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

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WTA Founders' Lecture
Big Sky Montana
March 3, 2011

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Harvard Medical School

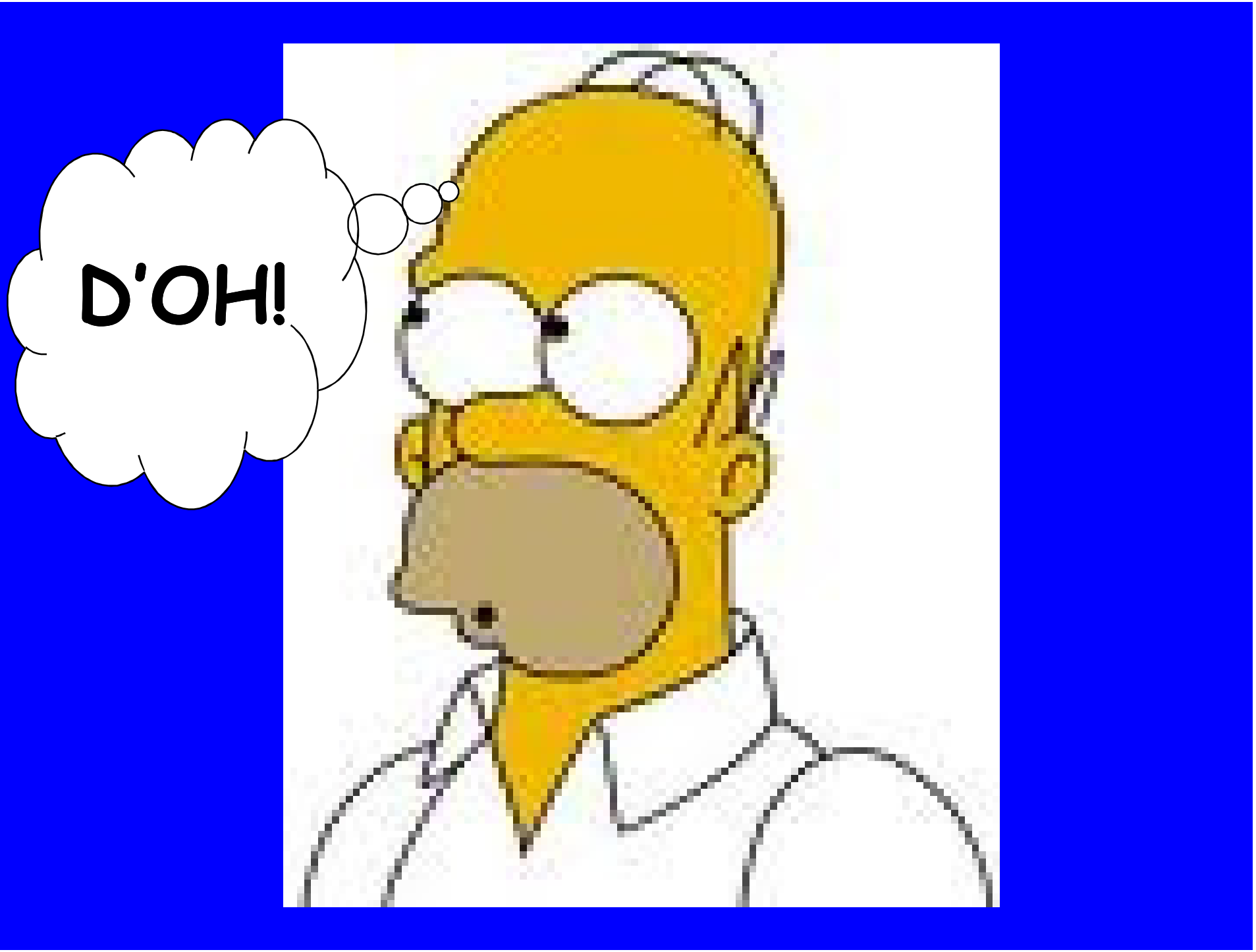
Disclosures / Competing interests

- 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***

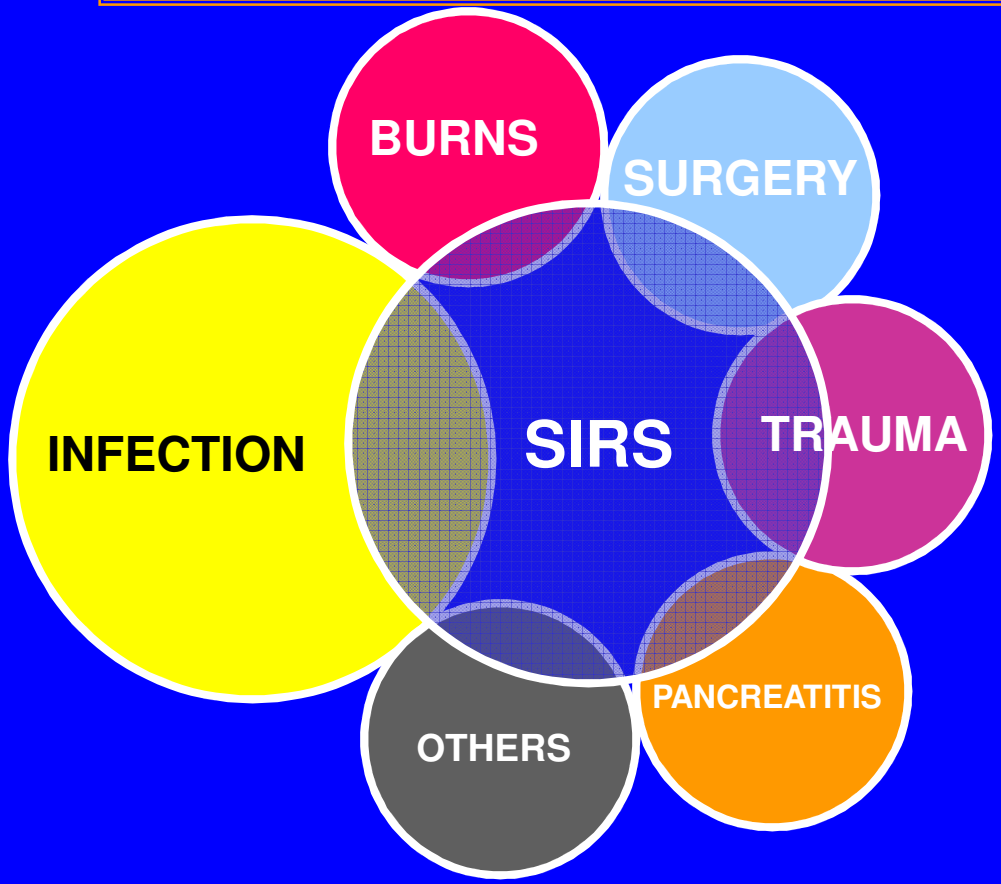
Mississippi, 1993

"Bubba"

- Morel-Lavallé
- MOF "due to sepsis"
- No 'source'
- I&D huge pelvic hematoma
- ***Every*** culture (-)
- Recovered


D'OH!

Systemic Inflammatory Response Syndrome (SIRS)



≥ 2 of the following:

- **Temp >38 ℃, <36 ℃**
- **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?

Post-op fever / SIRS

The "Three W's"

- Day 1 – ***W***IND (atelectasis)
- Day 3 – ***W***ATER (UTI)
- Day 5-7 – ***W***OUND (SSI's)

Textbook dogma …but how much is really truth?

Was it something in the fracture hematoma?

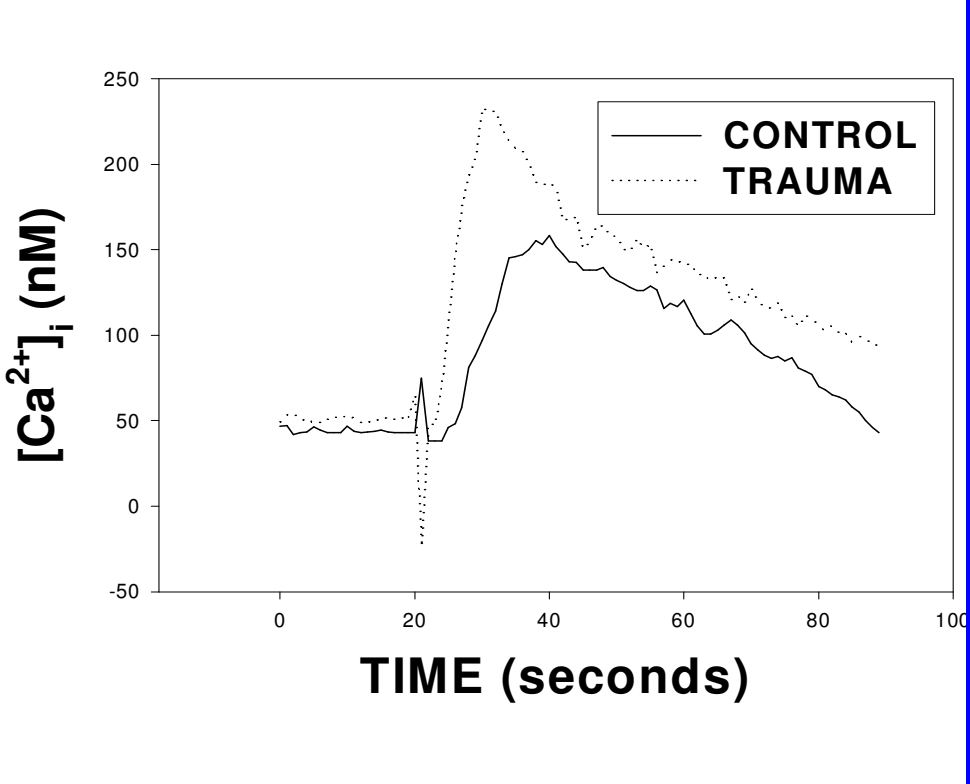
Living is easy
with eyes closed…
misunderstanding
all you see

To the lab !!

Hauser, AAST 1995

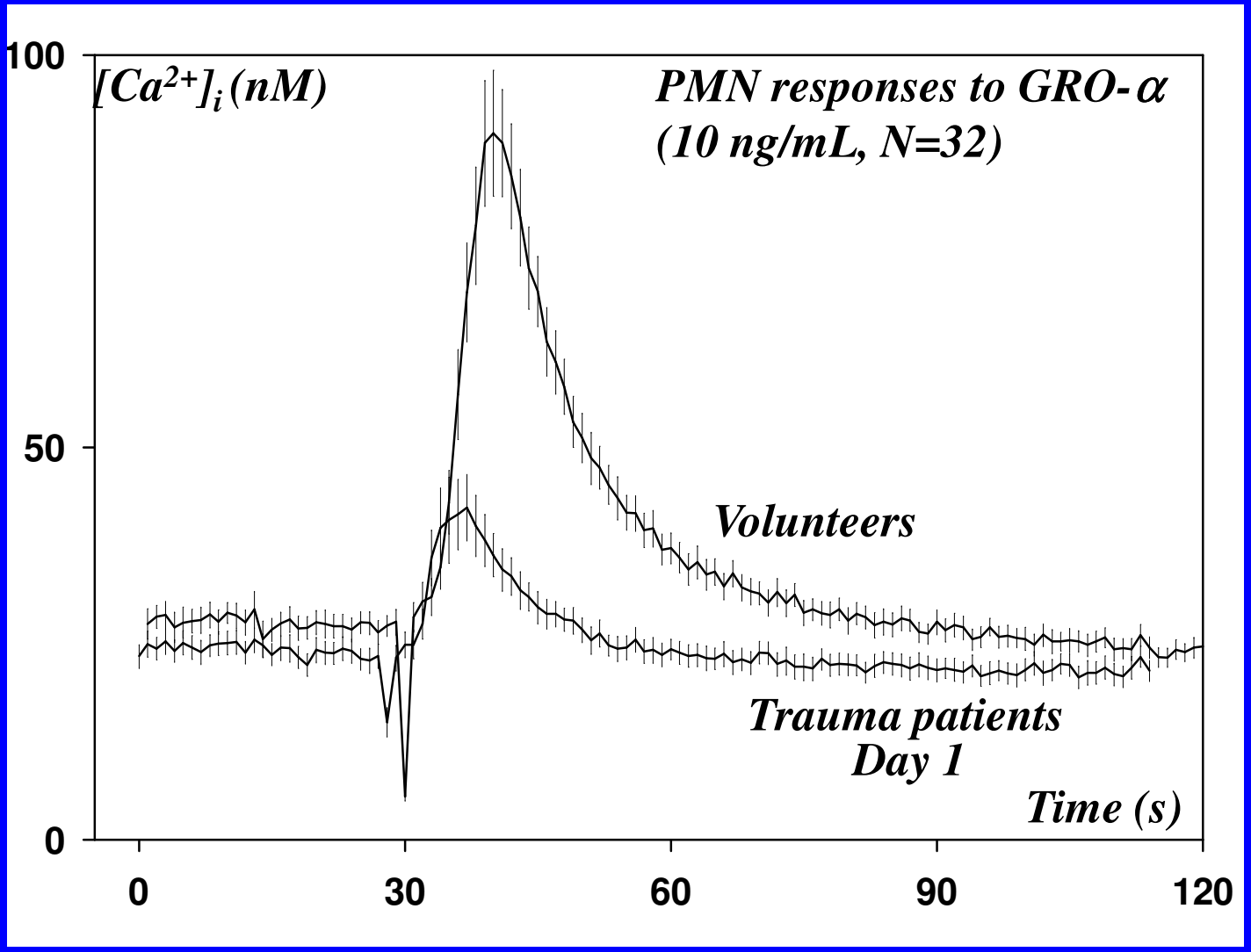
Femur fractures are an important source of systemic cytokines

Adams, Hauser. WTA 2000



Trauma altered responses to G-protein coupled cytokines, increasing responses to ***fMLP***

