Macrophage function in sepsis patients

Mihai G. Netea
Host defense during infections

Sepsis is the third leading cause of death in the U.S. after heart disease and cancer. 258,000 Americans die from Sepsis each year. 5+ million children worldwide die from Sepsis each year. 1.6 million cases of Sepsis in the U.S. every year. 55% of Americans have ever heard of the term “SEPSIS.”
New sepsis definitions

Patient with suspected infection

qSOFA ≥2? (see A)

No

Sepsis still suspected?

No

Monitor clinical condition; reevaluate for possible sepsis if clinically indicated

Yes

Assess for evidence of organ dysfunction

SOFA ≥2? (see B)

No

Monitor clinical condition; reevaluate for possible sepsis if clinically indicated

Yes

Sepsis

Despite adequate fluid resuscitation, 1. vasopressors required to maintain MAP ≥65 mm Hg AND 2. serum lactate level >2 mmol/L?

No

Yes

Septic shock

JAMA 2016

A qSOFA Variables
Respiratory rate
Mental status
Systolic blood pressure

B SOFA Variables
PaO₂/FiO₂ ratio
Glasgow Coma Scale score
Mean arterial pressure
Administration of vasopressors with type and dose rate of infusion
Serum creatinine or urine output
Bilirubin
Platelet count
Why do people die from sepsis?

Outer-membrane vesicles ("blebs")
Passive Immunization Against Cachectin/Tumor Necrosis Factor Protects Mice from Lethal Effect of Endotoxin

Beutler et al, Nature 1985
Hyperinflammatory activation of complement and coagulation system

van der Poll ... Netea, Nat Rev Immunol 2017
Increased Susceptibility of TNF-α Lymphotoxin-α Double Knockout Mice to Systemic Candidiasis Through Impaired Recruitment of Neutrophils and Phagocytosis of Candida albicans

Netea et al, J Immunol 1999
Immune paralysis in sepsis patients: lymphocytes

Immunosuppression in Patients Who Die of Sepsis and Multiple Organ Failure

Figure 5. Immune Effector Cells in Spleen Tissue

A Immunohistochemical staining for HLA-DR
Control patient Patient with sepsis

B Immunohistochemical staining for CD4
Control patient Patient with sepsis

C Immunohistochemical staining for CD8
Control patient Patient with sepsis

D T-cell counts

Boomer et al. JAMA 2011
Hyperinflammation and immune paralysis

- Acute phase: hyperinflammation
- Immunoparalysis
- Homeostasis/recovery
- Anti-inflammatory reactions
- Pro-inflammatory reactions

Time

Immune function

Death
Immune dysregulation in sepsis

Van der Poll et al, NEJM 2013
Defective monocyte-induced cytokines

Healthy controls

Severe sepsis patients
Long-term epigenetic reprogramming in sepsis

ChIP-seq
Histone tail modifications determine ‘activity’ by attracting TFs (we use 5 histone modifications)

ATAC-seq
Open chromatin (i.e. nucleosome-free regions) can be bound by TFs, which can be identified by motif sequence

ChIP-seq
Histone tail modifications determine ‘activity’ by attracting TFs (we use 5 histone modifications)

RNA-seq
Gene expression

WGBS – whole genome bisulfite sequencing
DNA methylation maintains DNA in a closed state
In vitro and in vivo study of human innate immune memory

**In vitro model**

- Buffy coat PBMCs
- Negatively-selected monocytes (to avoid activation)
- >95% CD14+ cells

**In vivo / ex vivo models**

**Tolerance**

- Healthy volunteers injected with LPS: temporary 'sepsis' state
- Monocytes

**Training**

- Healthy volunteers injected with BCG: ~ few weeks of trained phenotype

**Easy manipulation**

- Inhibitors/activators

**Fast readout**

- Cytokine release ELISA

**Easy collection of cells for sequencing**

Novakovic et al, Cell 2016
Large-scale chromatin changes
Mono-Macro differentiation and LPS response

Novakovic et al, Cell 2016
4 main clusters of H3K27ac change

Signal intensity at histone modification peaks (reads/peak)

Novakovic et al, Cell 2016
How does LPS exposure influence chromatin?

3,700 enhancers that gain H3K27ac (activation) only after LPS exposure, and retain memory of this exposure in the form of H3K4me1

- Some of these enhancers and promoters are associated with negative regulators of Inflammation.
- Therefore, LPS-Mf express repressors that are reducing inducibility of certain genes.

Novakovic et al, Cell 2016
Example gene with LPS induced H3K4me1 memory: IDO1 (Indoleamine 2,3-Dioxygenase 1)

IDO1 is a negative regulator of inflammation and is commonly associated with tolerance in cancer and sepsis

Novakovic et al, Cell 2016
Motif enrichment at tolerized genes

Motif enrichment 100bp sliding window

TFs associated with motif

G1) Tolerized
G2) Partially tolerized
G3) Responsive
Immune cell

- Glucose
  - Glycolysis
    - Pyruvate
    - 2 ATP
    - PDH
    - Oxidative phosphorylation
      - Krebs cycle
        - Electron transport
          - 36 ATP

