Epigenetics of Sepsis

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Objectives: Recent evidence from the fields of microbiology and immunology, as well as a small number of human sepsis studies, suggest that epigenetic regulation may play a central role in the pathogenesis of sepsis. The term "epigenetics" refers to regulatory mechanisms that control gene expression but are not related to changes in DNA sequence. These include DNA methylation, histone modifications, and regulation of transcription via non-coding RNAs. Epigenetic modifications, occurring in response to external stressors, lead to changes in gene expression, and thus lie at the intersection between genetics and the environment. In this review, we examine data from in vitro studies, animal studies, and the existing human sepsis studies in epigenetics to demonstrate that epigenetic mechanisms are likely central to the pathogenesis of sepsis and that epigenetic therapies may have potential in the treatment of sepsis and its associated organ failures.

Data Sources: Online search of published scientific literature via Pubmed using the term "epigenetics" in combination with the terms "sepsis", "infection", "bacterial infection", "viral infection", "critical illness", "acute respiratory distress syndrome", and "acute lung injury".

Study Selection: Articles were chosen for inclusion based on their relevance to sepsis, acute inflammation, sepsis-related immune suppression, and sepsis-related organ failure. Reference lists were reviewed to identify additional relevant articles.

Data Extraction: Relevant data was extracted and synthesized for narrative review.

Data Synthesis: Epigenetic regulation is a key determinant of gene expression in sepsis. At the onset of infection, host-pathogen interactions often result in epigenetic alterations to host cells that favor pathogen survival. In parallel, the host inflammatory response is characterized by epigenetic modifications in key regulatory genes, including *tumor necrosis factor* and interleukin-1β. In human sepsis patients, multiple epigenetic modifying enzymes show differential expression in early sepsis, suggesting a role for epigenetics in coordinating the response to infection. In the later stages of sepsis, epigenetic modifications accompany endotoxin tolerance and the immune-suppressed state. In animal models, treatment with epigenetic modifiers can mitigate the effects of sepsis and improve survival as well as reverse sepsis-associated organ injury.

Conclusions: Epigenetic modifications are associated with key phases of sepsis, from the host-pathogen interaction, to acute inflammation, to immune suppression. Epigenetic markers show promise in the diagnosis and prognosis of sepsis and epigenetic modifying agents show promise as therapeutic tools in animal models of sepsis. Human studies in the area of epigenetics are sorely lacking and should be a priority for sepsis researchers. (*Crit Care Med* 2020; 48:745–756)

Key Words: critical illness; deoxyribonucleic acid methylation; epigenetics; histone modification; microRNA; sepsis

SEPSIS AND MULTIPLE ORGAN DYSFUNCTION SYNDROME

Sepsis is a complex multisystem disorder characterized by a dysregulated host response to infection (1). This heterogeneous response can persist and evolve even after the inciting infection has been treated. The evolution of the clinical syndrome occurs through the altered transcription of thousands of genes. Widespread genetic reprogramming leads to disruption of fundamental cellular processes resulting in endothelial dysfunction, mitochondrial and metabolic derangement, immune failure, and cardiovascular collapse (2). Regulatory mechanisms underlying sepsis have proven difficult to elucidate, and it remains unclear whether defining molecular events are unique to sepsis or represent a common response to injury (3).

GENETIC REGULATION OF THE HOST RESPONSE

Although infection is the initiating event in sepsis, the primary cause of mortality is an inadequate, unbalanced,

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or/and uncoordinated host response. Adoption studies demonstrate that susceptibility to infection is only slightly correlated in biological siblings, while risk of death from infection is strongly correlated (4). This seminal work underscores that while susceptibility may be the product of exposure, outcomes are at least partially determined genetically. However, a recent genome-wide association study of sepsis survivors versus nonsurvivors identified only one gene variant associated with poor outcome (5). Analysis of a large number of studies also failed to find a robust association between gene variants and outcomes (6). The imperfection of a purely genetic model is consistent with additional biologic influences. An epigenetic explanation for this "missing heritability" is both biologically possible and conceptually compelling (7–9).

OVERVIEW OF EPIGENETICS

"Epigenetics" is a term encompassing regulatory mechanisms that govern gene expression but do not result from changes in DNA sequence (**Fig. 1**). These include DNA methylation, histone modifications, and non-coding RNAs (ncRNAs) (for a review of epigenetics and gene regulation see (10); for a review of research techniques in epigenetics see (11)).

Epigenetics lies at the intersection between genetics and the environment. Unlike the DNA code, epigenetic "marks" are frequently modified by environmental stressors, including disease (12, 13). Disease-specific epigenetic changes have been identified in conditions as diverse as preeclampsia (14), Down syndrome (15), Crohn's disease (16), asthma (17), and schizophrenia (18). In cancer, epigenetics is a central focus of current research, and epigenetic markers are being used for both diagnostic (19) and prognostic (20) purposes. Epigenetic therapies are also being investigated (21–23).

Although modifiable, epigenetic changes can be persistent and even heritable. Human studies reveal that individuals in utero at the time of the Dutch hunger winter of 1944–1945 developed higher rates of obesity, hypertension, coronary artery disease, and impaired glucose tolerance when compared with their biological siblings (24). These phenotypic changes were accompanied by specific epigenetic changes, namely hypomethylation of the *insulin-like growth factor 2* (IGF2) and *insulin-IGF2 read-through product* genes and hypermethylation of the *interleukin-10* (*IL-10*) and *leptin* genes (24). The same phenotypic changes persisted into the third generation, suggesting that epigenetic changes can impact phenotype across multiple generations (25).

Similar transgenerational epigenetic effects have been observed in a murine model of sepsis. Male (but not female) descendants of male sepsis survivors showed poorer survival in response to endotoxin, as well as lower plasma concentrations of IL-6, tumor necrosis factor (TNF), and IL-10 (26). Analysis of the paternal sperm revealed widespread changes in DNA methylation, with altered gene expression patterns that persisted into the subsequent generation (26).

Despite the critical importance of epigenetics to gene regulation, research in the epigenetics of sepsis is still in its infancy. However, evidence from the fields of microbiology and immunology and from a small number of human sepsis studies suggests that epigenetics may be central to the human sepsis response. In this review, we examine the evidence pointing to a central role for epigenetics in the pathophysiology of sepsis and examine its potential clinical applications.

