Inflammation after injury:
...sniffing the trail from femur fractures to formyl peptides

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Big Sky Montana
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Disclosures / Competing interests

- NIH / DOD / CIMIT
- No commercial funding
- Not now, never have been a member of Communist Party
- Never in jail (except overnight)
- 14 of the last 15 WTA meetings
Mississippi, 1993

- Morel-Lavallé
- MOF “due to sepsis”
- No ‘source’
- I&D huge pelvic hematoma
- **Every** culture (-)
- Recovered

“Bubba”
D'OH!
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
Burden of SIRS

• **Prevalence:**
  – 1/3 of all hospitalized patients
  – 1/3 of all trauma admissions
  – > **half** of all ICU patients
  – nearly **all** SICU patients
Sepsis or SIRS?

Aspiration or pneumonia?

Hematoma or pus?
The “Three W’s”

- Day 1 – WIND (atelectasis)
- Day 3 – WATER (UTI)
- Day 5-7 – WOUND (SSI’s)

Textbook dogma …but how much is really truth?
Was it something in the fracture hematoma?

Living is easy with eyes closed... misunderstanding all you see.
To the lab !!
Femur fractures are an important source of systemic cytokines.
Trauma altered responses to G-protein coupled cytokines, increasing responses to fMLP.
Trauma-suppressed responses to chemokines

Tarlowe and Hauser, SIS 2001
NUTS !!
Current understanding of SIRS

Infection

Fractures

Cytokines

SIRS

MOF

DEATH

‘DANGER’ molecules

Inadequate!
In sterile tissue Trauma

by Pattern Recognition Receptors (PRR)

Infection

PAMPs

TLR / GPCR

Fractures

Inflammatory program

immune activation

SIRS

MOF

DEATH

? Triggers / Signals / Events

Redundant cytokine cascade
Immune responses to ‘danger’

We have **two immune systems**

1) **Classical** - clonal expansion in response to new, non-self motifs

2) **Innate** – *pre-programmed* responses to evolutionarily conserved *danger* motifs
Adaptive ("classical") immunity

- Recent (vertebrates)
  - Clonal expansion of T and B-cells
  - Agonists non-self ‘antigens’
  - Receptors Ig-based
- Slow response (≥1 week)
  - Tumor, viruses
  - Acute infections, trauma
**Innate immunity**

- **Ancient** (invertebrates, multi-celled)
  - PMN, Mφ, DC, NKC (no clonal expansion)
- Use pattern recognition receptors
  - On germ-line (TLRs, GPCRs)
  - Rapid response to trauma, sepsis
- Target *conserved molecular motifs*
- Warn organism of “**DANGER**”
Exogenous infective agents (eg bacteria)

- **Pathogen associated** motifs (Janeway)
  - LPS, FPs, bacterial sugars, ‘CpG’ DNA, dsRNA, flagellin

- **PRRs [e.g. ‘Toll’ receptors] on immune cells** → activation → cytokines

- **Symptomatic infective SIRS** (*sepsis*)
  - NO$^\cdot$ → hypotension
  - PMN → EC → capillary leak
toll mutation in drosophila
TLR system
PRRs for PAMPs

TNF
IL-1

LPS, BACTERIA
LTA
BLPS
ZYMOS
Flageffin

MyD88
Rac
PI3K
TIRAP
TRAM
TRIF

MyD88
Cpg-DNA

IRAK4
AKT
NFκβ
MAPK

CD14
### Endogenous motifs - **DAMPs**

**Matzinger - “Damage”**

**Fewer DAMPs known than PAMPs**

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

- Can signal through **same PRR**
How can Trauma generate DAMPs?

1. ? Mechanical tissue injury
   • Cell destruction
   • ? Direct release of DAMPs

2. ? Hemodynamic tissue injury
   • ? I/R → gut inflammation
   • Other tissues / ? mechanisms
Mitochondria as DAMPs?

- Mitochondria were saprophytic bacteria
- Became *endo-symbionts*
- Evolved into organelles
- ? Could there be a ‘septic’ response to MT?
Potential mitochondrial DAMPs ??

- 13 ‘endogenous’ peptides
  - all begin with n-formyl-met
  - ? activation of FPRs like fMLP
- ‘Bacteria-like’ DNA
  - unmethylated ‘CpG’ repeats
  - ? activation of TLR-9
To the lab!!
Mt-FP from femur fractures activate PMN

Raoof, Hauser AAST 2008
Does *trauma* cause mitochondrial debris (MTD) to enter the circulation?
Plasma mtDNA after blunt trauma

Zhang, Hauser. Nature 2010
Shock, ischemia/reperfusion is a direct cause of SIRS … *but how?*

- Gut bacteria / endotoxins (Fine, Deitch) do **not** enter the portal vein (Moores)
- Gut lymph is inflammatory (Deitch, Moore) … *but how?*
- Do mitochondrial debris enter the circulation in hemodynamic shock?
Plasma mtDNA in Rat Hemorrhagic Shock

mtDNA (µg/ml)

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<tr>
<th></th>
<th>0.00</th>
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<tr>
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Zhang, Hauser, Shock 2010
BALF mtDNA after acid aspiration

Segal, B  Unpublished data
Do mitochondrial DAMPs (MTD) activate inflammatory cell signaling?
PMN activation by mtFPs suppresses chemokine \([\text{Ca}^{2+}]_i\) responses.
MTD activate PMN MAP-Kinases

MDP (μl) - 4 10 20 40

kDa 40 30 40 30 40 30

P-p38

p38

P-p44/42

p44/42
mt-DNA activates p38 MAPK

[Graph showing the activation of p-p38 and p38 with mtDNA treatment compared to control.]