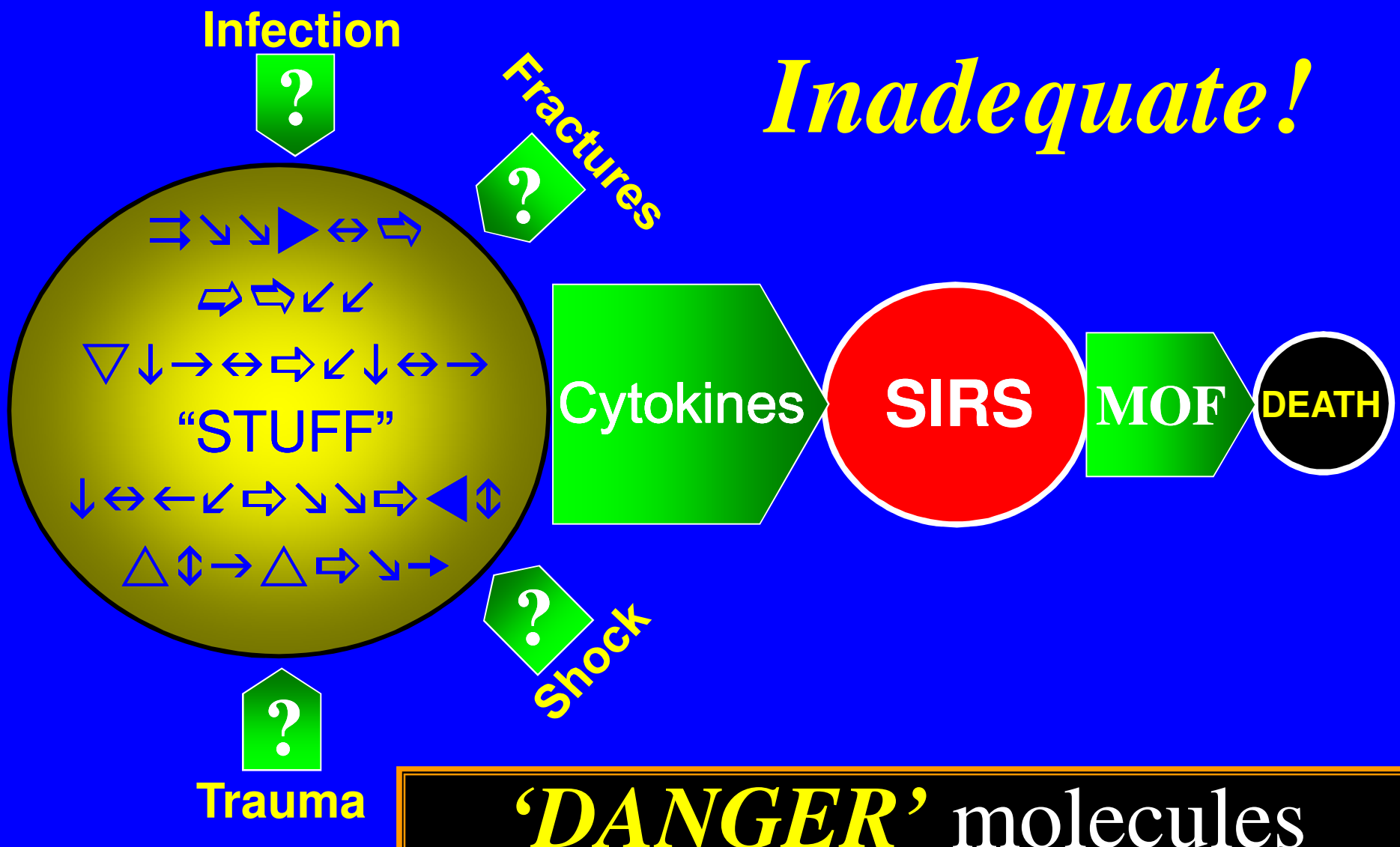
Trauma suppressed responses to chemokines



Tarlowe and Hauser, SIS 2001


NUTS !!

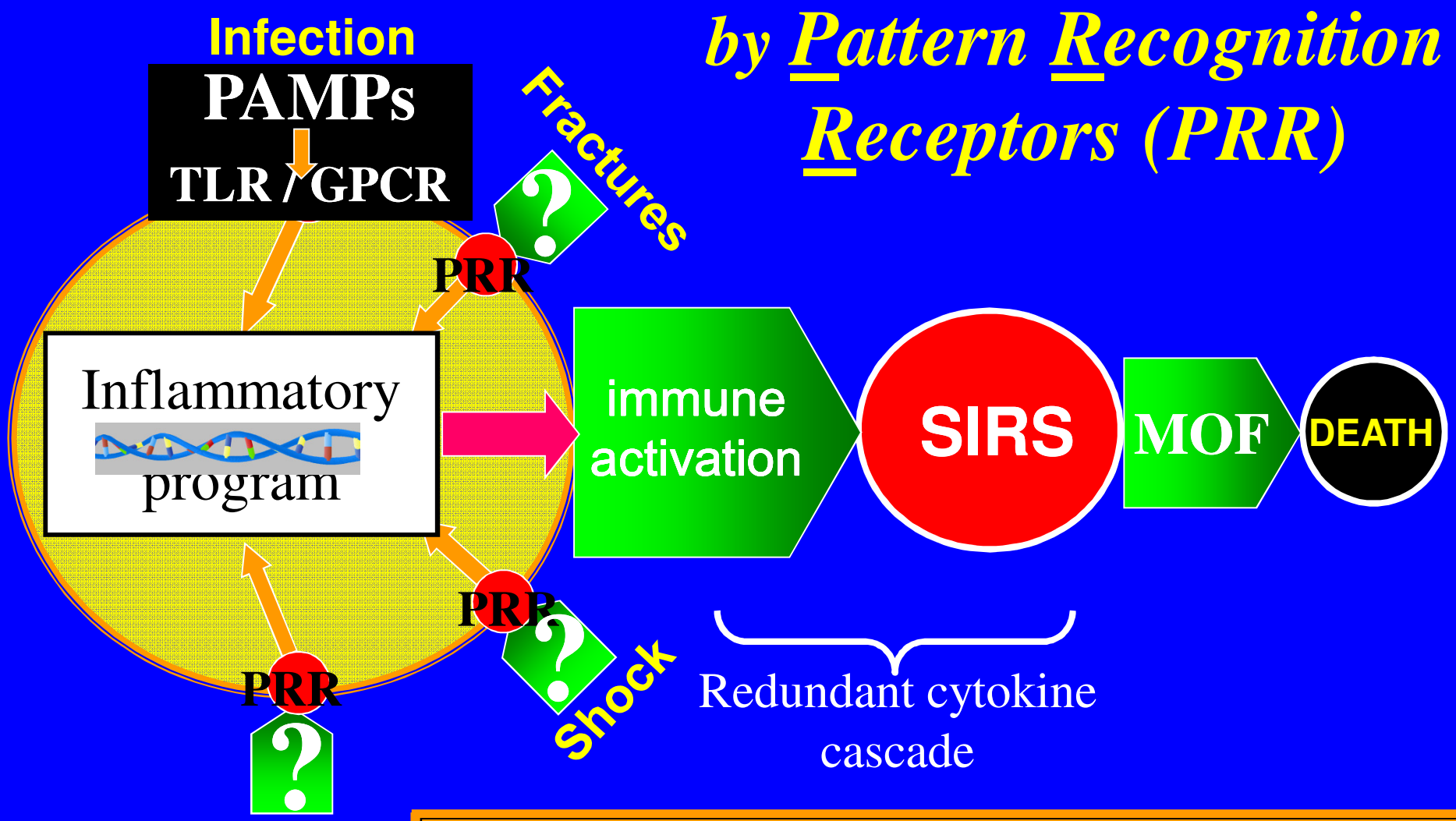
Current understanding of SIRS



'DANGER' molecules

In sterile tissue Trauma

by Pattern Recognition Receptors (PRR)



? Triggers / Signals / Events

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"*

Pathogen motifs - PAMPs

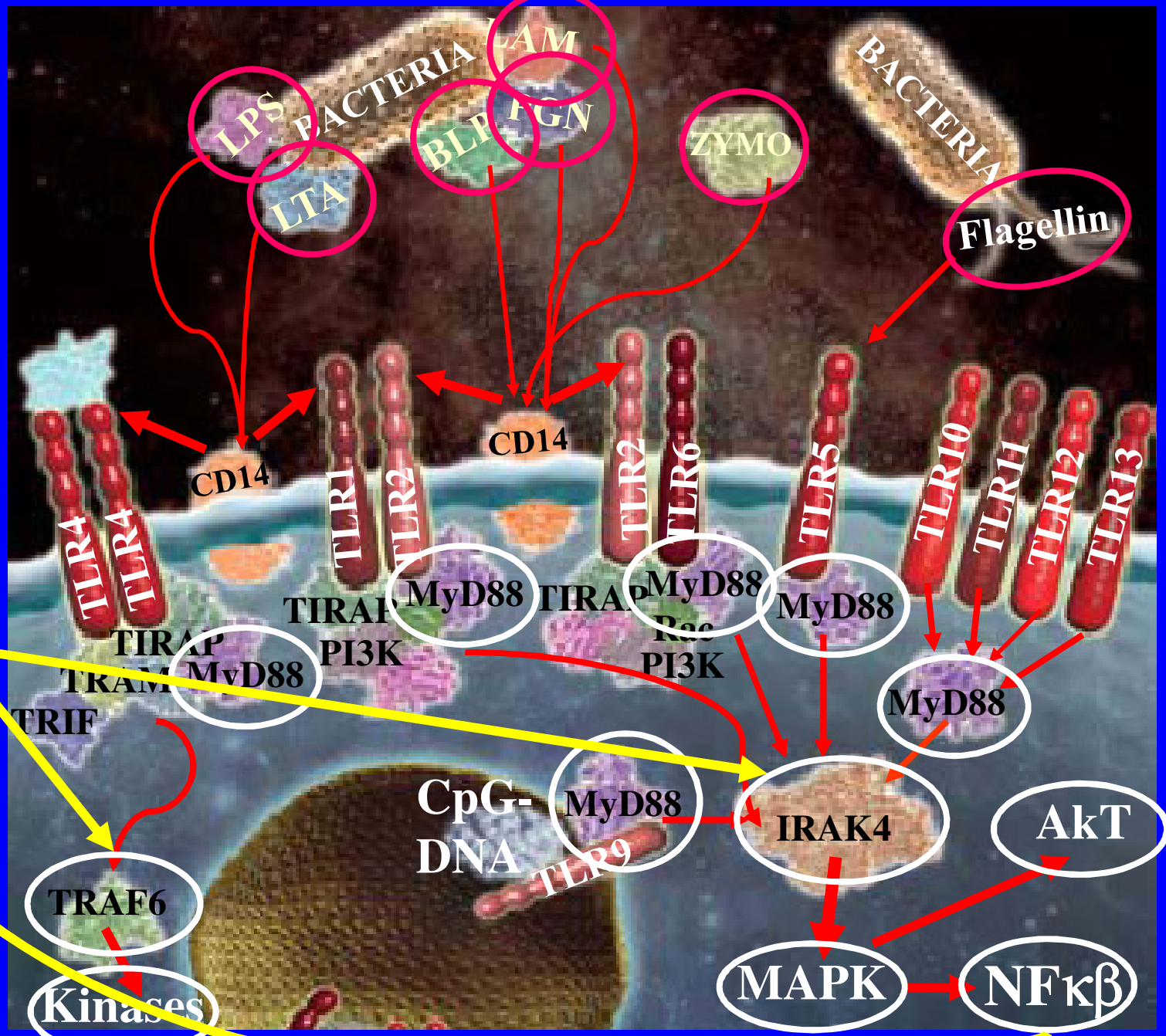
Exogenous infective agents (eg bacteria)

- *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 *(<u>sepsis</u>)*
  - ✓ $NO\cdot$ → hypotension
  - ✓ PMN → EC → capillary leak

toll mutation in drosophila

TLR system

PRRs for PAMPs



TNF

IL-1

Endogenous motifs - *DAMPs*

Matzinger - *"Damage"*

Fewer DAMPs known than PAMPs

Putative DAMP	PRR
HMGB-1	TLR4
S-100	RAGE
HSP 30/60	TLR4
B7-H3	TREM

- ✓ 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?*

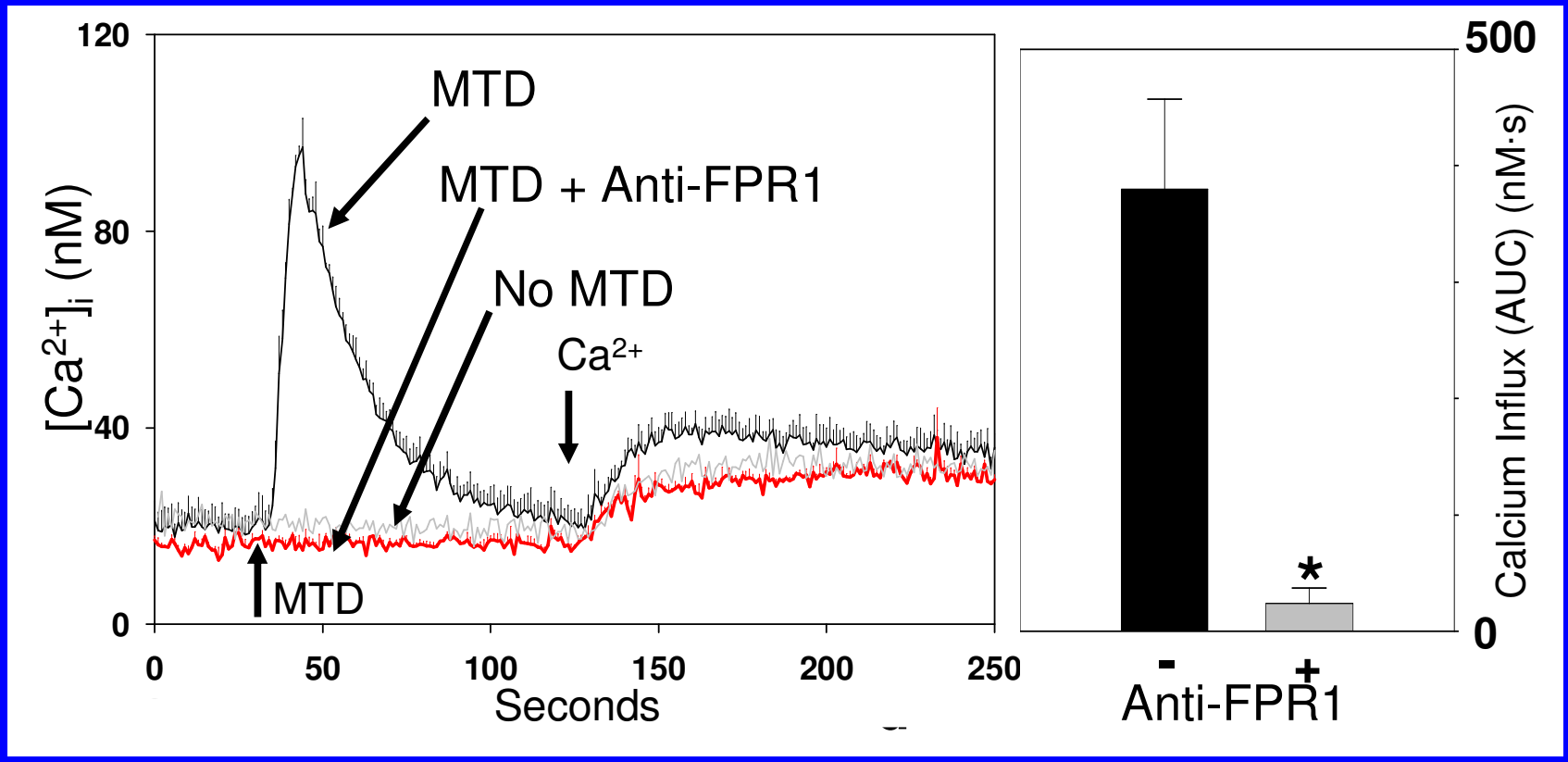
- Mitochondria were saprophytic bacteria
- Became *endo-symbionts*
- Evolved into organelles
- *? Could there be a 'septic' response to MT?*

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 !!