DNA Methylation

DNA methylation is the most widely studied epigenetic modification, likely because of the availability of quantitative genomic methods. Methylation of DNA occurs at cytosine-guanine dinucleotides and is mediated by DNA methyltransferase (DNMT) enzymes, while demethylation is catalyzed by the ten-eleven translocation enzymes (Fig. 1*A*) (27). The addition or removal of a methyl group to the DNA changes local chromatin structure resulting in changes in protein binding, thereby altering gene expression. Hypermethylation (increased methylation) of gene promoters and enhancers is often associated with gene repression while hypomethylation (decreased methylation) is associated with gene activation (28). This relationship is not consistent, however, making it challenging to anticipate the impact of DNA methylation at the level of individual genes (20, 29).

Histone Modifications

The core histone proteins, H2A, H2B, H3, and H4, form the nucleosome, around which the DNA double helix is wrapped. They are subject to a variety of covalent modifications, including methylation, acetylation, and phosphorylation, that alter their relationship to each other and to the DNA. Histone modification patterns are often predictive of gene expression—for example, H3K4me3 (trimethylation of lysine 4 of histone H3) and H3K27ac (acetylation of lysine 27 of histone H3) are often found at actively transcribed genes (10). Other marks, such as methylation of histone H3 at lysine 27 (H3K27me), are associated with gene repression (30). Histone modifications exert their transcriptional effect by two mechanisms: 1) by altering local chromatin structure leading to changes in DNA accessibility and 2) by regulating the binding of effector proteins that modulate transcription (31).

Histone modifications and DNA methylation are complementary processes that often jointly determine local gene expression patterns. For example, the demethylated tail of histone H3 recruits the DNA methyltransferase DNMT3A, leading to de novo methylation of DNA and local gene silencing (**Fig. 2*A***). This interaction is inhibited by methylation of H3K4, a histone mark associated with active transcription (32). Similarly, DNA methylation promotes deacetylation of histone H3 through recruitment of the methyl-CpG binding protein 2, which in turn recruits histone deacetylases (**Fig. 2*B***) (30). Histone deacetylation results in a tighter nucleosome and higher-order chromatin structures, thereby inhibiting gene expression (33). Thus epigenetic modifications work in concert to determine the state of gene expression.

Regulation by Non-Coding RNAs

ncRNAs are sequences transcribed from the genome that regulate the expression of other genes. The most widely studied ncRNAs are microRNAs (miRNAs), small sequences of 20–24 nucleotides that mediate posttranscriptional silencing of up to

