Epigenetics in Sepsis

Jean-Daniel Chiche, MD PhD

MICU & Dept of Host-Pathogen Interaction
Hôpial Cochin & Institut Cochin, Paris-F
Sepsis 2015: a litany of failed clinical trials?

- The dark side: RCTs
- The bright side
  - Improved ICU outcomes (better general care, less iatrogenic harm)
  - Even positive outcome RCTs from doing less (ventilation, sedation, …)
- Starting to understand what may be good for populations, but still looking for biomarkers to make decisions for the individual
- Concept: sepsis-induced immune dysfunction
Sepsis mortality & morbidity: Patterns are changing!
Less than 10% of deaths occur in the first 48h

Log-rank test $P$ value = 58
Hazard ratio: 1.05 (95% CI; 0.88-1.26)
Declining Case Fatality Rates for Severe Sepsis
Good Data Bring Good News With Ambiguous Implications

Theodore J. Iwashyna, MD, PhD; Derek C. Angus, MD, MPH

Figure. Potential Mechanisms of Decreasing Short-term Mortality Among Patients Across a Distribution of Illness Severity

<table>
<thead>
<tr>
<th>Current care</th>
<th>Significant morbidity</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full recovery</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Alternative mechanisms of potentially decreasing short-term mortality

<table>
<thead>
<tr>
<th>A  Overall improvement: more recovery, less morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full recovery</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B  Change viability threshold: same recovery, more morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full recovery</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C  Trade-off: less recovery, even more morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full recovery</td>
</tr>
</tbody>
</table>

High resources consumers
Nosocomial sepsis
A Paradigm for Sepsis Mortality

Hyper-inflammatory response vs. Homeostasis

*Early deaths due to overwhelming inflammation*

*Late deaths due to intractable inflammation-induced organ injury*

Innate vs. Adaptive

Recovery

Time (days)

A New Paradigm for Sepsis Morbidity

Early deaths due to overwhelming inflammation

Late deaths due to persistent immunosuppression and recurrent infections

Understanding the Inflammatory Cytokine Response in Pneumonia and Sepsis

Results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study

John A. Kellum, MD; Lan Kong, PhD; Mitchell P. Fink, MD; Lisa A. Weissfeld, PhD; Donald M. Yealy, MD; Michael R. Pinsky, MD; Jonathan Fine, MD; Alexander Krichevsky, PhD; Russell L. Delude, PhD; Derek C. Angus, MD, MPH; for the GenIMS Investigators

Arch Intern Med. 2007;167(15):1655-1663
A genomic storm in critically injured humans

- Severe stresses produce a global reprioritization affecting >80% of the cellular functions and pathways
- 5,136 genes exhibited at least a 2-fold change in expression over the time course
- Greatest changes seen within 12 h from injury
  - 3,051 genes with decreased expression
  - 2,085 genes with increased expression
A genomic storm in critically injured humans

XIAO W, J EXP MED 2011
Allocation to this subclass was independently associated with mortality (odds ratio = 2.7; CI$_{95}$ = 1.2–6.0; $P = 0.016$), and adjunctive corticosteroids prescribed at physician discretion were independently associated with mortality in this subclass (odds ratio = 4.1; CI$_{95}$ = 1.4–12.0; $P = 0.011$).
Understanding gene expression changes
The Complexity of Gene Expression & Transcription
Epigenetics includes all mechanisms that govern gene expression patterns without modifying the underlying DNA sequence

- MicroRNAs
- Chemical modifications of DNA
- Chemical modifications of associated histones
  ➔ Change the physical DNA accessibility to transcription factors
Epigenetics includes all mechanisms that govern gene expression patterns without modifying the underlying DNA sequence.

