immune genes wherein NF- κ B factor RelA/p65 rapid transactivator is replaced by NF- κ B factor RelB¹⁸. RelB then acts as a dual transcription regulator, repressing a set of genes by supporting formation of silent heterochromatin while activating euchromatin at another set of genes. The silent heterochromatin formation is aided by epigenetic mediators that alter proximal promoter histones and DNA, including G9a transmethylease for histone H3K9, HP-1 heterochromatin adaptor protein, H1 histone and its binding to high mobility group protein B1 (HMGB1), and DNA CpG methyltransferases DNA methyl transferases 3a and 3b (DNMT 3a/b). The molecular process by which RelB activates euchromatin of selective genes is poorly understood, but it clearly plays a role in sustaining expression of I κ B- α repressor of NF- κ B RelA/p65¹⁹. A seminal report indicates that thousands of specific genes that regulate immunity and metabolism are either increased or decreased during the acute inflammatory reaction¹². Of critical importance to understanding phenotype shifting and its clinical impact is that epigenetic reprogramming during the acute inflammatory hypo-inflammatory and potentially immunosuppressive state is reversible¹.

Epigenetic repression of pro-inflammatory genes during the acute systemic inflammatory process does not target just innate immunity phagocytes. It also reprograms dendritic cells²⁰ and may underlie changes in adaptive immunity that represses T cell function through excessive apoptosis, T cell exhaustion, and T regulatory repressor cell development (T-reg)²¹. Evidence shows that antagonizing the immunosuppressive phase of sepsis results in a reversal of T cell repressor cells and improve survival in rodent sepsis¹¹. The molecular epigenetic features underlying these changed components of adaptive immunity during acute systemic inflammation from sepsis or trauma are unclear.

Immunometabolic shifts during acute systemic inflammation

An emerging paradigm supports that epigenetic and post-translational processes integrate immunity and metabolism during inflammation and immunity at epigenetic and post translational levels¹. This important connection was first detected in chronic inflammation associated with obesity and diabetes, where the pro-inflammatory myeloid derived cells that dominates obese adipose tissue is linked to alterations in glucose metabolism, and which can be reversed if the anti-inflammatory myeloid derived cells becomes dominant^{22–24}.

The new field of integrated immunometabolism emphasizes the importance of cell nutrients (glucose, fatty acids, and amino acids) in regulating inflammation, innate and adaptive immunity. This is predicated on the need for distinct sources of energy to fuel inflammation. Increased use of glucose as fuel for immune effector cells is required to express pro-inflammatory genes and to kill invading microorganisms^{25,26}. This dominant glycolytic phenotype shifts to fatty acid oxidation was observed in repressor cells of adaptive immunity like CD4⁺ Treg^{27,28}. One of the early features of sepsis indicated a profound repression in adaptive immunity that regulates delayed type hypersensitivity and T cell effector and repressor, phenotypes that can persist for many days or weeks⁷. This emerging concept of reprogrammed immunometabolism has resulted in new ways to potentially treat chronic inflammatory diseases like obesity, diabetes, atherosclerosis, and Alzheimer's disease²⁹.

The concept of immunometabolism is equally important in acute systemic inflammation. We discovered a shift in use of glucose to fatty acids as fuel for innate immune phagocytes during sepsis²⁸, which develops concomitant with the epigenetic phenotype shifts depicted in Figure 4. To mount acute inflammation and competent innate immune response the phagocytes require glucose as a nutrient.^{30–32} The ATP surge required for the anabolic energy of acute inflammation is provided by glycolysis under normoxemic conditions, simulating the Warburg effect often seen in cancer³³. Glycolysis also activates the pentose phosphate pathway that controls microbicidal activity generated during NADPH oxidase activation and supports anabolic pathways of nucleic acid synthesis. Increased glucose fueling for cell anabolism requires elevated membrane expression of glucose transporter Glut1, as well as rises in expression of glycolysis regulatory genes. A concomitant drop in mitochondrial glucose oxidation with increased glycolysis during sepsis increases intra- and extracellular pyruvate and lactate. In innate immune phagocytes, increased glycolysis and decreased mitochondrial oxidation depend, at least in part, on hypoxia inducing factor 1 α (HIF-1 α) to transcribe glycolytic genes, which is essential for immune effector responses such as TNF- α , IL-1 β , other cytokines, chemokines and chemotactic mediators^{34,35}. The increased glucose flux and metabolism generates reactive oxygen species (ROS) needed for killing microbes and for supporting expression of pro-inflammatory genes. However, ROS, if poorly counterbalanced by antioxidant pathways, can generate cell death and reduce mitochondrial mass³⁶.