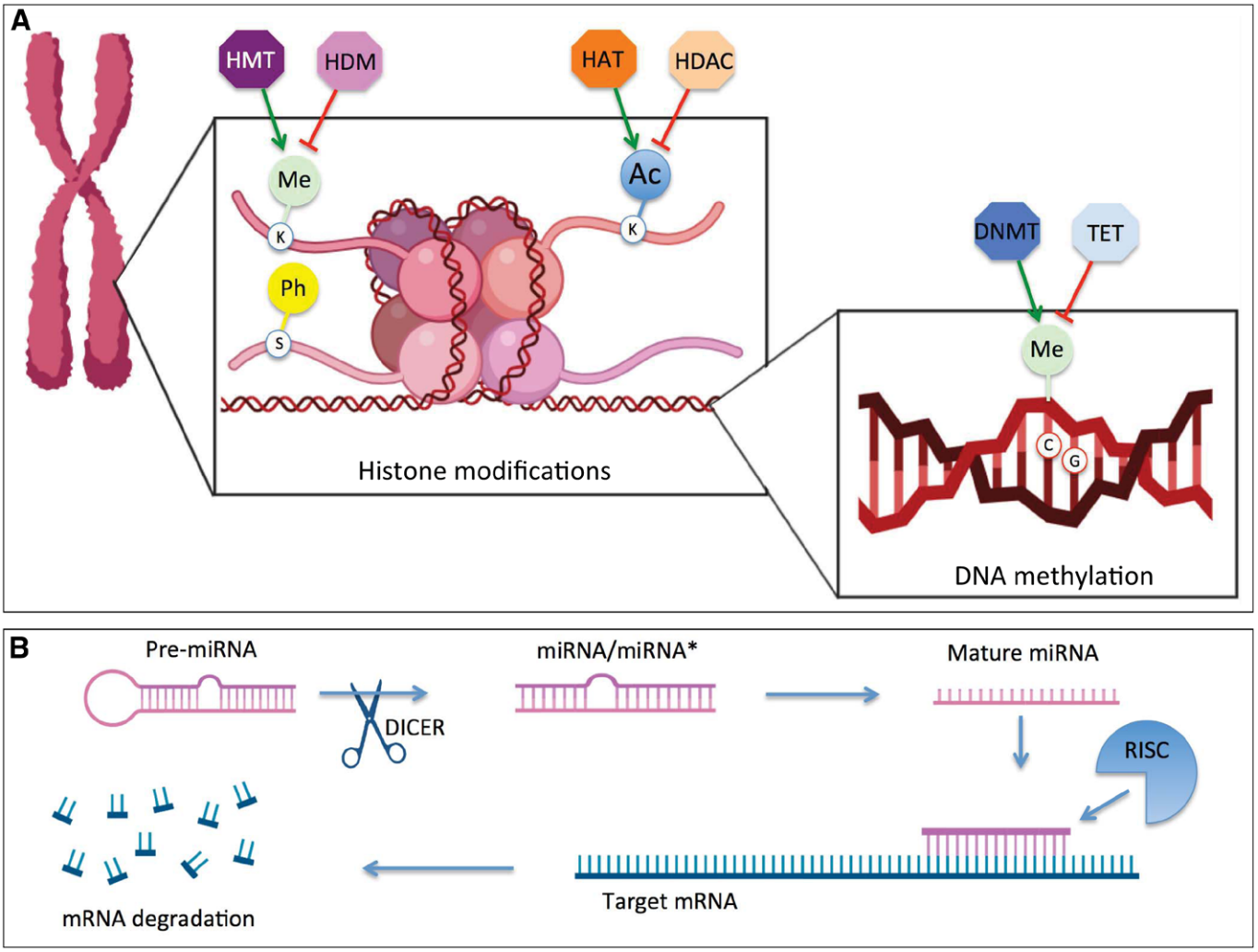

Figure 1. Epigenetic control of gene expression. **A**, Histone proteins determine the accessibility of the DNA strand to transcription factors and other transactivating proteins. The histone tails are subject to a wide variety of modifications including acetylation (Ac), methylation (Me), and phosphorylation (Ph), the effects of which are determined by location and type of modification. Histone Ac is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), while histone Me is mediated by histone methyltransferases (HMTs) and histone demethylases (HDMs). DNA Me occurs at cytosine-guanine dinucleotides (denoted by C and G) and is mediated by DNA methyltransferases (DNMTs) and ten-eleven translocation (TET) enzymes. The effects of DNA Me on transcription depend on its location relative to the gene. **B**, MicroRNAs (miRNAs) are small RNA particles involved in gene silencing. After transcription in the nucleus, the pre-miRNA forms a hairpin loop that is exported to the cytoplasm where it is recognized and cleaved by the Dicer enzyme. The miRNA/miRNA* duplex dissociates and the mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which mediates translational silencing, either through direct translational inhibition or through degradation of the target messenger RNA (mRNA).

60% of protein-coding genes (34). In cancer cells, miRNAs can act as both oncogenes and tumor suppressors and can themselves be epigenetically regulated (34). miRNAs function in the cytoplasm of the cell to cause translational inhibition or degradation of their target messenger RNAs (mRNAs) (Fig. 1*B*).

EPIGENETICS OF THE HOST-PATHOGEN INTERACTION

The initiating event in sepsis is invasion of the host by a pathogen. Many human pathogens, including bacteria, viruses, fungi, and parasites, use epigenetic mechanisms to manipulate the host response to infection, thereby favoring their own survival. Common strategies include production of epigenetic-modifying enzymes targeting the host chromatin, manipulation of host epigenetic modifying enzymes, and production of miRNAs that block expression of host cell proteins. Some of these epigenetic mechanisms contribute to the carcinogenic potential of viruses through the inhibition of tumor suppressor genes (35, 36).

A number of sepsis-associated pathogens exert targeted epigenetic effects on host cells. For example, *Listeria monocytogenes*, *Clostridium perfringens*, and *Streptococcus pneumoniae*, produce cholesterol-dependent cytolysins, a class of pore-forming proteins with epigenetic-modifying capabilities. The listeriolysin O (LLO) protein, produced by *L. monocytogenes*, induces dephosphorylation of histone 3 serine 10 (H3S10) and deacetylation of histone 4 at a subset of host genes, resulting in transcriptional reprogramming of host cells prior to bacterial invasion (37). The perfringolysin protein from *C. perfringens*