- Writers’ introduce histone marks, ‘erasers’ take them out and ‘readers’ can recognize a particular form of histone modification.
Chromatin access & transcription

A

Chromatin → Nucleosome
- Histone modifications
- DNA methylation

B

Gene “switched on”
- Active (open) chromatin
- Unmethylated cytosines (white circles)
- Acetylated histones

Gene “switched off”
- Silent (condensed) chromatin
- Methylated cytosines (red circles)
- Deacetylated histones

Transcription possible
Transcription impeded
DNA Methylation Patterns in Genome Regions

Physiological

Disease states

Unmethylated CpG island allowing transcription

Methylated CpG island

Unmethylated CpG island shore

Methylated CpG island shore

Methylated gene body

Unmethylated gene body

Methylated repetitive sequence

Unmethylated repetitive sequence

Transposition
Recombination
Genome instability
Histone modifications

- H1.4
- H2A
- H2B
- H3.1
- H4
Biochemical effects of methylation at histone H3

Histone 3 modifications

Green: histone marks associated with gene activation;
Red: histone marks associated with transcriptional repression
Green: histone marks associated with gene activation;
Red: histone marks associated with transcriptional repression
Nucleosome positioning patterns
From sepsis to epigenetic imprinting of immune progenitor cells
IRAK-M Regulates Chromatin Remodeling in Lung Macrophages during Experimental Sepsis

Kenneth Lyn-Kew¹, Eric Rich¹, Xianying Zeng¹, Haitao Wen², Steven L. Kunkel², Michael W. Newstead¹, Urvashi Bhan¹, Theodore J. Standiford¹*

A

Fold Increase TNP mRNA

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CLP</th>
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<tbody>
<tr>
<td>Unstim</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>LPS</td>
<td>0.0</td>
<td>0.0</td>
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B

Fold Increase IL-12 p40 mRNA

<table>
<thead>
<tr>
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<th>Sham</th>
<th>CLP</th>
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<tbody>
<tr>
<td>Unstim</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>LPS</td>
<td>5.0</td>
<td>5.0</td>
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</table>

C

Fold Increase iNOS mRNA

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CLP</th>
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<tbody>
<tr>
<td>Unstim</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>LPS</td>
<td>7.5</td>
<td>7.5</td>
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D

Fold Increase Lrp2 mRNA

<table>
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<th>Sham</th>
<th>CLP</th>
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<tbody>
<tr>
<td>Unstim</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>LPS</td>
<td>100</td>
<td>150</td>
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</table>
Systemic sepsis induces epigenetic silencing of cytokine gene expression in lung macrophages. IRAK-M appears to be a critical mediator of this response.
Epigenetic changes during sepsis

- GMP (Granulocyte Monocyte Progenitor)
  - Hyper Inflammatory Insult
  - Endotoxin shock
    - SEPSIS
      - H3K9me - IL-1β, TNFα promoters
      - HDAC expression/function (IL-8, CCL2)
      - AcH4, H3K4me3 promoters
      - KDM6B (JMJD3)
      - M1/M2 phenotype
      - H3K4me, H3K27me
      - IL-12 promoter
      - IL-12 production

- Macrophage
- Dendritic cell
Epigenetic changes during sepsis

CD4+CD62L+ T cell → CD4+CD62L+ T cell

↑ H3K27me (IFNγ, GATA-3 promoters)
Dysregulated Cytokine Expression

↑ H3K9ac (Foxp3 promoter)
↑ Kat2a mRNA
↑ T reg potential
Targeting Epigenetic Changes in Sepsis

Hyper-inflammatory response
Early

T_H1 cytokines and chemokines

Therapy
Modulate immune response

Death with acute organ dysfunction due to cytokine storm

Time for Epi-Drugs?

Hypo-inflammatory response
Late

Sepsis

Therapy
Block apoptosis or enhance immune function

Death due to primary infection or development of secondary infection

Apoptotic depletion of immune cells; T_H2 responses

Hotchkiss, Nature Med 2009
# Epigenetic modifications as therapeutic targets

Theresa K Kelly\(^{1,2}\), Daniel D De Carvalho\(^{1,2}\) & Peter A Jones\(^1\)