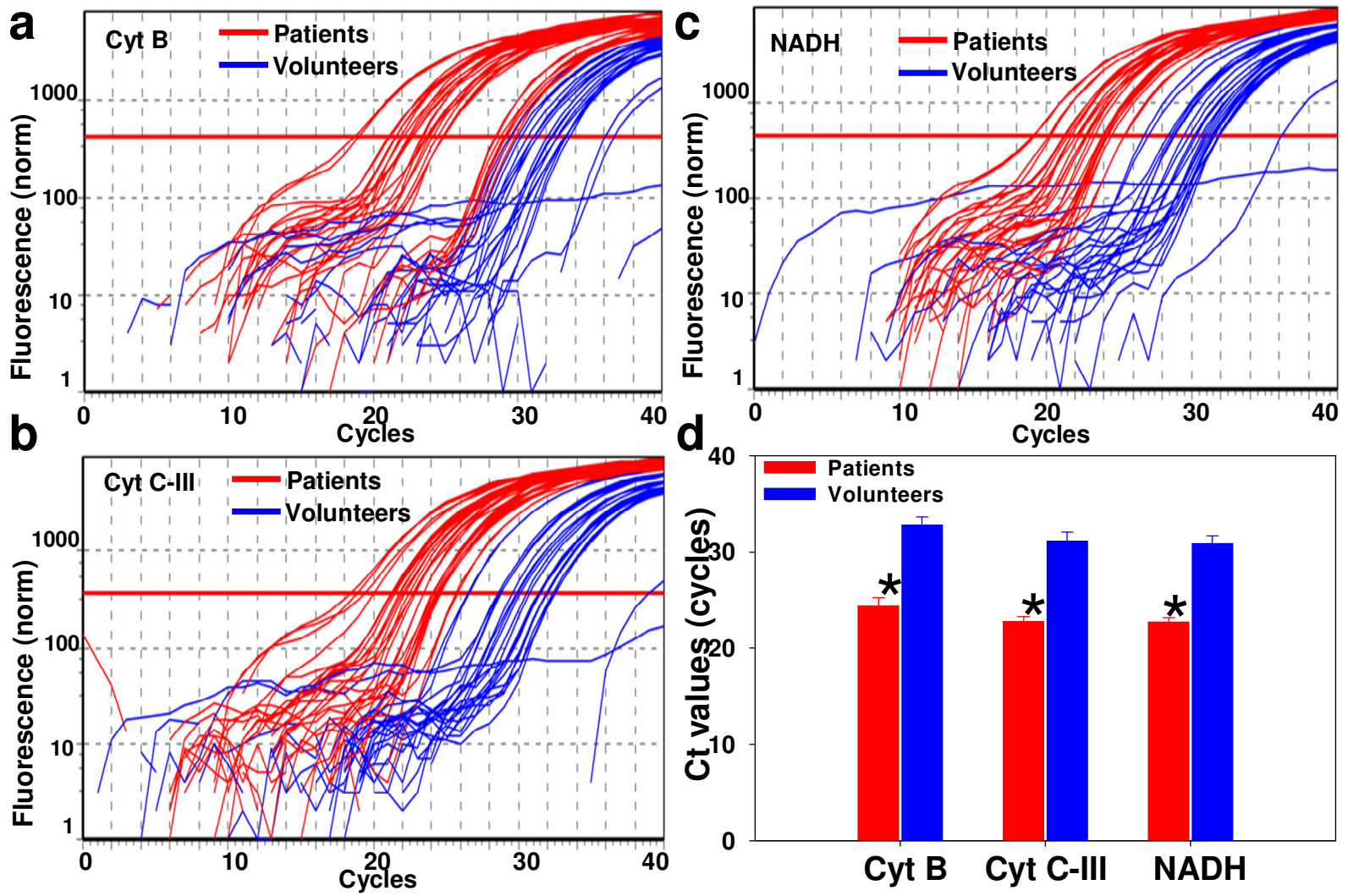
Mt-FP from femur fractures activate PMN



Raoof, Hauser AAST 2008

Does *trauma* cause mitochondrial debris (MTD) to enter the circulation ?

Plasma mtDNA after blunt trauma



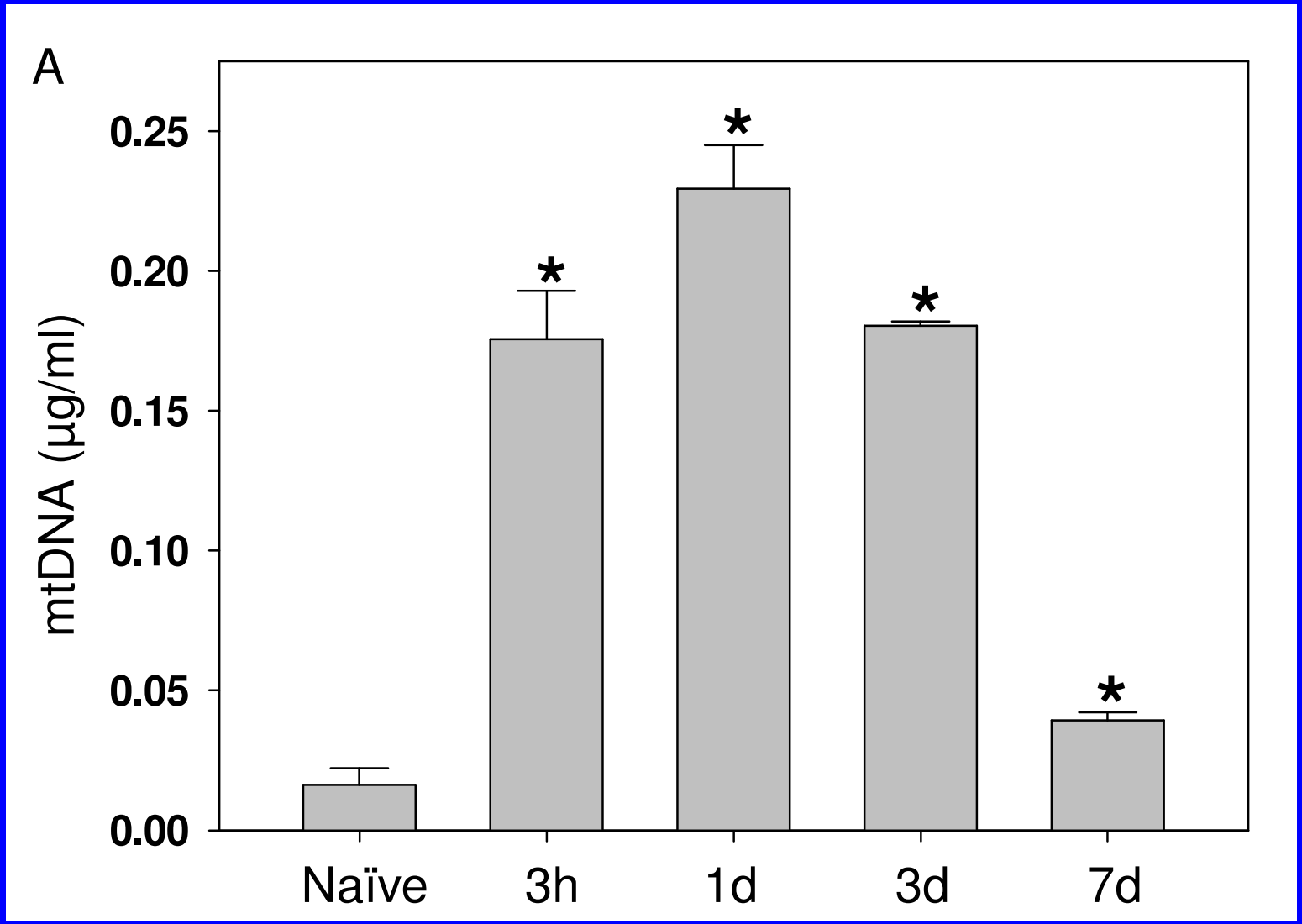
Zhang, Hauser. Nature 2010

Shock and circulating MTD

Shock, ischemia/reperfusion is a direct cause of SIRS …*but* ***how****?*

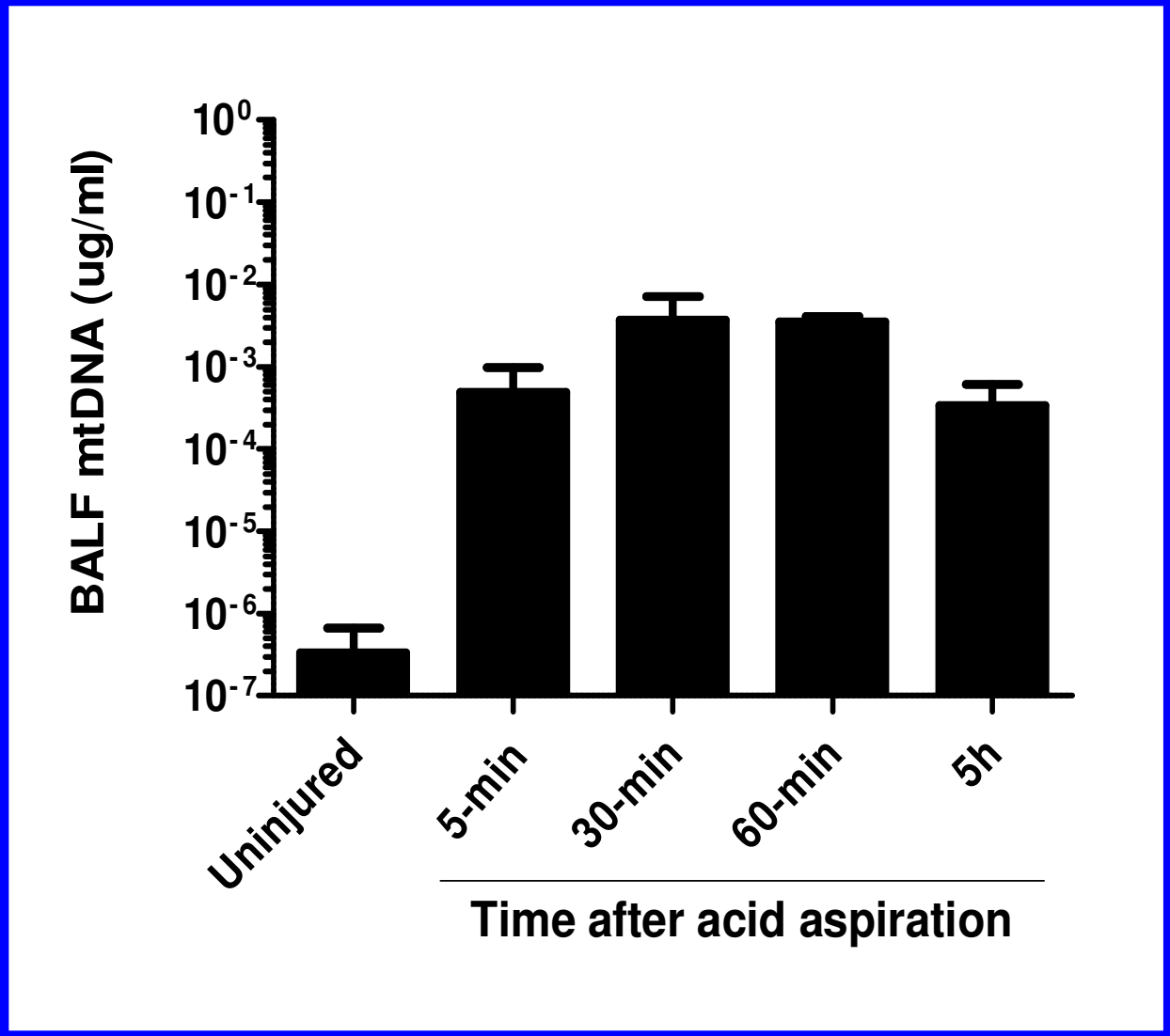
- Gut bacteria / endotoxins (Fine, Deitch) do ***not*** enter the portal vein (Moores)
- Gut lymph is inflammatory (Deitch, Moore) … *but how?*
- Do mitochondrial debris enter the circulation in hemodynamic shock?