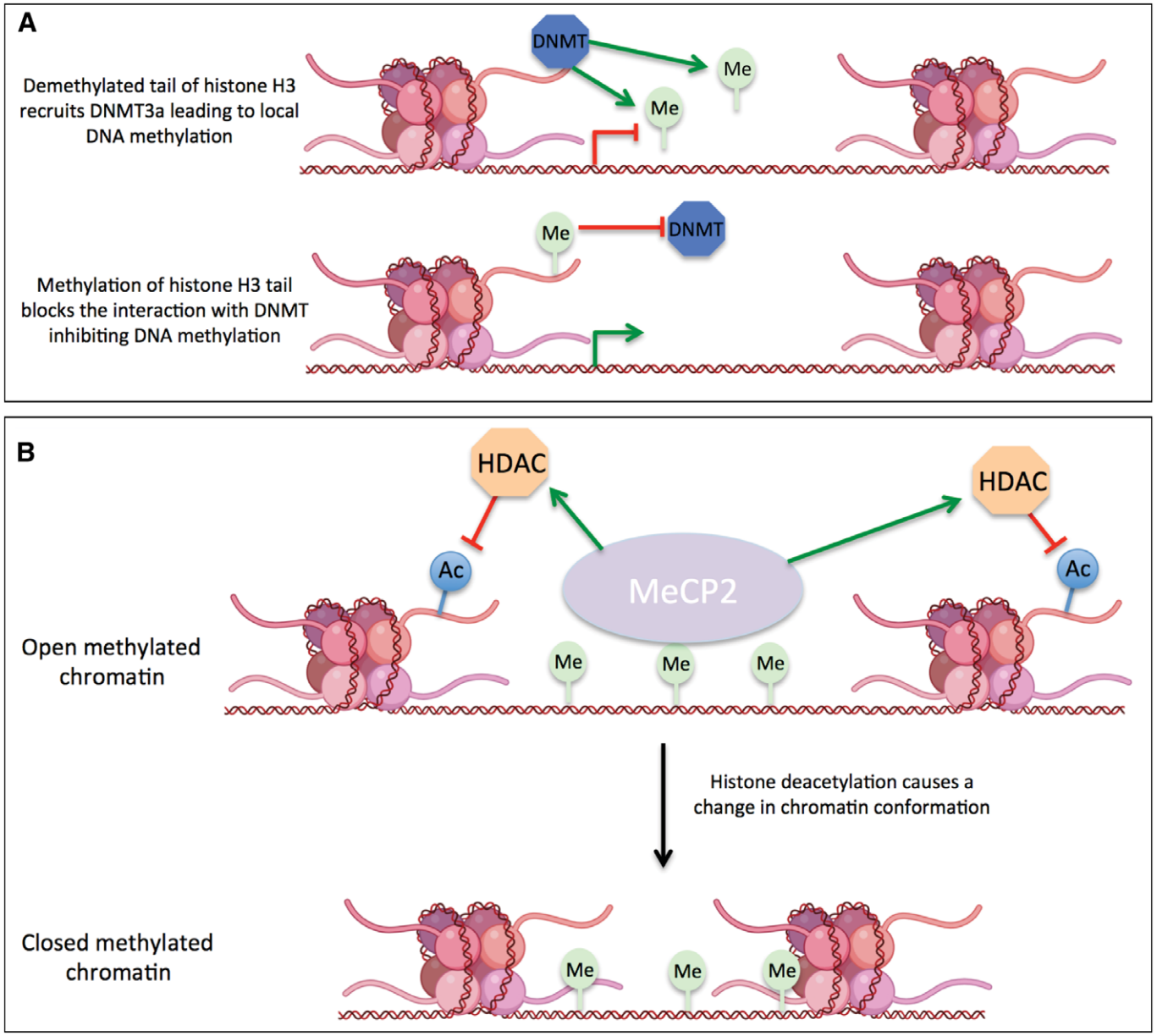

Figure 2. Epigenetic modifications act in concert to control gene expression. Examples of coordinated epigenetic modifications include (**A**) the demethylated tail of histone H3 recruits DNA methyltransferase 3a (DNMT3a) leading to DNA methylation (Me) of the adjacent DNA (*top*). Both changes are associated with silencing of gene expression. When histone H3 is methylated at lysine 4, recruitment of DNMT3a is inhibited and adjacent DNA remains unmethylated (*bottom*). Both changes are associated with activation of gene expression. **B**, Regions of methylated DNA are bound by the protein methyl-CpG binding protein 2 (MeCP2) that recruits histone deacetylases (HDACs). Removal of acetyl groups results in a tighter nucleosome and higher-order chromatin structures, leading to inhibition of gene expression. Ac = acetylation.

and the pneumolysin protein from *S. pneumoniae*, have a similar structure and function to LLO and also cause H3S10 dephosphorylation in target cells (37).

Escherichia coli is a frequent cause of intra-abdominal and urinary sepsis. As part of its invasion strategy, *E.coli* employs a type III secretion system to introduce bacterial proteins into host cells. These include the non-LEE-encoded effector C (NleC) protein, which was shown to degrade the histone acetyltransferase p300 in human colonic epithelial cells. p300 regulates the pro-inflammatory cytokine IL-8 via multiple mechanisms, including epigenetically (38). By downregulating IL-8 expression and thereby reducing neutrophil recruitment, *E.coli* likely favors its own survival. *Shigella flexneri* is another enteric pathogen that is closely related to *E.coli* and also uses a type-III injector system to introduce bacterial proteins into host cells. The *Shigella* phosphothreonine lyase OspF (OspF) protein was shown to migrate to the host cell nucleus, where it inhibits mitogen-activated protein-kinases (MAPKs). In the absence of MAPK activity, there was decreased H3S10 phosphorylation at a set of promoters regulated by the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB), leading to decreased expression of these genes (39). Mutation

of the OspF protein inhibited this effect in vitro and, in a rabbit model of Shigella infection, increased inflammation in the colonic epithelium (39). Thus, the epigenetic activity of OspF likely promotes immune system evasion by *S. flexneri*.

Many viruses are known to exert epigenetic effects on their hosts (40); however, limited data are available for viruses associated with sepsis. In an in vitro model of human airway epithelium, infection with H5N1 influenza or Middle Eastern respiratory syndrome coronavirus (MERS-CoV) resulted in down-regulation of interferon-stimulated genes, which are normally part of the cell's antiviral defense system (41, 42). The promoters of the downregulated genes showed decreased H3K4 methylation (an activating mark) and increased H3K27 methylation (a repressive mark), suggesting epigenetic regulation (42). In the case of influenza H5N1, the effect was dependent on the nonstructural protein 1, which is a histone H3 mimic (42, 43). Influenza and MERS-CoV infection also resulted in down-regulation of antigen-presenting genes. This effect was associated with increased DNA methylation of the gene promoters, once again suggesting that the viruses are using epigenetic strategies to interfere in host cell transcription (41)

The fact that multiple pathogens have evolved systems for epigenetically modifying host cells speaks to the likely importance of epigenetics in regulating the host response to infection. It also suggests the potential of this approach as a tool for modifying the early host response.

EPIGENETICS AND THE EARLY INFLAMMATORY RESPONSE

Early sepsis is characterized by widespread reprogramming of gene expression in circulating leukocytes. Signaling through the toll-like receptor 4 (TLR4) induces expression of hundreds of genes, including both pro- and anti-inflammatory pathways. One critical step is the activation of NF-κB, a transcription factor that promotes expression of multiple cytokines and inflammatory mediators including *TNF*, *IL-1*β, and *IL-8*.

Much of the data on the acute inflammatory response comes from studies of volunteers injected with bacterial endotoxin, a model of sepsis that manifests a more acute and pronounced increase in inflammatory cytokines than true human sepsis (44). Nonetheless, transcriptional and metabolic profiles of patients with sepsis show reasonable correlations with those of healthy volunteers exposed to endotoxin, suggesting similar pathways of gene activation and inhibition are present in both conditions (3, 45, 46).

Studies of human endotoxemia reveal changes in expression of more than 3,700 genes as early as 2 hours after exposure (47). At the epigenetic level, this is accompanied by changes in DNA methylation at several hundred genomic regions (48). Changes in histone marks have also been identified, with H3K27 acetylation and H3K4 methylation (both activating marks) predominating, particularly at genes involved in cytokine responses and interferon signaling (48). In vitro studies implicate DNA methylation in the pathogenesis of the early pro-inflammatory response. In human monocyte cell lines, stimulation with lipopolysaccharide (LPS) resulted in hypomethylation of the *tumor necrosis factor* (*TNF*) promoter, accompanied by displacement of nucleosomes from the binding site for NF-κB (49). This epigenetic modification allowed NF-κB to bind to the TNF promoter, resulting in up-regulation of *TNF* transcription (49, 50).

Studying the pathophysiology of sepsis in human sepsis patients is challenging. However, there is evidence from transcriptomic studies that epigenetics may play a role in the acute inflammation of sepsis. In an analysis of whole blood samples from patients with community-acquired pneumonia, Hopp et al (51) identified multiple epigenetic-modifying enzymes that were differentially expressed in early sepsis; these included both DNA methylation and histone-modifying proteins. The authors postulated that the transcriptional deregulation of chromatin-modifying enzymes in sepsis might drive chromatin re-organization, stimulating widespread transcriptional reprogramming (51).