Importantly, the onset of hypo-inflammation within hours after hyper-inflammation requires a shift in cell nutrients from glucose (and likely amino acids) to fatty acids²⁸. This switch increases expression of fatty acid membrane transporters (e.g., CD36), activates fat catabolism rather than storage, and promotes entry of fatty acids into mitochondrial to be

oxidized by the electron transport chain. A shift in immunometabolism from anabolism to catabolism occurs in cell models as well as innate immune phagocytes of murine and human sepsis²⁸. The innate immune cells of the hypo-inflammatory phase are in catabolic phase of low energy requirement and use fatty acid as predominant fuel³⁷. Similarly, the adaptive immune T repressor cells also exist in a low metabolic catabolic state with fatty acids as nutrients and fatty acid oxidation as the major source of ATP³⁸. Taken together, the congruent shifts in immunity and metabolism support that a master switch or a checkpoint might control the clinically relevant and epigenetically reprogrammed phenotypic shift during sepsis induced acute systemic inflammation. Figure 5 provides a schematic of immunometabolic shifts during sepsis, as introduced in Figure 4.

The microvascular interface (MVI) is an understudied and critically important component of acute systemic inflammation reprogramming

Microvascular interface (MVI) is the most important link between the circulating blood cells and the tissue beds that house local infections or receive physical injury and become physiologically compromised during acute systemic inflammation. The MVI, although recognized as important in inflammation for many years^{39,40} is poorly understood in context of *in vivo* phenotypic shifts, epigenetic reprogramming, and modifications in immunometabolism. Among the important features of previous MVI studies were changes in oxygen delivery and blood flow that occurs by altering arterioles after production of reactive nitrogen species (RNS), such as nitric oxide⁴⁰. While clearly important, these changes in perfusion do not directly deliver the immune response cells into tissue. Rather, leukocyte adhesion in the post capillary venules is the earliest marker and a rate determining factor of inflammatory response.⁴¹ This inflammatory response serves to of both, to defend as well as injure. Studies of the MVI are important in order to understand the epigenetic and immunometabolic concepts associated with phenotype shifts of acute systemic inflammatory diseases like sepsis and other acute inflammatory conditions.

Epigenetic regulation of endothelial cell adhesion molecules has been studied in chronic inflammation models of atherogenesis and hypoxia^{42–44}. Brahma (Brm) and brahma related gene 1 (Brg1) were recently found to modulate cell adhesion via chromatin modification⁴⁵. Leukocyte adhesion and its phenotypic shifting during acute systemic inflammation play a critical role in determining the sepsis response in mice^{46–50}. Increased leukocyte adhesion during early polymicrobial sepsis increases further in obese septic mice compared to lean mice with sepsis⁴⁷. Importantly, early⁴⁷ and late sepsis (in press) is reflected by change in the leukocyte adhesion at the MVI. Initial increases in MVI leukocyte adhesion in early sepsis are followed by decreases during late sepsis with immunosuppression. The phenomenon of endotoxin tolerance at the MVI in mouse sepsis can be used to mark the difference in the hyper-inflammation phenotype with leukocyte adhesion responsive to LPS challenge, and the hypo-inflammation and immunosuppressed phenotype, in which leukocyte adhesion is unresponsive (paralyzed) to endotoxin stimulation MVI. Immunoparalysis is critically important in impeding a return to homeostasis during sepsis. In support of this are findings that resolution in animals surviving sepsis is characterized by return of endotoxin responsiveness in the microcirculation (in press). Thus, *in vivo*