Legend:
- mtDNA: - , +
- p-p38
- p38

Density (p-p38)

Control  mtDNA

*
**TLR9 knockdown** blocks response to mtDNA (RAW macrophages)
MTD causes PMN IL-8 production
p38 activation by mtDNA

Can be blocked by CQ, ODNs
Do MTD activate inflammatory cell phenotypes?
PMN chemotaxis to MTD

Zhang, Hauser. Nature 2010
With $\alpha$-FPR1 (or $CsH$)
Chemotaxis to mt-FPs
PMN necrotaxis to mtFPs in vivo

McDonald, Science. 2011
mtDNA activates PMN-EC interactions
PMN adherence to EC

Itagaki, Hauser, unpublished
mtDNA activates expression of EC adhesins

Itagaki, Hauser, unpublished
Circulating MTD causes inflammatory organ injury
MTD induced ALI

Sham

i.v. mitochondria

(= 5% liver injury) at 6h

Zhang, Hauser, Nature 2010
MTD $\rightarrow$ PMN attack on lung

MMP-8 in lung

PMN in BALF
MTD activates lung injury

---

**C**

![Graph showing BALF TNF-α levels](chart)

- **Naïve**
- **Media**
- **MTD**

---

**D**

![Graph showing BALF IL-6 levels](chart)

- **Sham**
- **3h**
- **6h**
- **24h**

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**Albumin**

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<td>BALF albumin (µg/ml) *</td>
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**Wet/Dry**

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**BalF albumin (µg/ml)**

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**Lung wet/dry weight Ratio**

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**IL-6**

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<td>BALF IL-6 (pg/ml) *</td>
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Oxidant lung injury

4-HNE stains
Hepatic injury

**MMP-8**

Liver MMP-8 (Arbitrary unit)

- Naïve
- Media
- MTD

**p-p38**

Liver p-p38 (Arbitrary unit)

- Naïve
- Vehicle
- MTD

**IL-6**

IL-6 (pg/mg liver protein)

- Naïve
- Vehicle
- MTD

**TNF-α**

TNF-α (pg/mg liver protein)

- Naïve
- Vehicle
- MTD
**Peritonitis**

Figure 8: % of PMN Migration

A) Bar graph showing % PMN for different treatments:
- Saline
- CsH
- W-peptide
- MTD
- MTD/CsH

B) Bar graph showing % of PMN Migration for different stimuli:
- fMLF
- MTD
- IL-8
- mtDNA
- CpG DNA
- LPS

C) Image of cells

D) Image of cells
Evolutionary conservation of molecular patterns in bacteria and mitochondria contribute to the similarity of sepsis and SIRS
Many other mitochondrial and cellular DAMPs waiting to be discovered
What is ‘septic’ SIRS?

PAMPs from infection cause SIRS

PAMPs

INFECTION

SEPSIS

SIRS

BURNS

SURGERY

TISSUE TRAUMA

PANCREATITIS

OTHERS
What is traumatic SIRS?

Sepsis *perpetuates* SIRS → MOF → death
Profoundly alters our understanding of:

- **SIRS and MOF** after trauma
  - Lung, renal, cardiac, CNS, hepatic, metabolic
  - Fractures, crush injury, ischemia
- **SIRS** after surgery
  - Clinical febrile responses
  - Open vs ‘minimally invasive’ surgery
  - “Atelectasis”
- **Tumor surgery, vascular surgery**
  - Tumor lysis syndromes, febrile neutropenia
  - Revascularization
Management of SIRS

- PAMPs & DAMPs are *bio-markers*
  - PCRs for bDNA faster than cultures
  - PCR for mtDNA - confidence for SIRS
- Decrease *empiric antibiotic use*
  - resistance, toxicity, cost
- Treatments for SIRS
  - prevent MOF, catabolism
PCRs for bDNA / mtDNA

Universal bacterial primers

- **mt-Cytochrome B**
- **Bacterial 16s-DNA**

![Graph showing Ct values for different samples](image)

- **Volunteers**
- **Trauma Patients**
- **Fracture Fluids**
- **Water**

![Graph showing Ct cycles vs. [bacterial nucleic acids] (g/mL)](image)

- **Staphy**
- **E.coli**
- **B.Frag**
- **mtDNA**
- **water**

- **10^{-15}**
- **10^{-12}**
- **10^{-9}**
- **10^{-6}**
‘Chip-based’ PCRs
Sublethal E. coli sepsis (baboon)
Shiga-toxin infusion (baboon)

- mt-DNA Cyto B
- bact-DNA 16S
16s-DNA in anthrax infusion (baboon)
mt-DNA in anthrax infusion (baboon)
Tx of *infective SIRS*

1) **PAMP control**
   - *Drainage, source control*
   - Adjunctive antibiotics

2) **SIRS treatment**
   - *delay* anti-cytokine strategies
   - *delay* steroids, aPC, anti-inflammation
   - Wait until PAMP *biomarkers abate*
Treatment of *traumatic SIRS*

1) **Remove source of DAMPs**
   - *Debride / drain* anatomic sources
   - *Avoid* antibiotic use

2) **Prevent / treat SIRS early**
   - Anti-*DAMP* strategies (CsH, ODN)
   - Anti-*PRR* strategies (mAb’s)
   - Interrupt inflammatory *signaling*
D’OH!

NUTS!

MMMMM, DONUTS!