Studies of epigenetic modifying drugs in animal models reveal that the acute inflammatory response of endotoxemia can be inhibited via epigenetic mechanisms. In a mouse model of endotoxemia, exposure to decitabine, a chemotherapy agent and DNA methyltransferase inhibitor (DNMTi), reduced macrophage inflammation, migration, and adhesion (52). This was accompanied by down-regulation of key inflammatory genes including *TNF*, *IL-6*, *IL-1*β, inducible nitric oxide synthase, and the chemokines *C-C motif chemokine ligand 2*, *C-C motif chemokine ligand 5*, and *C-C motif chemokine ligand 9*. Procainamide, an antiarrhythmic agent and a DNMTi, has been tested in a rat model of endotoxemia, where it also decreased hypotension and hypoglycemia while improving survival (53). Similar results were obtained with hydralazine, an antihypertensive agent that is also a DNMTi (53). In all of these studies, the DNMTi was given simultaneously to, or prior to, LPS—suggesting that epigenetic inhibition may need to occur at or before the onset of inflammation to be effective.

EPIGENETICS AND SEPSIS-ASSOCIATED IMMUNE SUPPRESSION

Both pro-inflammatory and anti-inflammatory processes begin early in sepsis, but immune suppression is thought to predominate in later stages, contributing to the risk of secondary infection and late mortality. Sepsis-associated immune suppression is a complex phenomenon. Depletion of mononuclear cells, including T cells, B cells, and dendritic cells, occurs via apoptosis (54). Monocytes show diminished secretion of inflammatory cytokines but maintain secretion of anti-inflammatory cytokines including IL-1RA and IL-10 (55). There is down-regulation of major histocompatibility complex class II gene expression accompanied by reduced antigen presentation (55). Neutrophil numbers are increased but neutrophil function is impaired, correlating with an increased risk of nosocomial infection (54).

One of the hallmarks of sepsis-associated immune suppression is "endotoxin tolerance"—a failure of the innate immune system to respond to bacterial endotoxin. In vitro evidence suggests that epigenetic modifications are key to the establishment

of endotoxin tolerance. In a series of elegant experiments, El Gazzar et al (49) showed that in resting monocytes, the *TNF* promoter was methylated and transcriptionally inactive. Upon initial exposure to endotoxin, the *TNF* promoter was rapidly demethylated, resulting in repositioning of nucleosomes and exposure of an NF-κB binding site (49). As the cells progressed to endotoxin tolerance, however, the *TNF* promoter was bound by the histone methyltransferase G9a, resulting in dimethylation of histone 3 lysine 9 (H3K9) and recruitment of DNMTs, leading to recurrent methylation of the *TNF* promoter. After this "re-methylation" event, the *TNF* promoter was no longer sensitive to stimulation by endotoxin (56). The cytokine IL-1β, which is also downregulated during endotoxin tolerance, showed a similar increase in H3K9 dimethylation at its promoter, suggesting that both cytokines may be regulated by a common epigenetic mechanism (57).

miRNAs may also play a role in endotoxin tolerance. Several miRNAs from endotoxin-tolerant monocytes have shown activity against *TNF*. Expression of miR-221, miR-579, miR125b, and miR146a is induced by TLR4 signaling after endotoxin exposure (58). miR-221, miR-579, and miR125b contain binding sites for the 3' untranslated region of *TNF*, suggesting they act directly to prevent TNF translation (58). miR146a does not contain a *TNF* mRNA binding site but is required for the association of the *TNF* mRNA with the miRNA-induced silencing complex, suggesting it acts indirectly to suppress *TNF* expression (59).

At the epigenome-wide level, endotoxin tolerance is accompanied by widespread changes in histone marks. In vitro studies have demonstrated that active histone marks are missing at the promoters and enhancers of genes that are transcriptionally downregulated in endotoxin-tolerant monocytes (48). This effect can be partially reversed by exposing the endotoxin-tolerant monocytes to β-glucan, a fungal wall component that induces "trained immunity" in monocytes. After β-glucan treatment, the monocytes showed partial reversal of the endotoxin tolerant phenotype accompanied by histone modification at distal enhancer elements. These experiments suggest that tolerance can be reversed at the epigenetic level leading to transcriptional reactivation of unresponsive genes (48).

The state of endotoxin tolerance is characterized by a transition from high-energy glycolysis to low-energy lipolysis, mimicking starvation. Epigenetics may play a role in regulating this transition as well. The metabolism sensor *sirtuin1* (*SIRT1*) is a histone deacetylase that senses the metabolic state of the cell due to its dependence on the co-enzyme nicotine adenine dinucleotide (NAD^+). When NAD^+ is in short supply, *SIRT1* promotes formation of heterochromatin at key inflammatory genes such as *TNF* and *IL-1*β (60). It also deactivates NF-κB via multiple mechanisms. Thus, *SIRT1* may be a central regulator of the immune-suppressive phase of sepsis. In recent studies, Vachharajani et al (61) and Martin et al (62) treated immune-tolerant mice after cecal ligation and puncture with a selective SIRT1 inhibitor, EX-527; SIRT1 inhibition caused a transition from tolerant to active phenotype in key immune cell types, including neutrophils, dendritic cells, and CD4+ T cells. It also reduced mortality from 40% to 0% when administered 24 hours post insult (63).

EPIGENETICS AND ACUTE LUNG INJURY

Acute lung injury (ALI) is a frequent complication of sepsis and studies suggest that DNA methylation plays a role in its pathogenesis. In a rat model of LPS-induced ALI, DNMT1 levels were shown to be elevated in the lungs (53). This was accompanied by an increase in 5-methylcytosine levels, confirming an increase in DNA methylation (53). Epigenome-wide analysis of lung tissue revealed differential methylation of 1,721 genes, including many associated with the hyperinflammatory response (64). A further 42 differentially methylated genes were associated with MAPK signaling, of which seven have previously been associated with ALI/acute respiratory distress syndrome (65). The epigenetic effects of LPS exposure on lung tissue could be partially reversed with DNMT1 inhibition; pretreatment of the rats with procainamide, a DNMT1 inhibitor, resulted in up-regulation of 141 genes that were downregulated by LPS alone (53).

