PAMPs, DAMPs and our evolving understanding of Sepsis and SIRS

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Gulf War Subcommittee

Disclosures / Competing interests

FUNDING

• NIH
• DoD (CDMRP)
• CIMIT
• No commercial funding
**Systemic Inflammatory Response Syndrome (SIRS)**

≥ 2 of the following:
- Temp >38°C, <36°C
- Pulse >90
- RR >20, PCO2 <32
- WBC >12,000, <4000 or >10% bands

Inflammatory response to illness of any source

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**Burden of SIRS**

1/3 of all hospitalized patients
- More than half of all ICU patients
- Nearly all SICU patients
- Morbidity and mortality 2° organ failure
  - Lung (ALI / ARDS) > liver/kidney
Inflammation can reflect Infection or ‘Sterile SIRS’

Hemoperitoneum vs bacterial peritonitis

Aspiration vs bacterial pneumonia

Mechanistic understanding of SIRS

'\textit{DANGER}' molecules

Infection vs Fractures vs Shock vs Trauma

WRONG!
**In non-infective conditions**

In the setting of infections

- PAMPs
  - TLR / GPCR
  - Recognized by Pattern Recognition Receptors (PRR)

In non-infective conditions

- Ancient (invertebrates, multi-celled)
  - PMN, Mφ, DC, NKC

- No clonal expansion
  - PRR on germ-line (TLRs, GPCRs)
  - multi-functional

- Immediate response to danger motifs

- Rapid responses in trauma, sepsis

**Innate immunity**

- Fractures
- Shock
- Inflammation

- Redundant cytokine cascade

*Injury → DAMPs → PRR*
Exogenous *infective* motifs

(LPS, FPs, bacterial sugars, ‘CpG’ DNA, dsRNA, flagellin…)

- **Bind PRRs → immune activation**
  - **Cytokines etc**
- **Symptomatic infective SIRS (“sepsis”)**
  - ↑ NO• release → vasodilatation
  - ↑ PMN-EC interactions → capillary leak

**PAMPs**

?? DAMPs…

*Non-infective* motifs

? **Endogenous** products of tissue injury
  - Intracellular motifs released by mechanical injury
  - Membrane motifs changed by toxins
  - New motifs 2’ to metabolic, I/R stress

*Bind PRRs → immune activation*

*Cytokines etc*

?? …symptomatic *non-infective SIRS*
**Intracellular DAMPs**

<table>
<thead>
<tr>
<th>Putative DAMP</th>
<th>PRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGB-1</td>
<td>TLR4</td>
</tr>
<tr>
<td>S-100</td>
<td>RAGE</td>
</tr>
<tr>
<td>HSP 30/60</td>
<td>TLR4</td>
</tr>
<tr>
<td>B7-H3</td>
<td>TREM</td>
</tr>
</tbody>
</table>

- Few known
- Signal through PRR’s like PAMPs

**Mitochondria as DAMPs**

**...why are clinical sepsis and SIRS so often indistinguishable?**

- Mitochondria were saprophytic bacteria
  - Became endo-symbionts
  - Evolved into organelles

- ‘Septic’ response to MT?
Do mitochondria contain DAMPs?

- 13 ‘endogenous’ peptides
  - begin with n-formyl-met
  - *Do they activate FP receptors*
- ‘Bacteria-like’ DNA
  - *Unmethylated ‘CpG’ repeats*
  - *Do they activate TLR-9*

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Does mechanical tissue injury cause circulation of mitochondrial debris? (MTD)
Do shock / ischemia-reperfusion injury result in circulation of MTD?
### Plasma mtDNA in rat HS

<table>
<thead>
<tr>
<th></th>
<th>Naïve</th>
<th>3h</th>
<th>1d</th>
<th>3d</th>
<th>7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA (µg/ml)</td>
<td>0.00</td>
<td>0.05</td>
<td>0.10</td>
<td>0.15</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*P<0.01 vs vol plasma (ANOVA)

**mtDNA appears in plasma of FFx patients**

Plasma Cyto B in Femur Fx

<table>
<thead>
<tr>
<th></th>
<th>Volunteer Plasma</th>
<th>Patient Plasma</th>
<th>Reaming Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct count (cycles)</td>
<td>30</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

2^{14} -fold increase

*P<0.01 vs vol plasma (ANOVA)
Do MTD activate inflammatory cell signaling?

mtFPs activate $[\text{Ca}^{2+}]_i$ (via FPR1)

Zhang, Hauser, Nature 2010
mtDNA activates p38 via TLR9

mtDNA (μg/ml) 5 10
ODN - + - +
p-p38
p-p38
p38

TLR9 blocked by CQ, ODNs

Zhang, Hauser, Nature 2010

Does MTD activate inflammatory cell phenotypes?
**MTD activates cytokine production**

- **MTD (µg/ml)**: 0, 10, 20, 40, 100, 200, 400
- **LPS (µg/ml)**: 1, 10

- **IL-8 (µg/ml)**
  - **4hr**: MTD activates cytokine production
  - **24hr**: MTD activates cytokine production

**mtDNA activates PMN / EC interactions**

- Zhang, Hauser *Nature* 2010
Do mitochondrial DAMPs activate innate immunity in vivo?

MTD → PMN attack on lung

MMP-8 in lung

PMN in BALF

Zhang, Hauser *Nature* 2010
MTD causes ALI

Zhang, Hauser Nature 2010
**MTD ALI is oxidant-related**

4-HNE stains

Zhang, Hauser *Nature* 2010

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**Evolutionary conservation of PAMPs and DAMPs in bacteria and mitochondria cause many similarities between sepsis and SIRS**

*Nature editorial March 4, 2010*
So what is ‘septic’ SIRS?

PAMPs from infection cause SIRS

What is non-infectious SIRS?

2° Sepsis perpetuates SIRS → MOF → death
Treatment of *infective* SIRS

1) Remove PAMPs (bio-markers)
   - Antibiotic Tx
   - Drainage, source control
2) Rx SIRS *after* source control
   - Target PRR, signal cascades
   - Steroids, aPC, anti-cytokine Tx
   - (All *dangerous* w/o source control)

Treatment of *endogenous* SIRS

1) Remove DAMPs (bio-markers)
   - *Debride / drain* sources
   - *Avoid* antibiotics
2) *Prevent / treat* SIRS *early*
   - Target DAMPs and PRR
   - Interrupt inflammatory signaling
   - Safe w/o infection (*but ??healing*)
Acknowledgements

Hauser Lab
Kiyoshi Itagaki
Qin Zhang
Mustafa Raoof
Tolga Sursal

Junger Lab
Yu Chen
Yuka Sumi

London
Karim Brohi