endotoxin tolerance or MVI immunoparalysis is the most prominent feature of a phenotypic shift from hyper-inflammation to immune repression between blood and tissue, during leukocytes cannot enter local sites of infection is not cleared. Further, endotoxin tolerance of blood leukocytes studied *ex vivo* in humans is a biomarker of poor outcome sepsis⁵¹.

We deem phenotype shifting of leukocyte adhesion at the MVI as a major indicator of the changing epigenetic axis of immunity and metabolism. MVI reprogramming in mice temporally correlates with epigenetic phase shifts observed in a human cell model of sepsis¹ in human and mouse bone marrow macrophages assessed *ex vivo*, and inflammatory shifts in humans given endotoxin *in vivo*⁵². Further defining molecular controls at the MVI in septic mice and in humans, when possible, should clarify the critical role of restoring homeostasis during sepsis resolution.

SIRT-1 controls immunometabolic and bioenergy phenotypic shifts during acute inflammation

Accumulated evidence strongly supports nuclear sirtuin 1 (SIRT-1) as a master regulator of homeostasis in cells and organisms⁵³. First described in yeast, the Sir2 gene was linked to replicative lifespan of budding yeast as a generator of reversibly silent heterochromatin, which acts by sensing NAD⁺ and deacetylating nearby histones⁵⁴. There are 7 mammalian homologues of Sir2 proteins SIRT-1-7. SIRT-1, SIRT-6 and SIRT-7 are primarily nuclear, SIRT-3, SIRT-4 and SIRT-5 are mitochondrial and SIRT-2 is cytosolic⁵⁴. Generation of NAD⁺ within specific compartments of the cells where the sirtuins are located is essential in determining sirtuin function. Nicotinamide phosphoribosyltransferase (NAMPT) is the rate limiting enzyme for activating sirtuins in humans, but other sources from *de novo* production exist in other species and might at times be important in humans. The NAD⁺ cycle and its inhibitory feedback loop are depicted in Figures 4 and 5.

Our laboratory has identified a critical role for SIRT-1 in balancing immunometabolic processes associated with acute systemic inflammation^{28,55}. Mechanistically, increases in available NAD⁺ and its sensing by SIRT-1 fosters formation of facultative heterochromatin at promoters of genes that initiate sepsis (e.g. TNF- α and IL-1 β)^{56,57}. This process requires changes in expression of NAD⁺ rate limiting enzyme NAMPT and SIRT-1⁵⁵. During the initiation phase of acute inflammation, SIRT-1 directly binds to the NF-KB RelA/p65 proximal promoter of the acute pro-inflammatory genes like TNF- α to inactivate its transcription by deacetylating lysine 310 (K310). Simultaneously, it induces RelB transcription and supports its binding to putative NF-kB sites no longer occupied by RelA/p65. SIRT-1 also modifies histones by deacetylating histones (e.g., H1) that further promote heterochromatin formation at pro-inflammatory genes, and possibly HIF1- α and other genes that control glycolysis. Concomitantly, it activates NF-kB RelB and PGC1- α to promote expression of genes supporting fatty acid oxidation. Sirtuin family members other than SIRT-1 participate in immune and metabolic reprogramming during acute inflammation. Nuclear sirtuins, SIRT-1 and SIRT 6, combine to epigenetically support the switch in early acute systemic inflammation myeloid phagocytes from early TLR4 glycolysis rises and reduced glucose oxidation by mitochondria to mitochondrial fatty acid β oxidation as the dominant cell nutrient during the hypo-inflammatory and immunosuppressed phenotype of

acute systemic inflammation associated with murine and human sepsis²⁸. Activation of SIRT-1 and SIRT-6 may also support the reduction in pyruvate entry and metabolism in mitochondria, thereby limiting glucose oxidation in the tricarboxylic acid cycle as mitochondrial fueling shifts to fatty acid oxidation²⁸. A SIRT-1 nuclear to mitochondrial communication involve RelB dependent increases in expression of master mitochondrial regulator, SIRT-3, to support increased mitochondrial biogenesis and activation of the electron transport system ATP generation (manuscript under review). The role of SIRT-1 and SIRT-6 in shifting immune and metabolic pathways is depicted in Figure 4.