In a separate mouse model of LPS-induced ALI, mice were pretreated with the DNMT inhibitors decitabine or azacitidine (66). Mice that received no pretreatment showed histological changes typical of ALI, including alveolar edema, hemorrhage, and neutrophil infiltration. By comparison, treated mice showed preserved lung architecture, low lung wet-to-dry ratios, decreased serum levels of TNF, IL-1β, and low levels of tissue myeloperoxidase (a neutrophil product that aggravates inflammatory damage in the lungs) and malondialdehyde (a marker of oxidative stress) (66).

In a mouse model of intratracheal LPS-induced lung injury, posttreatment with the DNMT inhibitor azacitidine accelerated recovery, possibly via up-regulation of the protein forkhead box protein 3 (Foxp3) in regulatory T cells (Treg) (67). Treg are a subset of CD4+ T cells that suppress innate and adaptive immune. Azacytidine treatment resulted in increased pulmonary Treg in the lung as well as increased Foxp3 expression (67). Similar findings were seen in a model of murine influenza (67).

Histone acetylation may also be important in ALI. In a mouse model of sepsis, pretreatment with the histone deacetylase inhibitors (HDACi) trichostatin A or sodium butyrate improved lung injury, survival, and decreased circulating IL-6 levels during sepsis (68). It is unclear whether these results indicate a direct role for HDACi in reversing ALI or a more general role in mitigating the inflammatory response to sepsis (see below).

EPIGENETIC MARKS AS SEPSIS BIOMARKERS

Biomarkers are crucial in determining diagnosis, estimating prognosis, and monitoring response to therapy. Given the relative ease of DNA isolation and the stability of DNA methylation marks, DNA methylation patterns may prove useful as biomarkers. One study found that DNA methylation of the

procalcitonin (calcitonin-related polypeptide α [CALCA]) gene promoter correlated with bacterial sepsis in preterm newborns (69). The methylation state of the CALCA promoter was dependent on whether sepsis was caused by a Gram-positive or Gram-negative pathogen, suggesting differential regulation of procalcitonin at the epigenetic level depending on the type of infection. These findings have yet to be validated in other studies.

A small study of epigenome-wide DNA methylation changes in whole blood samples from six neonates (three with sepsis vs three healthy controls) also identified 81 DNA methylation marks, corresponding to 64 unique genes that correlated with sepsis status (70). Functional analysis revealed enrichment for protocadherin genes, a family of highly conserved cell adhesion molecules, all of which were hypermethylated in the septic neonates (70). Although this strategy holds promise for identifying sepsis biomarkers, the study was very small.

We have recently completed the first epigenome-wide analysis of DNA methylation profiles in adult sepsis patients (71). A total of 134 adult ICU patients (66 septic and 68 nonseptic patients, matched for severity of illness) were characterized. Using whole blood samples, we identified 668 differentially methylated regions in 443 genes, including genes associated with sepsis such as complement component 3, angiopoietin 2, myeloperoxidase, lactoperoxidase, human leukocyte antigen (HLA)-A, HLA-DRB1, HLA-C, and HLA-DQB1. Functional analysis revealed enrichment for antigen processing and presentation, methyltransferase activity, cell adhesion, and cell junctions. We also identified co-methylation modules (i.e., methylation sites with coordinated methylation levels) that were associated with important clinical traits including severity of illness, need for vasopressors, and length of stay. These results demonstrate that measurable epigenetic differences exist between infectious and noninfectious inflammation in human subjects. They also serve as proof of principle that epigenetic information can be collected at the bedside and that this information may have utility in the diagnosis and prognosis of sepsis and its associated organ failures.

miRNAs have also been proposed as sepsis biomarkers. In addition to functioning intracellularly, miRNAs are released into the circulation where they remain stable (72). In critically ill patients, plasma levels of miR-133a were shown to be higher in patients with sepsis relative to those with noninfectious inflammation and were predictive of both ICU and long-term mortality (73). miR-133a levels also correlated with markers of disease severity; however, it was less discriminating of sepsis versus nonsepsis status than routinely used biomarkers such as procalcitonin and C-reactive protein (73). Two other miRNAs, miR-146a and miR-223, were shown to be decreased in sepsis relative to noninfectious systemic inflammatory response syndrome (74). Area under the receiver operating characteristic curves (AUCs) for these two miRNAs were 0.804 and 0.858, respectively, significantly greater than C-reactive protein (74). Levels of mi-223 also correlated with sepsis outcome, with an AUC of 0.748 for distinguishing survivors from nonsurvivors (75). When combined with five additional miRNAs (miR-15a, miR-16, miR-122, miR-193b, and miR-483-5p), the AUC increased to 0.953, which was significantly better than the Sequential Organ Failure Assessment score (0.782), Acute Physiology and Chronic Health Evaluation II score (0.752), and procalcitonin (0.689) (75). Thus far, no individual miRNA biomarker has emerged as the frontrunner; however, the concept has clear potential (72, 76).

EPIGENETIC MODIFIERS AND SEPSIS THERAPEUTICS

Animal studies suggest that epigenetic modifiers may have potential for sepsis therapy. As mentioned above, treatment of rats with LPS followed by the DNMT1 inhibitor procainamide reversed many of the clinical features of sepsis (53). Similarly, pretreatment of mice with HDACi prior to cecal ligation and puncture reduced plasma IL-6 levels and improved survival, as well as reducing lung injury and neutrophil infiltration of the lungs (68). In a separate study, pretreatment of mice with the HDACi suberoylanilide hydroxamic acid (SAHA) followed by LPS exposure followed by repeat treatment with SAHA reduced levels of inflammation (77). The authors further examined the effects of HDACi on gene expression and found 208 genes differentially expressed between animals treated with LPS alone versus those treated with LPS + SAHA (78). Survival improved from 0% at 7 days (with LPS alone) to 80% (with LPA + SAHA), showing that expression changes translated into significant phenotypic improvement (77)

The mechanism by which DNMTi and HDACi reverse some of the genotypic and phenotypic features of sepsis is unknown. It may be significant that these inhibitors prevent epigenetic modifications, thereby preserving the "status quo" and presumably inhibiting changes in gene expression. In the animal models described above, the inhibitors were given either prior to the onset of sepsis/endotoxemia or shortly thereafter. The only study to show benefit from late administration of epigenetic modifying agents (24 hr after sepsis onset) was using a SIRT1 inhibitor (61). Since SIRT1 is thought to promote the immune suppression phase of sepsis (see above), it seems logical that delayed administration of a SIRT1 inhibitor would be beneficial.