## Table 3  Epigenetic cancer therapies under commercial development (either in safety and efficacy trials or approved)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sponsor</th>
<th>Indication</th>
<th>Clinical status</th>
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<tbody>
<tr>
<td><strong>DNMT inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Aza-CdR (Dacogen)</td>
<td>Eisai (Tokyo)</td>
<td>MDS</td>
<td>Approved May 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AML</td>
<td>Phase 3 in 480 patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1(^{st}) line CML</td>
<td>Phase 2 in 19 patients</td>
</tr>
<tr>
<td>5-Aza-CR (Vidaza)</td>
<td>Celgene (Summit, NJ, USA)</td>
<td>MDS</td>
<td>Approved May 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AML</td>
<td>Phase 3 targeting 480 patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hematologic cancer</td>
<td>Phase 2</td>
</tr>
<tr>
<td>S110 (dinucleotide prodrug of decitabine)</td>
<td>SuperGen (Dublin, CA, USA)</td>
<td>MDS and AML</td>
<td>New Drug Application</td>
</tr>
<tr>
<td><strong>HDAC inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romidepsin (Istodax; a cyclic depsipeptide)</td>
<td>Celgene</td>
<td>CTCL</td>
<td>Approved November 2009</td>
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<tr>
<td></td>
<td></td>
<td>NHL</td>
<td>Phase 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTCL</td>
<td>Approved October 2006</td>
</tr>
<tr>
<td>Vorinostat (Zolinza; suberoylanilide</td>
<td>Merck (Whitehouse Station,</td>
<td>Mesothelioma</td>
<td>Phase 3 targeting 660 patients</td>
</tr>
<tr>
<td>hydroxamic acid)</td>
<td>NJ, USA)</td>
<td>MDS, NHL, brain cancer and NSCLC</td>
<td>Phase 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple myeloma</td>
<td>Phase 2 and 3 targeting 742 patients</td>
</tr>
<tr>
<td>Vorinostat + bortezomib (Velcade)</td>
<td>Novartis (Basel)</td>
<td>Hodgkin's lymphoma</td>
<td>Phase 3 in 367 patients</td>
</tr>
<tr>
<td>Panobinostat (LBH589; hydroxamate analog)</td>
<td>Spectrum Pharmaceuticals (Irvine, CA, USA)</td>
<td>CML, AML and MDS</td>
<td>Phase 2/3</td>
</tr>
<tr>
<td>Panobinostat + bortezomib + dexamethasone</td>
<td>Spectrum Pharmaceuticals (Irvine, CA, USA)</td>
<td>Multiple myeloma</td>
<td>Phase 3 targeting 676 patients</td>
</tr>
<tr>
<td>Belinostat (PXD10; hydroxamate analog)</td>
<td>MethylGene (Montreal, QC, Canada)</td>
<td>AML, CTCL, MDS, NHL and ovarian cancer</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Mocetinostat dihydrobromide (MGCD0103;</td>
<td>Spectrum Pharmaceuticals (Waltham, MA, USA)</td>
<td>AML, CLL, Hodgkin's lymphoma, NHL, pancreatic cancer and thymic carcinoma</td>
<td>Phase 2</td>
</tr>
<tr>
<td>aminopyrimidine analog)</td>
<td>Synthax Pharmaceuticals (Waltham, MA, USA)</td>
<td>Breast cancer, Hodgkin's lymphoma and NSCLC</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Entinostat (SNDX-275; synthetic benzamide</td>
<td>PCI-24781 (CRA-024781; hydroxamic acid</td>
<td>Hematologic cancer and sarcoma</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>derivative)</td>
<td>Pharmacyclics (Sunnyvale, CA, USA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Peregrine Pharmaceuticals (Tustin, CA, USA)</td>
<td>Glioblastoma multiforme</td>
<td>Phase 2 in 40 patients</td>
</tr>
</tbody>
</table>
I-BET, a specific antagonist that mimicks acetylated histones & suppresses a specific subset of LPS-inducible genes
Suppression of inflammation by a synthetic histone mimic