The reversible nature of epigenetic reprogramming of immunity and inflammation is emerging as critically important in designing new treatments for sepsis. Most strikingly, we have found that blocking SIRT-1 after the shift from hyper-inflammation to hypo-inflammation and immunosuppression in septic mice rescues virtually all mice from death and reverses MVI leukocyte adhesion dysregulation (in press).

Thus, the SIRT-1 likely occupies an essential checkpoint for sensing and restoring deviations in homeostasis that accompany both early acute hyper-inflammation of sepsis and later immunosuppression and chronic organ injury. This homeostasis function of SIRT-1, however, is compromised if the SIRT-1 levels are too low before or during early sepsis, or remains too high during late sepsis with chronic disease and continued high mortality. We support the concept that sirtuins provide evolutionarily conserved checkpoints that shift and then restore the defend and mend concept of homeostasis. If further substantiated, this concept introduces a paradigm shift for understanding, preventing, and treating the uncontrolled immunometabolic responses of acute systemic inflammation.

Expert Commentary

We are entering an exciting new era in understanding the inflammatory process. First is the reversible plasticity in innate and adaptive immune effector and repressor cells, a concept that can be directly linked to identifying new treatment approaches for diseases like obesity and sepsis. Second is the emerging concept that epigenetic reprogramming of immunity, metabolism, and mitochondrial bioenergetics directs the course of inflammation. Third is the recognition of energy and redox sensing by homeostasis sensors and modifiers of sirtuin family and other energy sensors like cyclic AMP dependent kinase (AMPK) may inform new therapies for acute inflammation.

Five year view

Applying treatment approaches based on epigenetic programming of immunometabolic pathways linked to acute systemic inflammation from sepsis and other non-infectious phenotypes like trauma hold considerable promise. The most important potential opportunity lies in treating the diseases by rebalancing homeostasis with SIRT-1 activators during the early (before 24 hours after onset) or SIRT-1 inhibitors during the late (during immunosuppression) phenotypes. To accomplish this, the concept must be embraced by the pharmacologic industry and NIH granting agencies, extensively tested in animals, and carefully applied to humans.

Among the important challenges for better understanding this new concept include: identifying receptor and signaling pathways that inform the epigenetic pathways linked to the homeostasis axis; further elucidating control of altered homeostasis at microvascular interface among specific organs; discovering molecular markers that reliably identify phenotype shifts in homeostasis; and assessing genome-wide phenotype shifting by large scale state-of-the-art tools (RNA sequencing; chromatin landscape mapping; proteomics, metabolomics). We expect in the near future to see better translation and implementation of knowledge of how to modify potentially lethal acute systemic inflammation than heretofore realized.

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Key issues

- Acute inflammation during sepsis kills millions annually. Currently there is no treatment that targets sepsis pathophysiology
- Epigenetic programming of thousands of genes decides the fate of the organism during acute inflammation including sepsis
- Sepsis is an immunometabolic disease.
- Epigenetic regulator and homeostat SIRT1 is reprograms inflammatory and metabolic pathways during sepsis
- Modifying SIRT 1 activity provides a new target for preventing early or treating late sepsis.

Figure 1.