Importantly, several epigenetic drugs including DNMTi and HDACi, are approved as monotherapy for cancer treatments (21, 23, 79). Furthermore, epigenetic therapies are gaining traction for the treatment of cachexia and muscle wasting (80, 81), as a possible treatment for psychiatric and mood disorders (82), and as personalized therapy for autoimmune disorders (83). The human epigenetic drug database provides data on epigenetic drugs categorized by drug, target, or disease; however, data from human sepsis patients is lacking (84).

CONCLUSIONS

Epigenetic modifications are critical determinants of gene expression in human health and disease. Growing evidence suggests that epigenetics is at the heart of the gene expression changes associated with sepsis (**Tables 1–3** for summary of

TABLE 1. Overview of Epigenetic Data From In Vitro and In Vivo Animal Experiments

References	Sepsis Phase	Epigenetic Change	Model	Finding
Agarwal et al (85)	Immune activation	DNA methylation	CD4+ mouse T cells in vitro	Lineage-specific DNA demethylation of the interferon-gamma, IL-2, IL-4, and IL-13 genes occurs after activation of naïve T-helper cells to Th1 or Th2 phenotype
Huang et al (66)	Immune activation	DNA methylation	Mouse model of endotoxemia	DNMT inhibition with decitabine or 5-azacitidine 1 hr prior to LPS exposure reduced serum TNF and IL-1b levels as well as reducing levels of myeloperoxidase and malondialdehyde, indicated reduced production of reactive oxygen species
Bruniquel et al (86)	Immune activation	DNA methylation	CD4+ mouse T cells in vitro	Demethylation of the *IL-2* gene promoter occurs after T cell activation and is necessary and sufficient to enhance IL-2 transcription in
Cao et al (52)	Immune activation	DNA methylation	Mouse macrophage cell line	Exposure to decitabine (a DNMT inhibitor) reduces macrophage inflammation, migration, and adhesion
Shih et al (53)	Immune activation	DNA methylation	Rat model of endotoxemia	Treatment with procainamide (a DNMT inhibitor) at 1 hr after LPS administration reduced hypotension, hypoglycemia, superoxide production, multiple organ dysfunction, and improved survival from 31% to 42% at 6 hr
Martin et al (62)	Immune tolerance	Histone acetylation	Mouse cecal ligation and puncture model of sepsis	Inhibition of SIRT1 (a histone deacetylase) 24 hr after cecal ligation and puncture restores the active phenotype of innate and adaptive immune cells
Vachharajani et al (61)	Immune tolerance	Histone acetylation	Mouse cecal ligation and puncture model of sepsis	Inhibition of SIRT1 (a histone deacetylase) 24 hr after cecal ligation and puncture (but not at 0 or 12 hr) improves survival at 7 d from 40% to 100%
Bomans et al (26)	Immune tolerance	DNA methylation	Mouse cecal ligation and puncture model of sepsis	Male descendants of sepsis survivor fathers showed poorer survival in response to endotoxin and lower plasma levels of IL-6, TNF, and IL-10. Sperm analysis revealed widespread DNA methylation changes in the paternal DNA
Zhang et al (64)	Acute lung injury	DNA methylation	Rat model of LPS-induced acute lung injury	1,721 genes are differentially methylated in rat lung tissue with LPS-induced acute lung injury
Shih et al (53)	Acute lung injury	DNA methylation	Rat model of LPS-induced acute lung injury	LPS-induced acute lung injury is associated with increased lung levels of DNMT1. Treatment with a DNMT inhibitor (procainamide) 1 hr after LPS infusion resulted in decreased DNMT1 levels and decreased lung injury
Huang et al (66)	Acute lung injury	DNA methylation	Mouse model of LPS-induced acute lung injury	Pretreatment of mice with the DNMT inhibitors decitabine or 5-azacitidine, 1 hr prior to LPS exposure, prevented endotoxemia-induced acute lung injury in mice
Singer et al (67)	Acute lung injury	DNA methylation	Mouse model of LPS-induced acute lung injury	Posttreatment of mice with the DNMT inhibitor decitabine, at 24 hr after LPS-exposure, improved recovery from acute lung injury via activation of regulatory T cells
Zhang et al (68)	Acute lung injury	Histone acetylation	Mouse cecal ligation and puncture model of sepsis	Pretreatment with histone deacetylase inhibitors, 30 min prior to cecal ligation and puncture, resulted in decreased lung injury and increased survival

DNMT = DNA methyltransferase, IL = interleukin, LPS = lipopolysaccharide, SIRT1 = sirtuin 1, TNF = tumor necrosis factor.