Plasma mtDNA in Rat Hemorrhagic Shock



Zhang, Hauser , Shock 2010

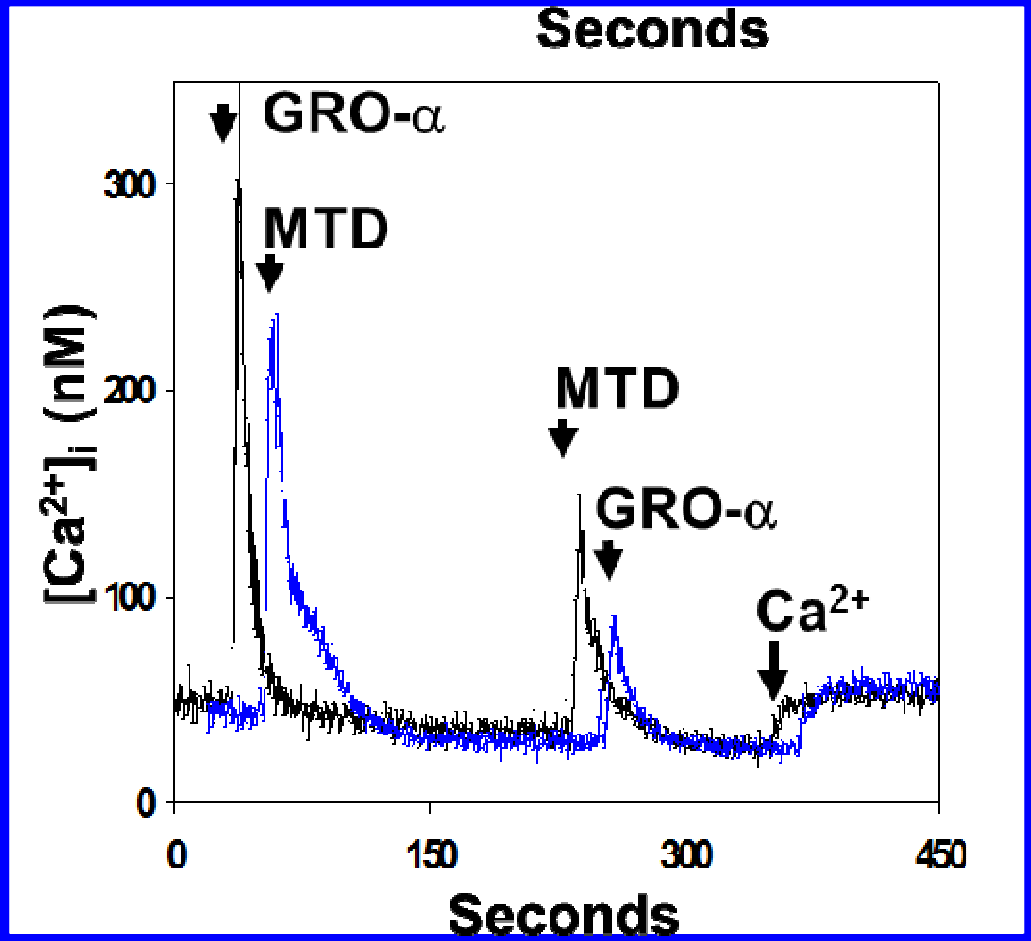
BALF mtDNA after acid aspiration



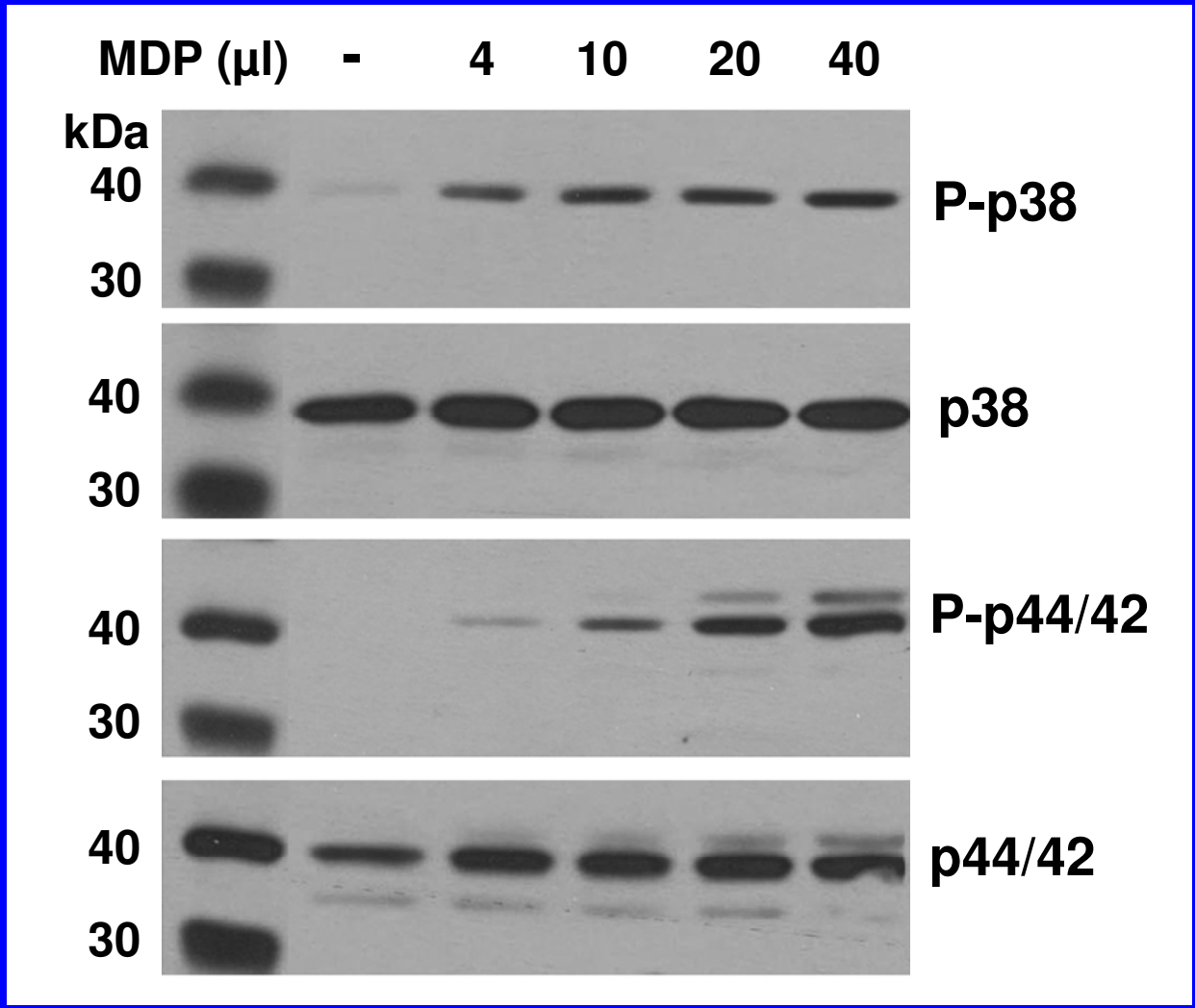
Segal, B Unpublished data

Do mitochondrial DAMPs (MTD) activate inflammatory cell signaling ?

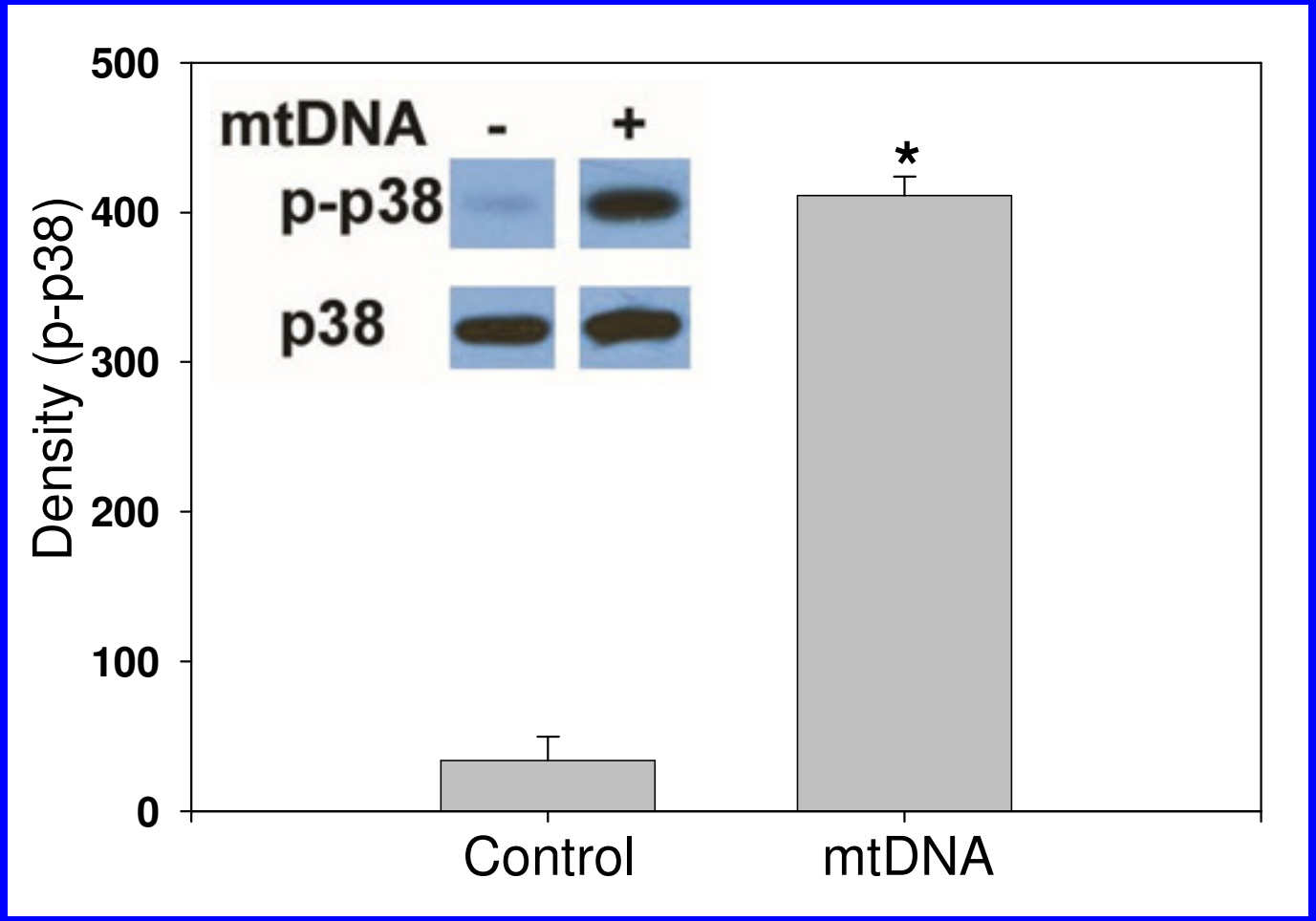
PMN activation by mtFPs suppresses chemokine $[Ca^{2+}]_i$ responses