Epigenetic chromatin modifications: Chromosomes are tightly packaged DNA wound around nucleosome histones. Epigenetic reprogramming across chromosomes follows cell sensing and signaling and can either activate euchromatin transcription or reversibly repress it by forming compacted heterochromatin. Heterochromatin is inaccessible to transcription factors due to epigenetic histone and DNA modifiers, which methylate histone tails and/or DNA (not shown). In contrast, activated euchromatin exposes specific DNA sequences to multiple transcription factors by acetylating histone tail lysines (K), as well as phosphorylating and dephosphorylating histone serine/threonine residues (not shown).

Figure 2.

Panel A. Marked changes in mRNA levels of multiple genes accompany acute systemic inflammation. Both mild and severe acute systemic inflammation depart from homeostasis by increasing (gene activation) and decreasing (gene repression) thousands of mRNAs of the same sets of genes, indicating that mild and severe sepsis with high mortality rates are directed by magnitude and persistence of transcription and/or mRNA degradation. These observations support that quantitative rather than qualitative changes in gene expression are responsible for exceeding the homeostatic threshold needed to promptly resolve acute systemic inflammation.

Panel B. Epigenetic reprogramming directs phenotype shifting during acute systemic inflammation. During mild or severe acute systemic inflammation, a specific set of acute pro-inflammatory genes (e.g. $\text{TNF-}\alpha$ and $\text{IL-1}\beta$) initiating the hyper-inflammatory phenotype are epigenetically reprogrammed by a rapid (within hours) shift from activated euchromatin to silent heterochromatin at acute proinflammatory genes. Epigenetic reprogramming between active euchromatin and silent heterochromatin cannot be inferred from steady-state levels of transcribed mRNA. Another critical distinction is that epigenetic mechanisms drive the magnitude of hyper-inflammation, which correlates with how long the shifted hypo-inflammatory phenotype and immunosuppression last.

Figure 3.

Epigenetic reprogramming occurs during acute systemic inflammation with sepsis. At sepsis onset, pro-inflammatory gene promoters exist in a "poised" euchromatin state, which immediately shifts these genes to activated euchromatin by NF-κB p65 transactivation by acetylation as well as acetylation of histone lysines (not shown). A complex feed-forward process replaces NF-κB p65 with NF-κB factor RelB, which by recruiting a multicomplex epigenetic repressosome condenses euchromatin to silent heterochromatin and shifts hyper-inflammation to hypo-inflammation. It is not known if RelB and other co-factors also activate euchromatin of other sets of genes with overlapping or distinct functions (e.g. protein synthesis, metabolism and cytoskeleton). Importantly, the epigenetic reprogramming associated with mild or severe sepsis is reversible.

Figure 4.

Shifts in metabolism and immunity are integrated during inflammation. Rapid increases in glucose uptake by the glucose transporter GLUT1 and amplified glycolysis are required for both optimal innate and adaptive immune responses. During this time, pyruvate mitochondrial uptake and oxidation are diminished and ATP is provided by the high level of glycolysis. Concomitant with the shift from hyper-inflammation to hypo-inflammation is a nutrient switch from glucose to increased fatty acids, with increased uptake by CD36 and elevated mitochondrial fatty acid oxidation. This shift from a glycolytic high energy demanding state of anabolism to a lower catabolic state of fatty acid mitochondrial oxidation occurs in both mild and severe sepsis, but is greater in magnitude and more prolonged during severe disease. NAD⁺ activation of sirtuin family members underlies immunometabolic reprogramming.

Figure 5.

NAD⁺ generation and control of sirtuin function. NAD⁺ sensed by members of the sirtuin family of deacetylases and ribosyl transferases play important roles in regulating the dominant phenotypes associated with acute systemic inflammation. In mammals, the dominant source of NAD⁺ generation for sirtuin activation is the rate-limiting salvage pathway controlled by NAMPT, which controls production of NAD⁺ and its metabolites. Compartmentalization of NAD⁺ and specific sirtuins determines changes in cell physiology. SIRT-1 is a critically important homeostat for controlling inflammation phenotypic shifts during immunometabolic reprogramming.