TABLE 2. Overview of Epigenetic Data From In Vitro Human Studies

References	Sepsis Phase	Epigenetic Change	Model	Finding
Menachery et al (41)	Pathogen-host interaction	DNA methylation	Human airway epithelial cell line	Influenza infection increases DNA methylation of antigen-presenting genes and decreases expression
Hamon et al (37)	Pathogen-host interaction	Histone phosphorylation and acetylation	Human cervical cancer cell line	The LLO protein from *Listeria monocytogenes* induces dephosphorylation of H3S10 and deacetylation of H4 at a subset of host genes prior to bacterial invasion (37). The perfringolysin protein (*Clostridium perfringens*) and the pneumolysin protein (*Streptococcus pneumoniae*), have a similar structure and function to LLO (37)
Shames et al (38)	Pathogen-host interaction	Histone acetylation	Human intestinal epithelial cell line	*Escherichia coli* non-LEE-encoded effector C protein binds the histone acetyltransferase p300 leading to its degradation and resulting in down-regulation of IL-8 expression
Arbibe et al (39)	Pathogen-host interaction	Histone phosphorylation	Human intestinal epithelial cell line	The Shigella protein phosphothreonine lyase OspF inhibits H3S10 phosphorylation at NF-κB-regulated gene promoters leading to decreased expression of immune response genes including IL-8
Menachery et al (42)	Pathogen-host interaction	Histone methylation	Human airway epithelial cell line	The influenza nonstructural protein 1 is a histone mimic and downregulates interferon-stimulated genes via H3K27 methylation
Novakovic et al (48)	Immune activation	DNA methylation	Primary human monocytes	Endotoxin exposure results in widespread DNA methylation changes (primarily demethylation) at 2,700 demethylated regions containing a minimum of four differentially methylated CpGs with a minimum of 30% change in methylation
Novakovic et al (48)	Immune activation	Histone methylation	Primary human monocytes	Endotoxin exposure results in H3K27 acetylation and H3K4 methylation at the promoters of cytokine and interferon signaling genes
El Gazzar et al (56)	Immune tolerance	Histone methylation and DNA methylation	Human monocyte cell line THP-1	In endotoxin-tolerant macrophages, the TNF promoter is bound by the histone methylase G9a leading to dimethylation of H3K9 and recruitment of DNA methyltransferases that methylate the promoter region. After methylation, the promoter is no longer sensitive to stimulation by endotoxin
ElGazzar et al (59)	Immune tolerance	MicroRNA	Human monocyte cell line THP-1	In endotoxin-tolerant monocytes, miR146a regulates binding of the inhibitor RelB to the TNF promoter, causing gene silencing. It also regulates posttranscriptional silencing of TNF by promoting the binding of TNF messenger RNA to the microRNA-induced silencing complex
ElGazzar et al (59)	Immune tolerance	MicroRNA	Human monocyte cell line THP-1	miR-221, miR-579, miR125b, and miR146a are induced by toll-like receptor 4 signaling and contribute to silencing of TNF
Chan et al (57)	Immune tolerance	Histone methylation	Human monocyte cell line THP-1	In endotoxin tolerant cells the IL-1β gene shows increased H3K9 dimethylation along with decreased NF-κB binding and decreased levels of gene expression

H3K9 = histone 3 lysine 9, H3K27 = histone 3 lysine 27, H3S10 = histone 3 serine 10, IL = interleukin, LLO = listeriolysin O, NF-κB = nuclear factor kappa B, TNF = tumor necrosis factor.

TABLE 3. Overview of Epigenetic Data From Human Sepsis Studies

References	Sepsis Phase	Epigenetic Change	Model	Finding
Tendl et al (69)	Clinical sepsis	DNA methylation	Neonates with sepsis (9) vs neonates with isolated infections (5)	DNA methylation at the procalcitonin promoter correlates with the presence or absence of sepsis in neonates and also with gram-positive vs gram-negative causes of sepsis
Dhas et al (70)	Clinical sepsis	DNA methylation	Neonates with sepsis (3) vs neonates without sepsis (3)	Epigenome-wide analysis of whole blood samples reveals 81 differentially methylated CpG sites in 64 genes that distinguish neonates with and without sepsis
Hopp et al (51)	Clinical sepsis	DNA methylation and histone methylation	Adult patients with community-acquired pneumonia (180)	Expression of epigenetic-modifying enzymes, including DNA methyltransferases, ten-eleven translocation enzymes, histone methyltransferases, and histone demethylases, varies with sepsis severity
Binnie et al (71)	Clinical sepsis	DNA methylation	Adults patients with sepsis (66) vs nonseptic critical illness (68)	DNA methylation profiling reveals 668 differentially methylated regions distinguishing patients with sepsis from those with nonseptic critical illness. Functional analysis reveals enrichment for antigen processing/presentation, methyltransferase activity, cell adhesion, and cell junctions. Co-methylation modules (methylation sites with coordinated methylation) were associated with important clinical traits including severity of illness, vasopressors, and length of stay
Tacke et al (73)	Clinical sepsis	MicroRNAs	Adult sepsis patients (176) vs nonseptic critically ill patients (85)	Serum miR133a levels are increased in patients with sepsis relative to nonseptic critical illness and correlate with severity of illness scores as well as mortality
Wang et al (74)	Clinical sepsis	MicroRNAs	Adult sepsis patients (50) vs cardiac surgery patients (30) vs healthy controls (20)	Serum levels of miR146a and miR-223 are decreased in human sepsis patients relative to patients with noninfectious systemic inflammatory response syndrome and healthy controls. In ROC analysis, the AUC for miR223 in the diagnosis of sepsis was 0.858 (0.748–0.968) and for miR-146a was 0.804
Wang et al (75)	Clinical sepsis	MicroRNAs	Adult sepsis patients divided into survivors (117) and nonsurvivors (97)	In ROC analysis, serum levels of 6 microRNAs (miR-15a, miR-16, miR-122, miR-193b, miR-223, and miR-483-5p) distinguished sepsis survivors from nonsurvivors at 24 hr after sepsis diagnosis with an AUC of 0.953

AUC = area under the receiver operating characteristic curve, ROC = receiver operating characteristic.

data presented in this review). This is supported by the fact that pathogens have evolved epigenetic strategies to manipulate the host response to infection. Although much of the evidence linking sepsis and epigenetics remains circumstantial, it is increasingly apparent that epigenetic regulation lies at the heart of the cell-specific, bioenergetic, and immunologic responses of sepsis. Testing of epigenetic inhibitors in animal models of sepsis reveals that epigenetic manipulation can block or reverse the gene expression changes associated with sepsis, and this correlates with significant improvements in morbidity and mortality.

Epigenetic biomarkers have also shown promise in the diagnosis and prognosis of sepsis. At a technical level, epigenetic biomarkers may have benefits relative to RNA markers in terms of stability and ease of measurement. Furthermore, technology (e.g., Qiagen PAXgene tubes) exists to collect blood samples at the bedside. Further work will be required to validate epigenetic biomarkers in human sepsis populations and to develop point of care diagnostic tools for ease of use. Epigenetic markers might also be useful in stratifying patients according to their "sepsis response state" (87), thereby leading to individualized treatment plans.

Most of the data linking epigenetics and human sepsis comes from in vitro studies and animal models. Data from human sepsis patients is sorely lacking. Coordination between clinical and translational researchers will be required to generate annotated cohorts of human sepsis patients with accompanying epigenetic samples for large-scale analysis. Fortunately,

the cancer research community has paved the way in terms of technical approaches and clinical understanding of epigenetics and disease. The sepsis community should take advantage of this wealth of accumulated knowledge to accelerate research in this important new area.

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