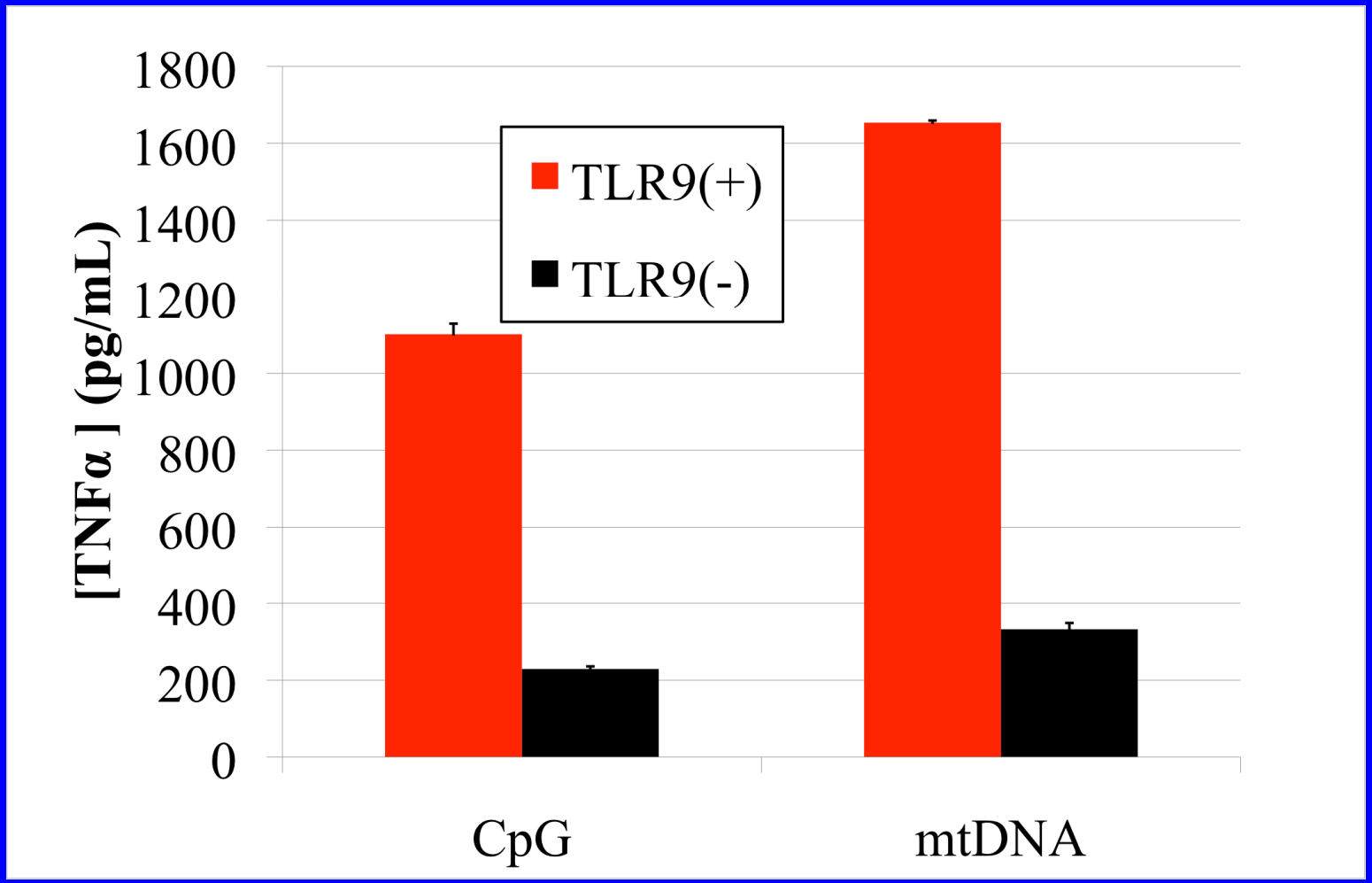
MTD activate PMN MAP-Kinases



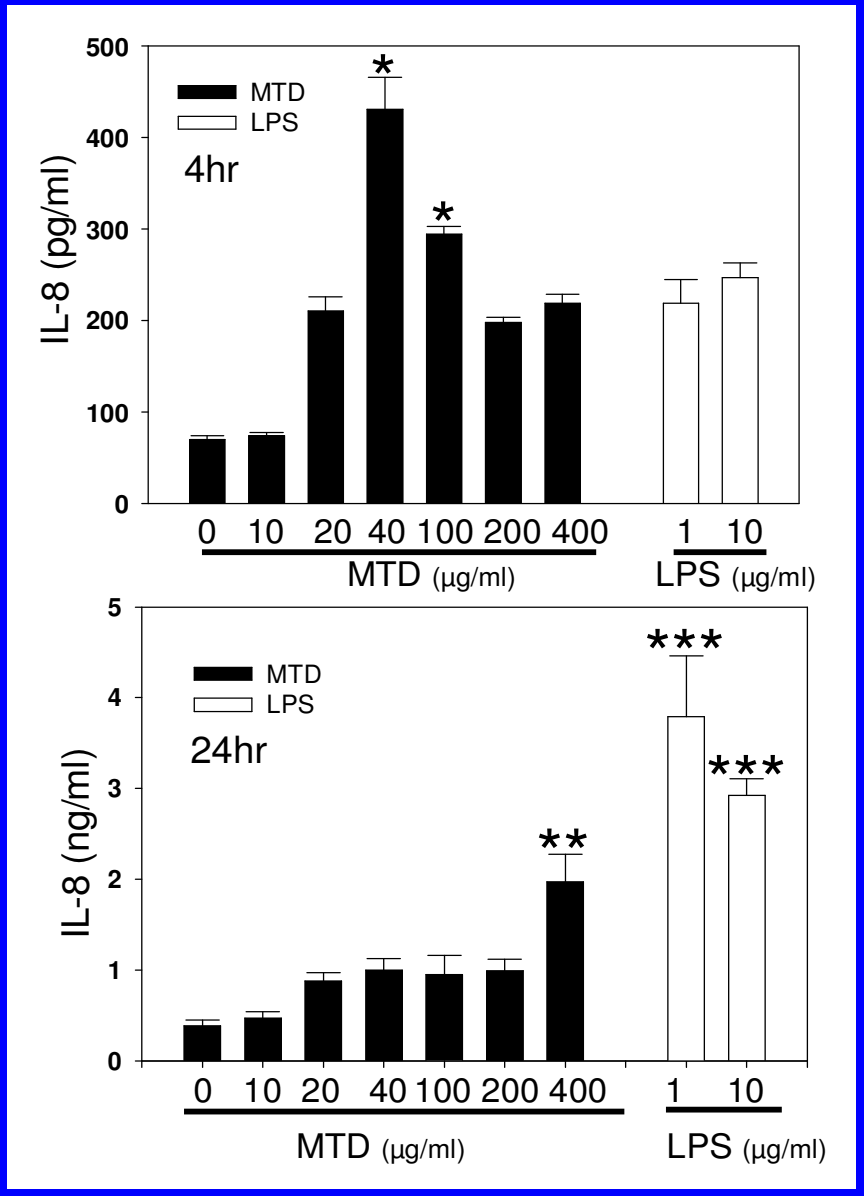
mt-DNA activates p38 MAPK



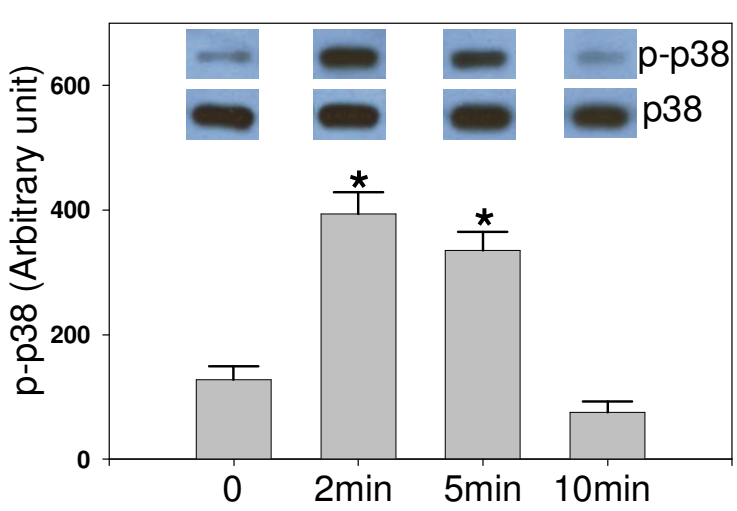
TLR9 knockdown blocks response to mtDNA (RAW macrophages)

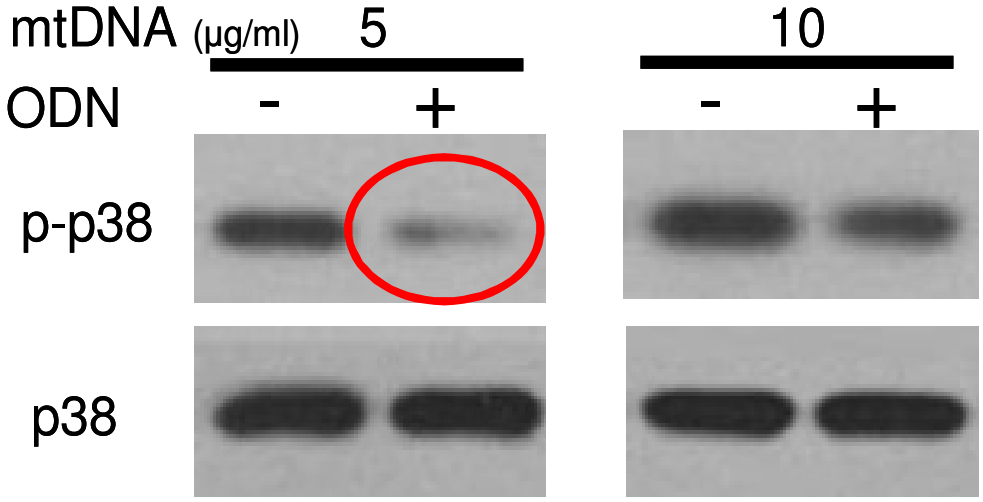


MTD causes PMN IL-8 production



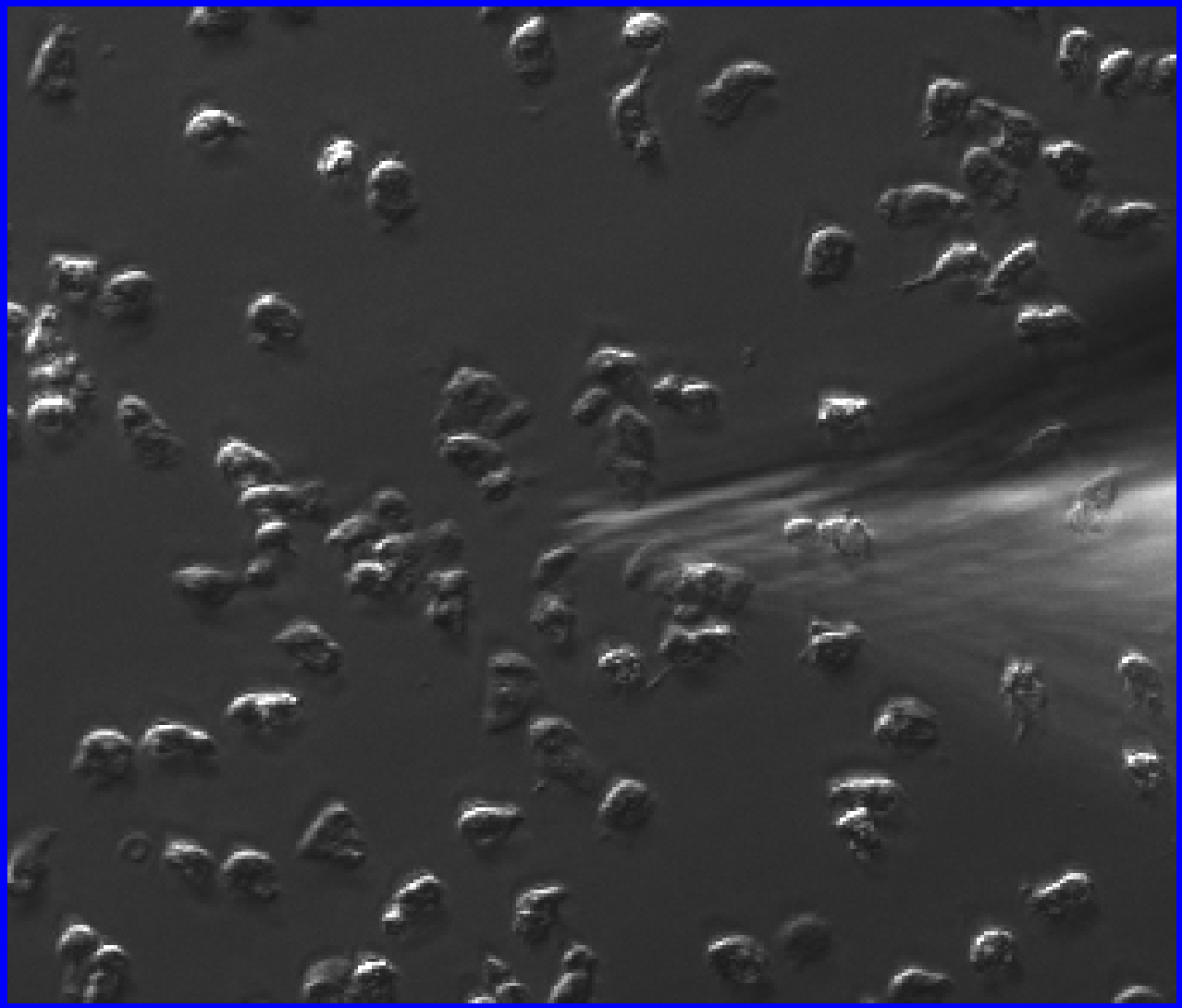
p38 activation by mtDNA





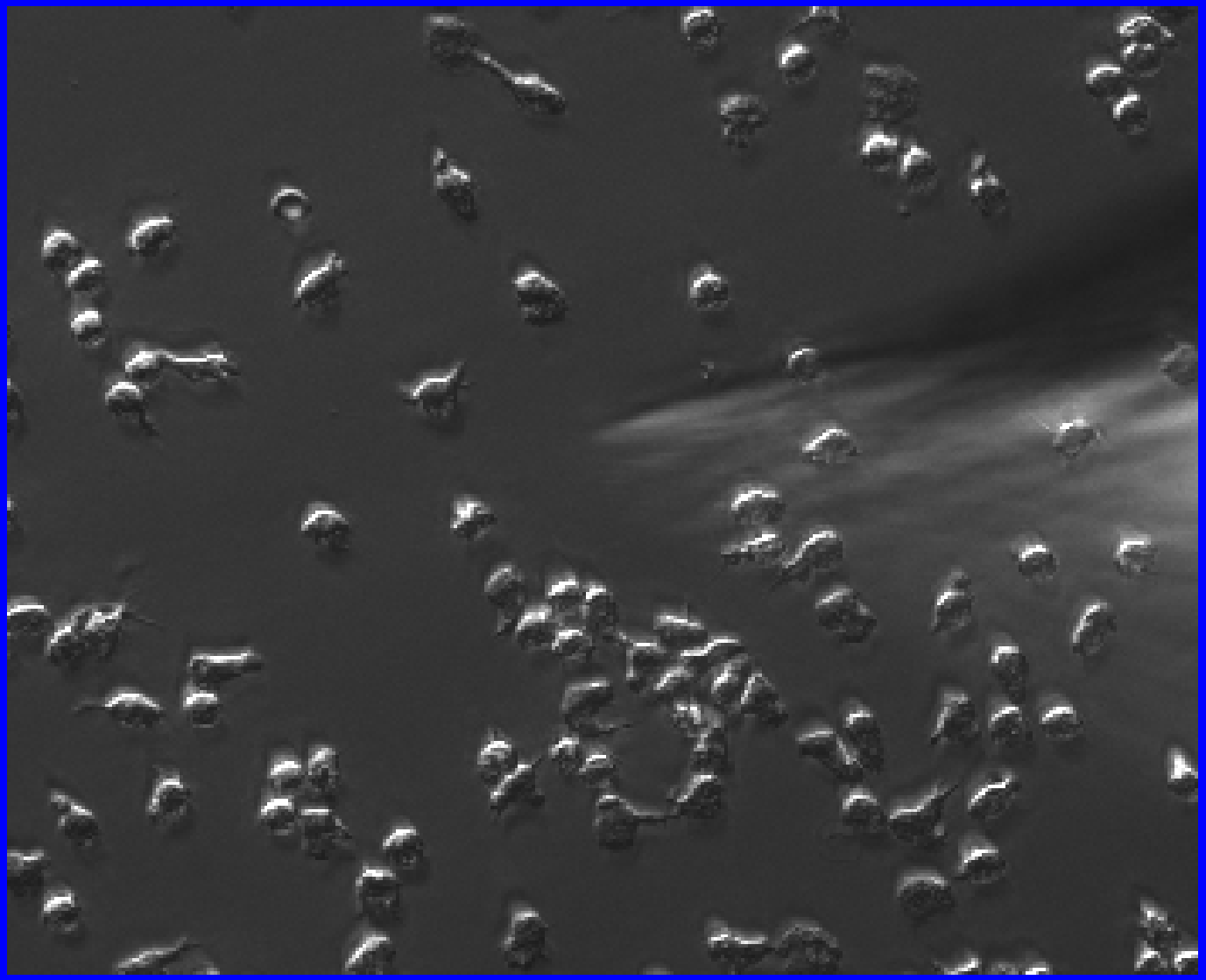
Can be *blocked* by CQ, ODNs

Do MTD activate inflammatory cell phenotypes ?