Edwige Nicodeme¹*, Kate L. Jeffrey²*, Uwe Schaefer²*, Soren Beinke³*, Scott Dewell⁴, Chun-wa Chung⁵, Rohit Chandwani², Ivan Marazzi², Paul Wilson⁵, Hervé Coste¹, Julia White³, Jorge Kirilovsky¹, Charles M. Rice⁵, Jose M. Lora³, Rab K. Prinjha³, Kevin Lee³ & Alexander Tarakhovsky²
Surviving lethal septic shock without fluid resuscitation in a rodent model

Yongqing Li, MD, PhD, a Baoling Liu, MD, a Eugene Y. Fukudome, MD, a Ashley R. Kochanek, BS, a Robert A. Finkelstein, MD, CM, ab Wei Chong, MD, PhD, a Guang Jin, MD, PhD, a Jennifer Lu, BS, a Marc A. deMoya, MD, a George C. Velmahos, MD, PhD, a and Hasan B. Alam, MD, FACS, a Boston, MA

IP LPS injection + post treatment with SAHA
(suberoylanilide hydroxamic acid)
Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection

Thierry Roger,1 Jérôme Lugrin,1 Didier Le Roy,1 Geneviève Goy,1 Matteo Mombelli,1 Thibaud Koessler,2 Xavier C. Ding,1 Anne-Laure Chanson,1 Marlies Knaup Reymond,1 Isabelle Miconnet,3 Jacques Schrenzel,2 Patrice François,2 and Thierry Calandra1

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Inhibition by TSA and VPA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TSA</td>
</tr>
<tr>
<td>IL-1α</td>
<td>+</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>+</td>
</tr>
<tr>
<td>IL-1β</td>
<td>+</td>
</tr>
<tr>
<td>IL-4</td>
<td>-</td>
</tr>
<tr>
<td>IL-6</td>
<td>+</td>
</tr>
<tr>
<td>IL-7</td>
<td>-</td>
</tr>
<tr>
<td>IL-8</td>
<td>-</td>
</tr>
<tr>
<td>IL-10</td>
<td>+</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>+</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>+</td>
</tr>
<tr>
<td>TNF</td>
<td>+</td>
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<tr>
<td>RANTES</td>
<td>+</td>
</tr>
<tr>
<td>IP-10</td>
<td>+</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>+</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>+</td>
</tr>
<tr>
<td>MCP-1</td>
<td>+</td>
</tr>
<tr>
<td>G-CSF</td>
<td>+</td>
</tr>
<tr>
<td>TGFα</td>
<td>+</td>
</tr>
<tr>
<td>IFNγ</td>
<td>+</td>
</tr>
</tbody>
</table>

Impact of TSA on gene modulation by LPS or Pam3CSK4:

- enhancement
- no effect
- inhibition

(Blood. 2011;117(4):1205-1217)
Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection

Thierry Roger, Jérôme Lugrin, Didier Le Roy, Geneviève Goy, Matteo Mombelli, Thibaud Koessler, Xavier C. Ding, Anne-Laure Chanson, Marlies Knaup Reymond, Isabelle Miconnet, Jacques Schrenzel, Patrice François, and Thierry Calandra

HDAC inhibition increases mortality to nonsevere infection with *K. pneumonia* & *C. albicans*
Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection

Thierry Roger, Jérôme Lugrin, Didier Le Roy, Geneviève Goy, Matteo Mombelli, Thibaud Koessler, Xavier C. Ding, Anne-Laure Chanson, Marlies Knaup Reymond, Isabelle Miconnet, Jacques Schrenzel, Patrice François, and Thierry Calandra

HDAC inhibition protects from lethal toxic shock and severe sepsis.
Modulation of Acetylation: Creating a Pro-survival and Anti-Inflammatory Phenotype in Lethal Hemorrhagic and Septic Shock
Modulation of Acetylation: Creating a Pro-survival and Anti-Inflammatory Phenotype in Lethal Hemorrhagic and Septic Shock

Summary

- Epigenetic changes contribute to the phenotypic changes associated with sepsis, hemorrhagic shock and MOF
  - Chemical modifications of DNA
  - Histone modifications
  - Nucleosome positioning patterns
  - microRNAs

- Epigenetic changes can be targeted by Epi-drugs in experimental models of sepsis, ALI and hemorrhagic shock