- Akt
  - Lipid synthesis
    - AcetylCoA
      - Histone acetyltransferase

- mTOR
  - Autophagy

- HIF-1α
  - TLR stimulation
  - Immune cell stimulation

- Naïve cells
  - O2 not present
    - NAD+
    - Lactic acid

- Active cells
  - O2 present
  - NADH
  - HIF-1α

- Naïve cells
  - TLR stimulation
  - Immune cell stimulation

- Oxidative phosphorylation
  - Krebs cycle
    - Electron transport
      - 36 ATP

- Akt
  - Lipid synthesis
    - AcetylCoA
      - Histone acetyltransferase
Immunometabolic shifts during monocyte reprogramming

Saeed, Quintin et al, Science, 2014
Microarray analysis from Gram-negative sepsis patients

Lactate production as a biomarker for prediction of immunoparalysis

Lower cytokine and lactate production in LPS tolerant monocytes

Mitochondrial maximum capacity is reduced in LPS tolerant monocytes

Immunometabolic function in monocytes from sepsis patients

IFNγ partially restored immunometabolic function of LPS tolerant monocytes

Figure 6


- Acute phase: hyperinflammation
- Immunoparalysis
- Pro-inflammatory reactions
- Homeostasis/recovery
- Anti-inflammatory reactions
- Glycolysis
- TCA-cycle
- Triglyceride metabolism

Acute phase: ↑ glycolysis, ↓ TCA-cycle
Immunoparalysis: ↓ glycolysis, ↓ TCA-cycle, ↓ triglyceride metabolism
Recovery: = glycolysis, = TCA-cycle, = triglyceride metabolism
Metabolic status and epigenetic programming decides the long-term activation fate of the monocyte.

- Inflammatory stimuli
  - Immune signaling
    - Epigenetic enzymes
      - Histone modifications
        - Long-term immune paralysis
  - Sustained metabolic reprogramming
    - Metabolic paralysis:
      - Myofunctional dysfunction
      - Decreased glycolysis
      - Maintains immune paralysis status
The double-edge fate of immune response in sepsis

Sepsis

Protective immunity

Localized innate immune response
- Release of pro-inflammatory mediators
- Leukocyte recruitment
- Complement activation
- Coagulation activation

Pro-inflammatory response

Homeostasis

Anti-inflammatory mechanisms

Local repair mechanisms
- Inhibition and resolution of inflammation
- Tissue repair
- Return to homeostasis

Immune suppression

Leukocytes and parenchymal cells
- Release of pro-inflammatory mediators
- Cell injury with release of DAMPs

Endothelium
- Release of pro-inflammatory mediators
- ↑ Adhesive and procoagulant properties
- ↓ Barrier function

Platelets
- Release of pro-inflammatory mediators
- Activation of neutrophils and the endothelium
- Microvascular thrombi

Others
- Coagulation activation (microvascular thrombosis)
- Complement activation

CD4+ T cells
- ↑ Apoptosis
- Exhaustion
- T\(^{1/2}\) cell polarization

CD8+ T cells
- ↑ Apoptosis
- Exhaustion
- ↓ Cytotoxic function

Neutrophils
- ↓ Apoptosis
- ↑ Immature cells with decreased antimicrobial functions

Antigen-presenting cells
- Reprogramming of macrophages to an M2 phenotype
- Reduced HLA-DR expression

Lymph node
- Apoptosis of B cells and follicular DCs

Others
- Expansion of regulatory T cell and MDSC populations

van der Poll ... Netea, Nat Rev Immunol 2017
Immune-based classification of sepsis patients

van der Poll … Netea, Nat Rev Immunol 2017
# Future development of drug therapies in sepsis

## Discovery and development

### Current
- In vitro models and a limited number of acute animal models using young healthy animals

### Future
- In vitro models and a variety of animal models that include different infectious sources and pathogens, and consider age, comorbidity and supportive therapies

## Phase III clinical trial

### Identification of pathway X as therapeutic target

### Clinical validation of target

<table>
<thead>
<tr>
<th>Current</th>
<th>Future</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Evaluation of expression of pathway X in patients in time</td>
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</table>

### Early clinical trials

<table>
<thead>
<tr>
<th>Current</th>
<th>Future</th>
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<tbody>
<tr>
<td>Treatment dose and duration based on limited animal studies and small pharmacodynamic studies in humans.</td>
<td>Evaluation of treatment dose and duration based on changes in biomarker X.</td>
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### Efficacy trials

<table>
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<tr>
<th>Current</th>
<th>Future</th>
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<tbody>
<tr>
<td>Patient selection based on clinical severity (“all-comers”).</td>
<td>Patient selection based on expression of biomarker X.</td>
</tr>
<tr>
<td>No treatment monitoring</td>
<td>Treatment monitoring based on expression of biomarker X</td>
</tr>
<tr>
<td>Endpoint: 28 -day mortality</td>
<td>Adaptive trial design</td>
</tr>
</tbody>
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van der Poll … Netea, Nat Rev Immunol 2017
What may a realistic approach?

• Assess immune competence with simple assays:
  • **Hyperinflammation**: MAS-like criteria
  • **Immune paralysis**: HLA-DR expression, lymphocyte numbers, proinflammatory cytokine production, IgA

• Assess in proof-of-principle trials which interventions modulate function: immune, epigenetic, metabolic, etc

• Perform clinical trials with 2 or 3 interventions that have an impact on immune status *on personalized level*
Thank you!

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