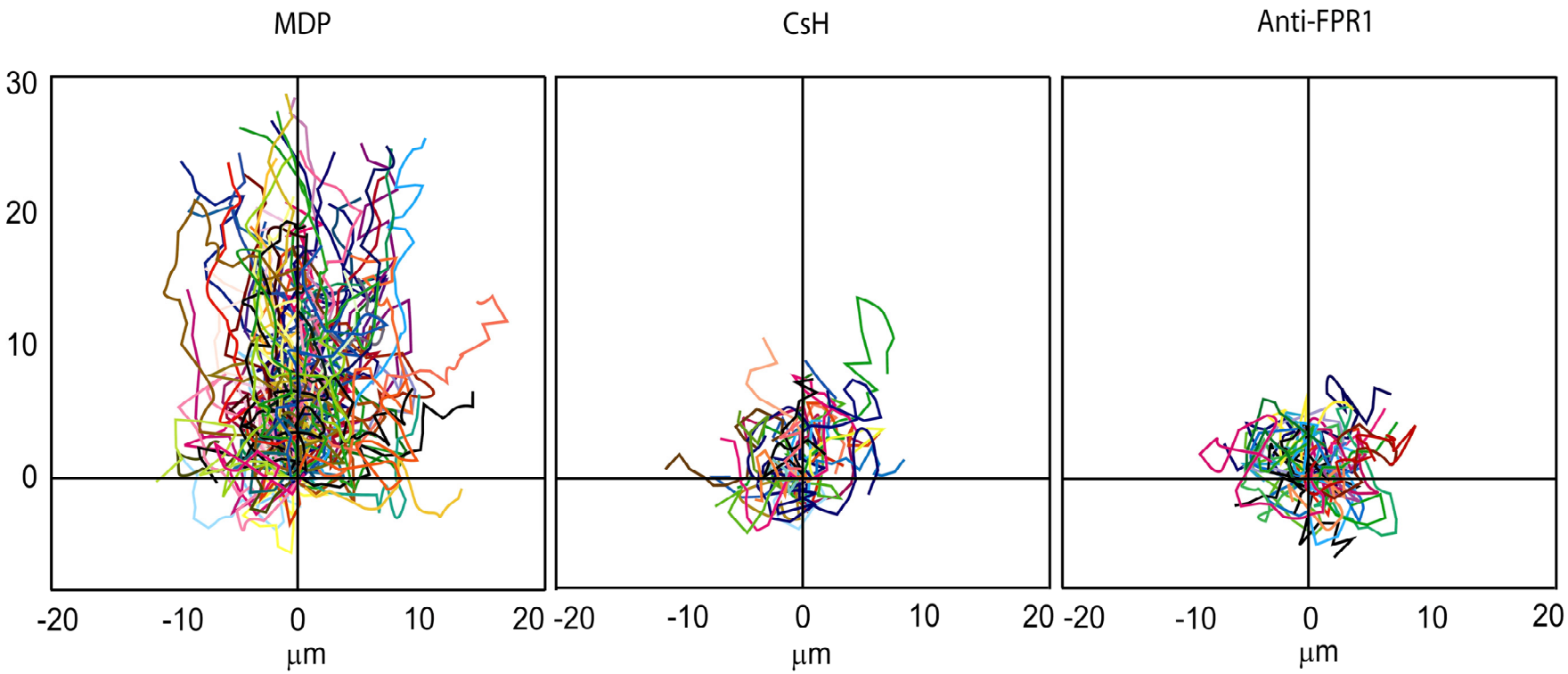
PMN chemotaxis to MTD

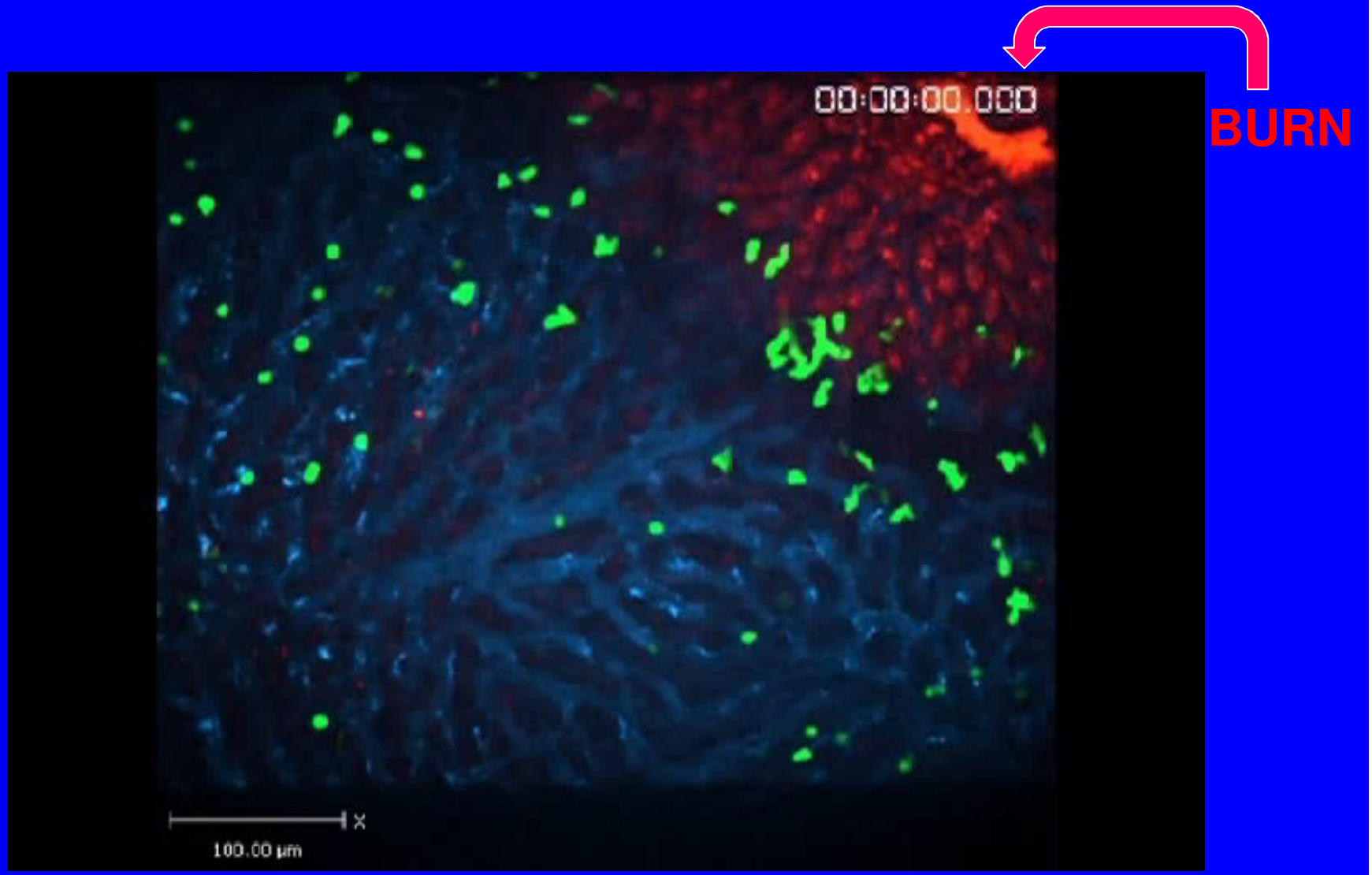
Zhang, Hauser. Nature 2010



With α-FPR1 (or ***CsH***)

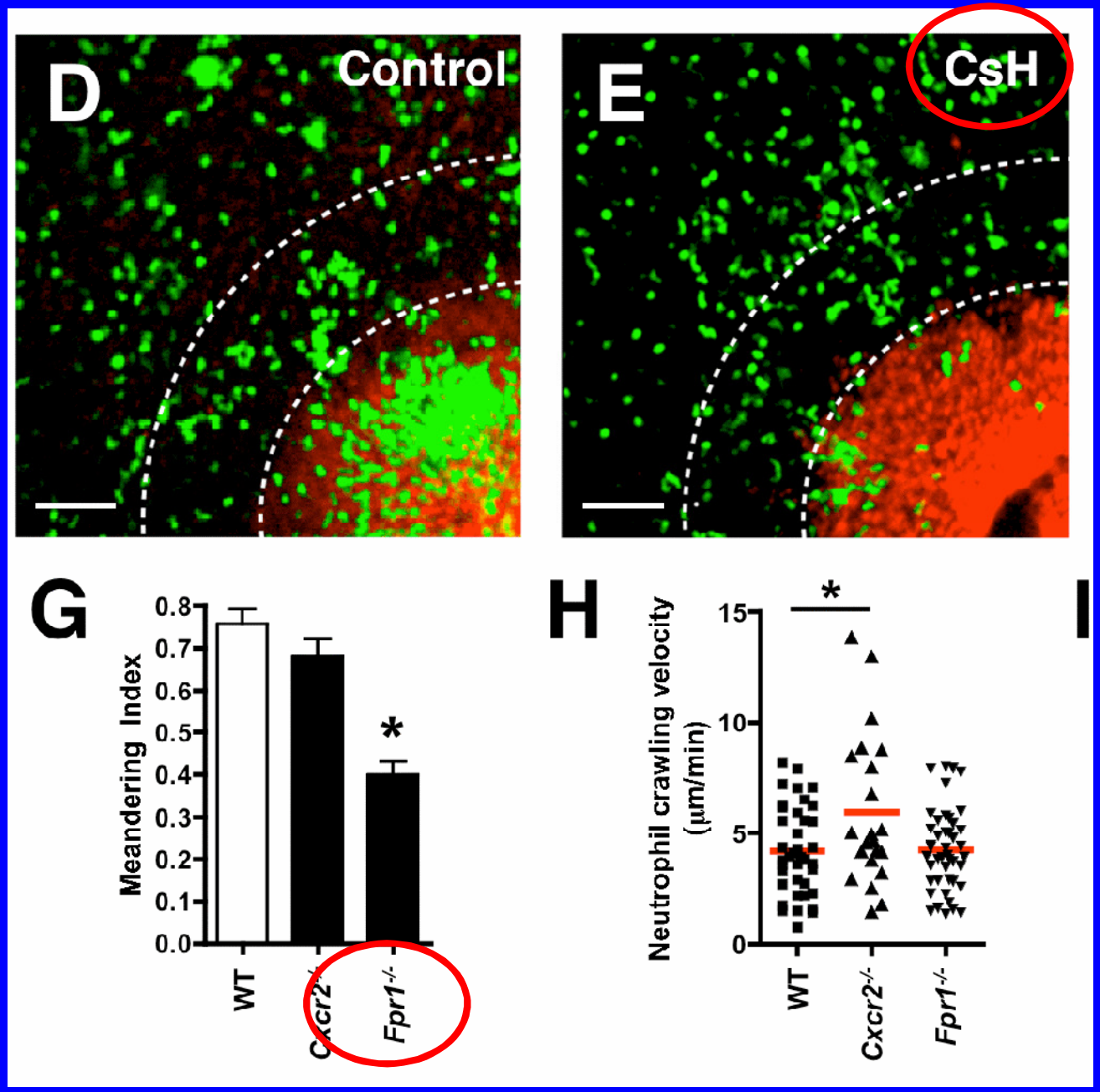
Chemotaxis to mt-FPs





PMN necrotaxis to mtFPs in vivo

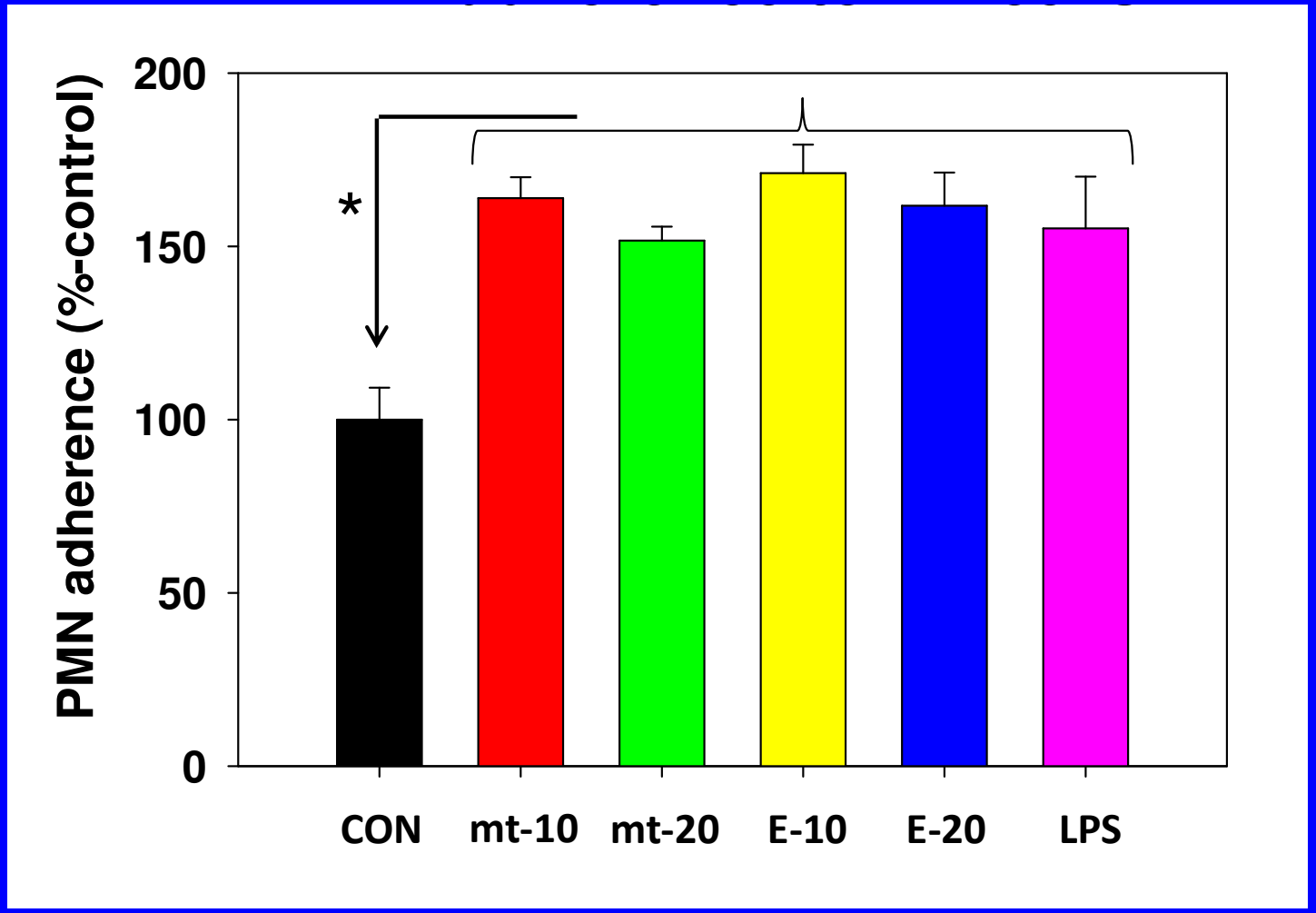
McDonald, Science. 2011



McDonald, Science 2011

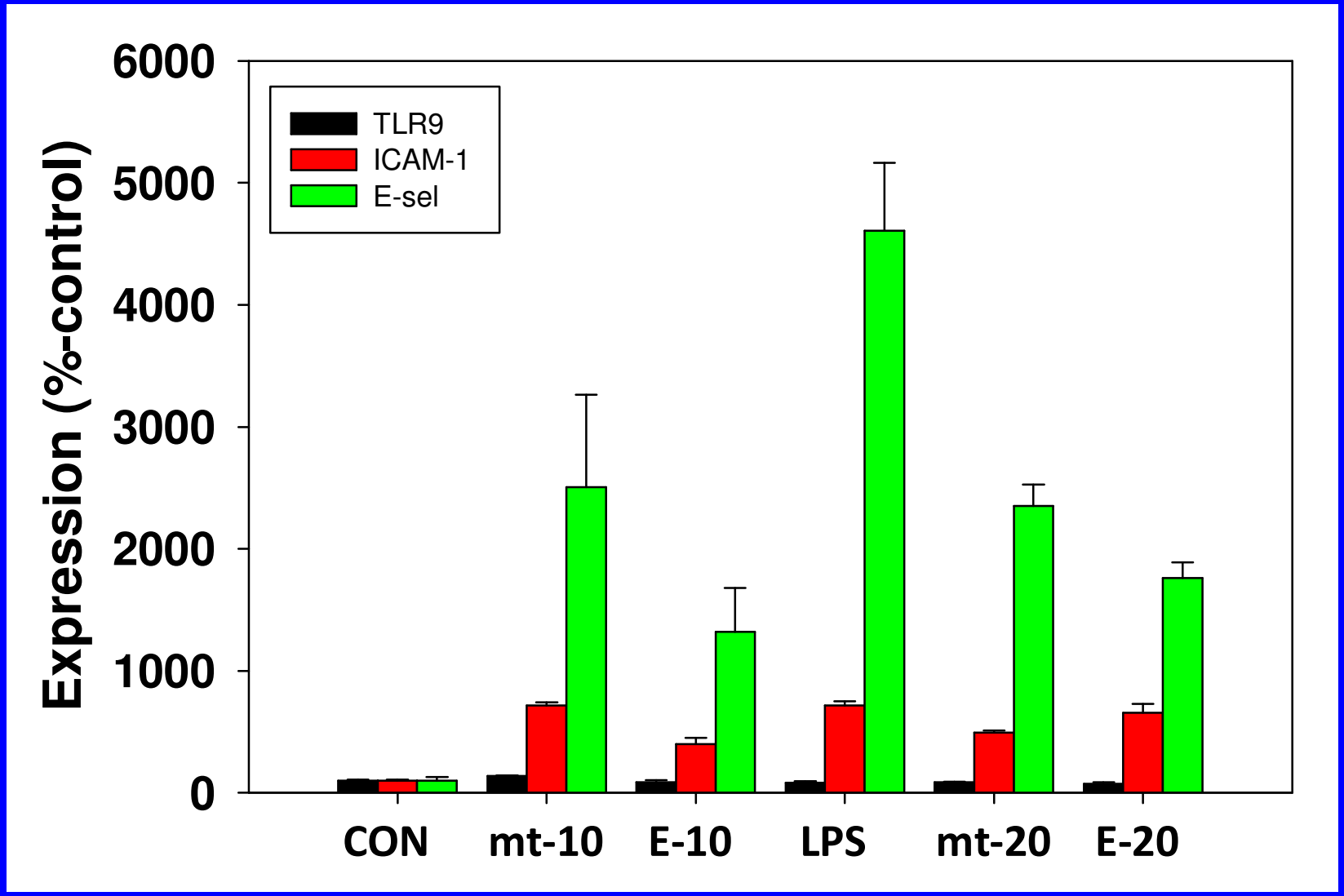
mtDNA activates PMN- EC interactions

PMN adherence to EC



Itagaki, Hauser, unpublished

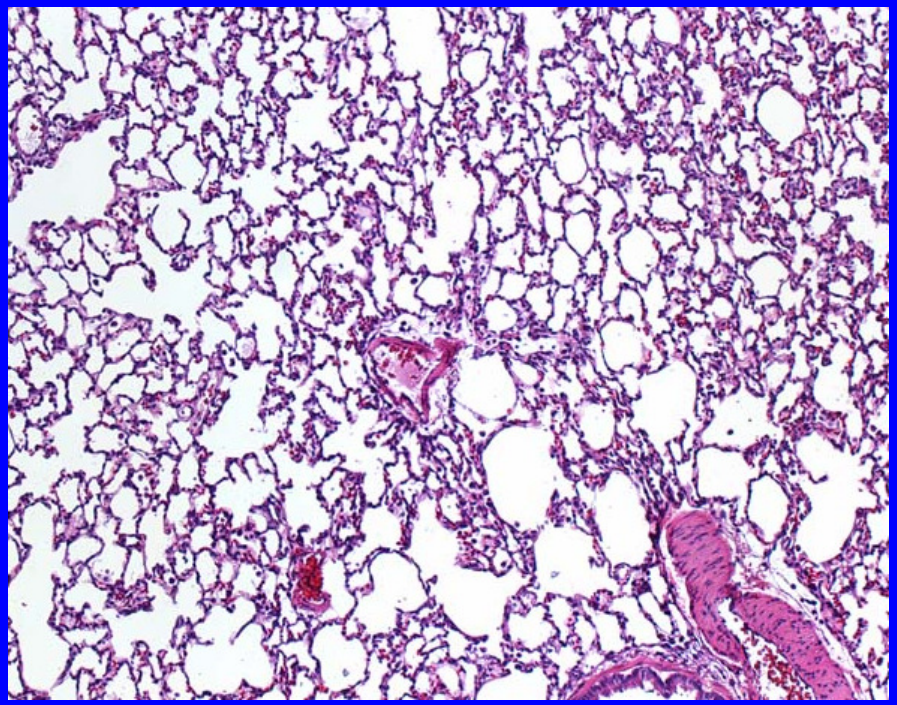
mtDNA activates expression of EC adhesins



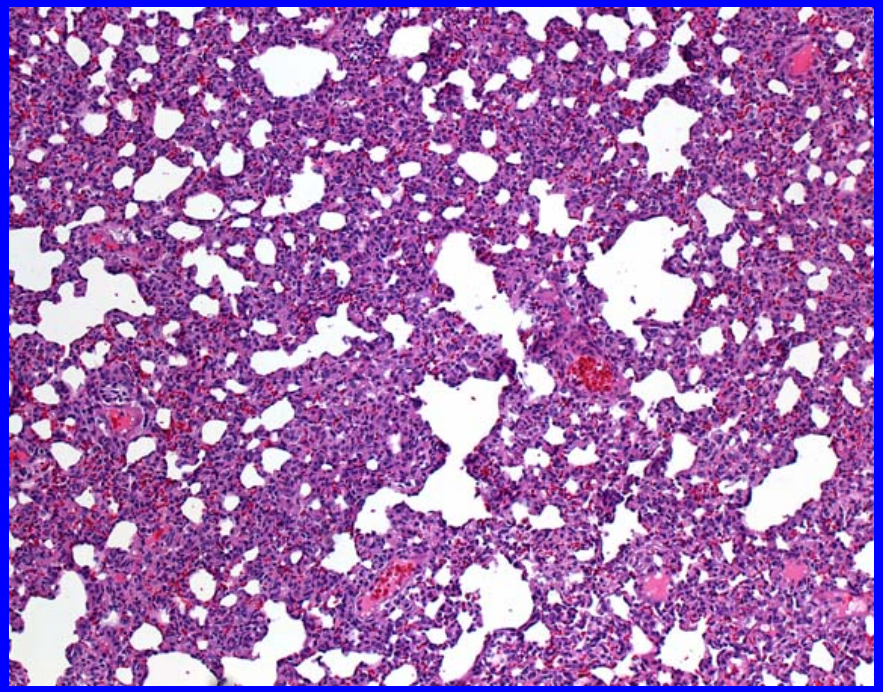
Itagaki, Hauser, unpublished

Circulating MTD causes inflammatory organ injury

MTD induced ALI



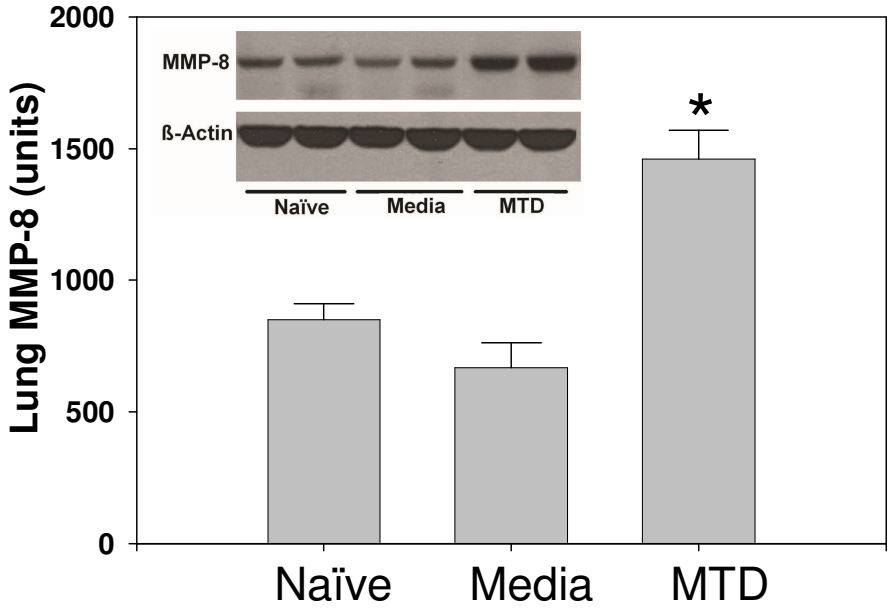
Sham

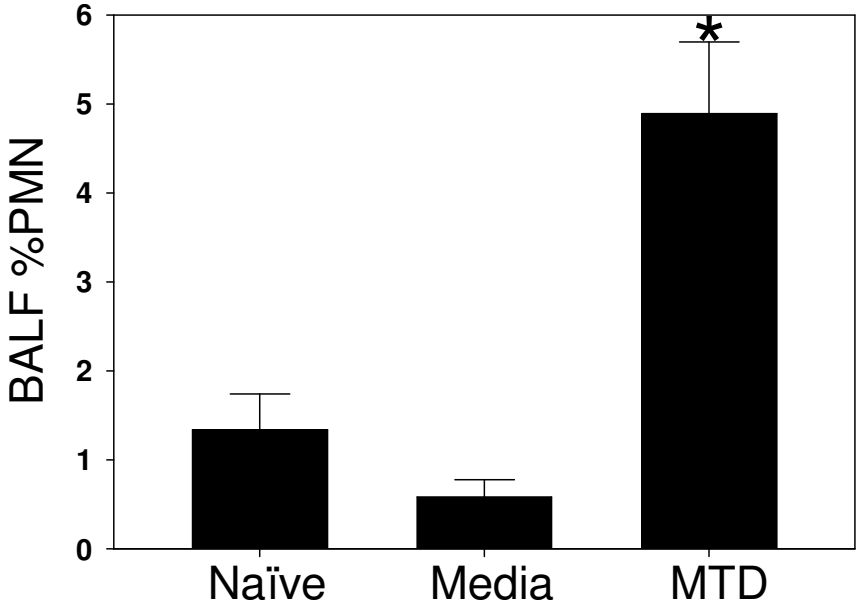


i.v. mitochondria
*(= **5% liver injury**)* at 6h

Zhang, Hauser, Nature 2010

MTD → PMN attack on lung

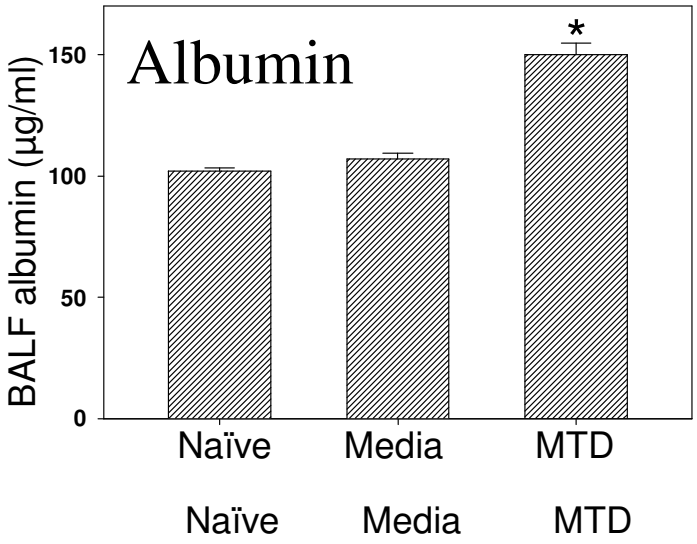


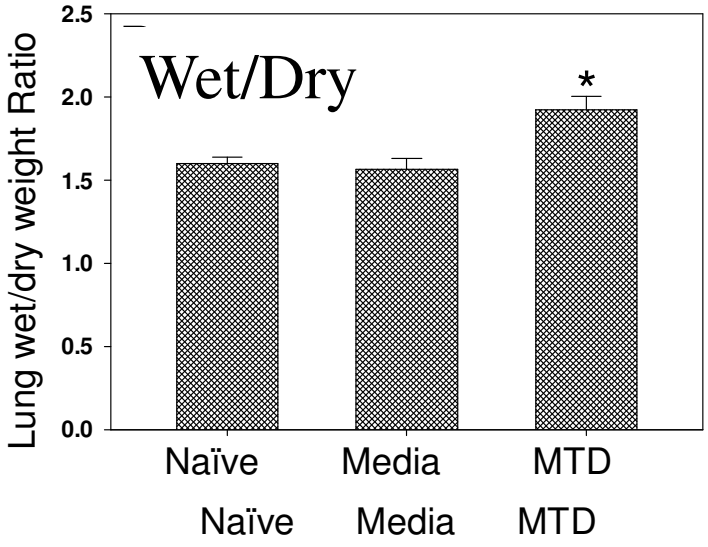


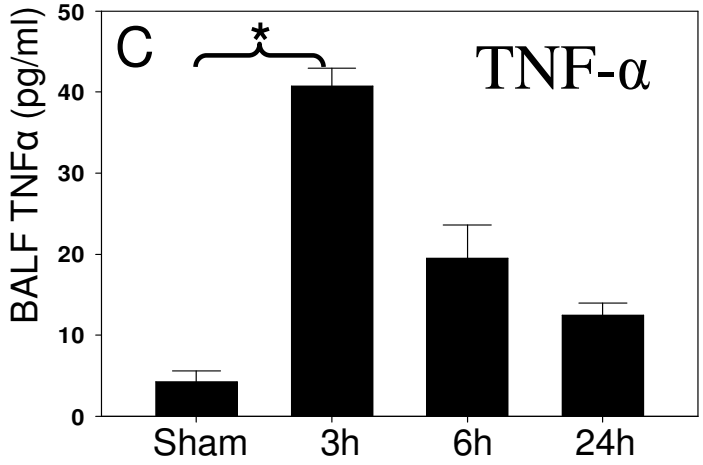
MMP-8 in lung

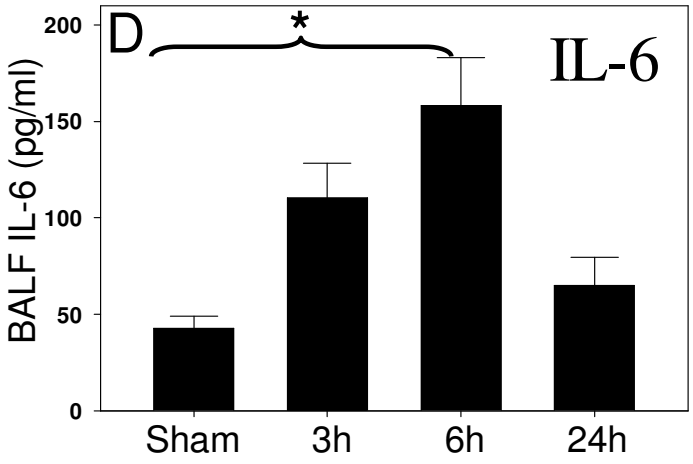
PMN in BALF

MTD activates lung injury

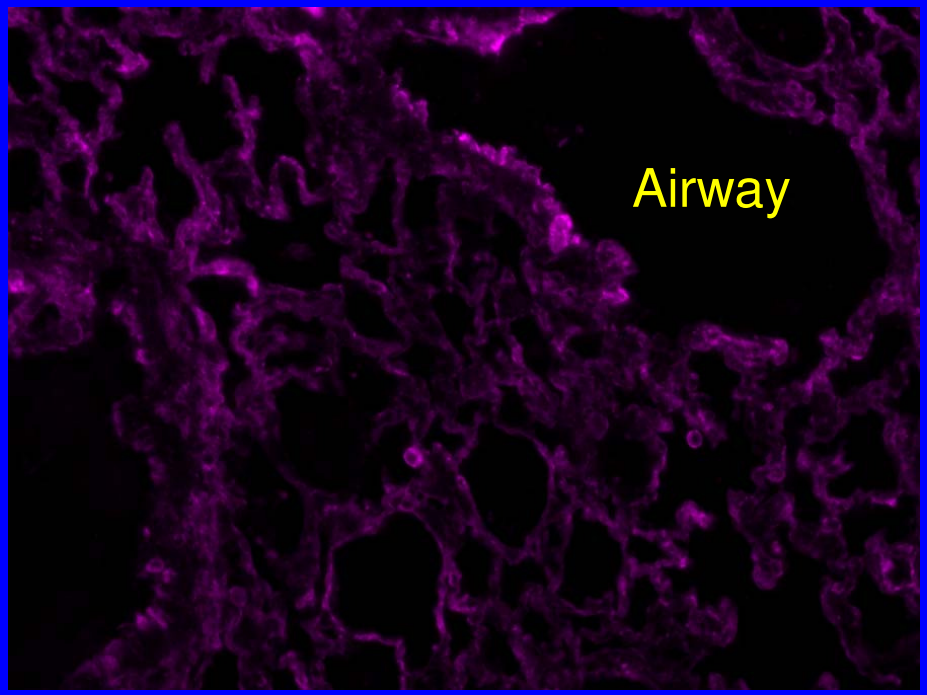


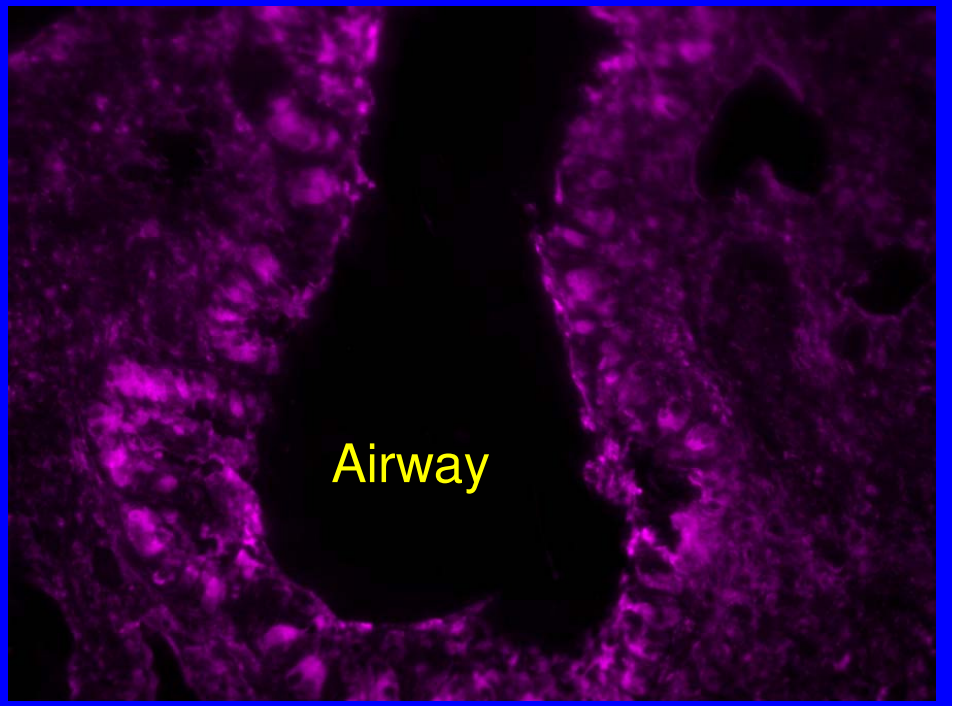






Oxidant lung injury



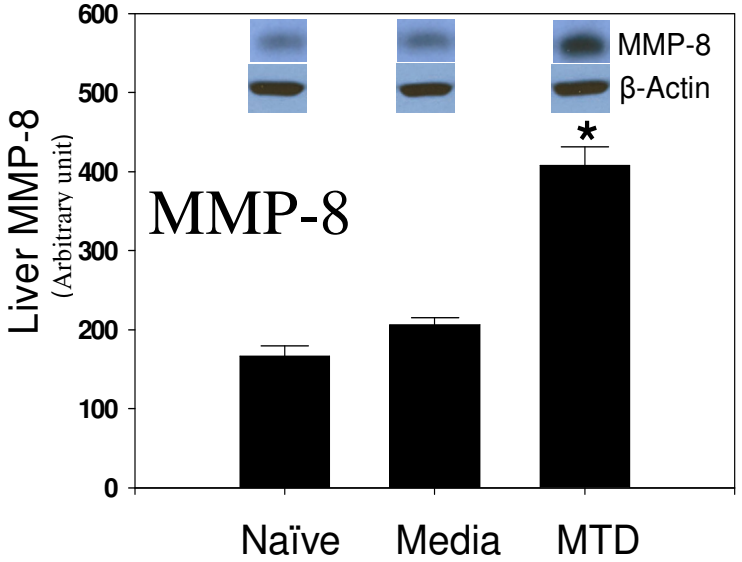


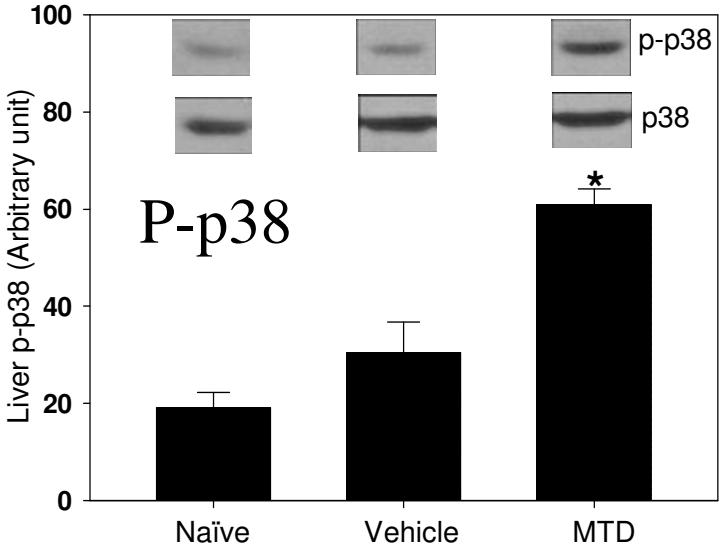
Media

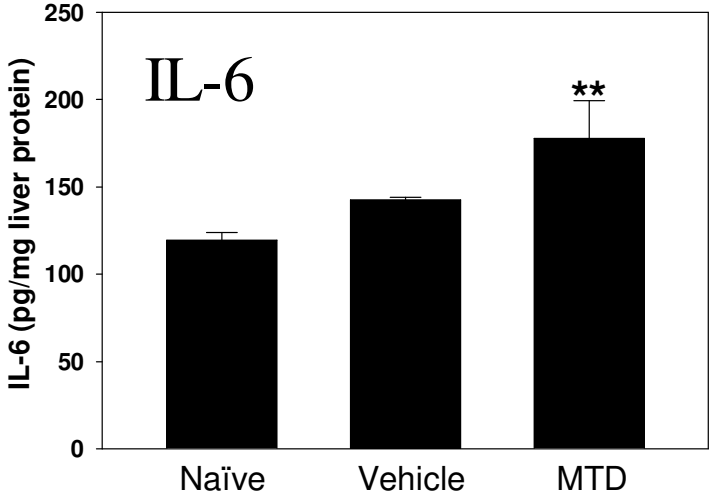
MTD

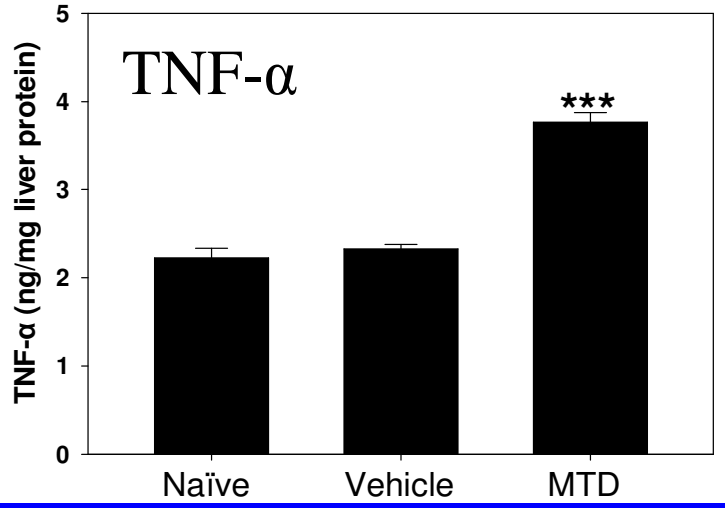
4-HNE stains

Hepatic injury

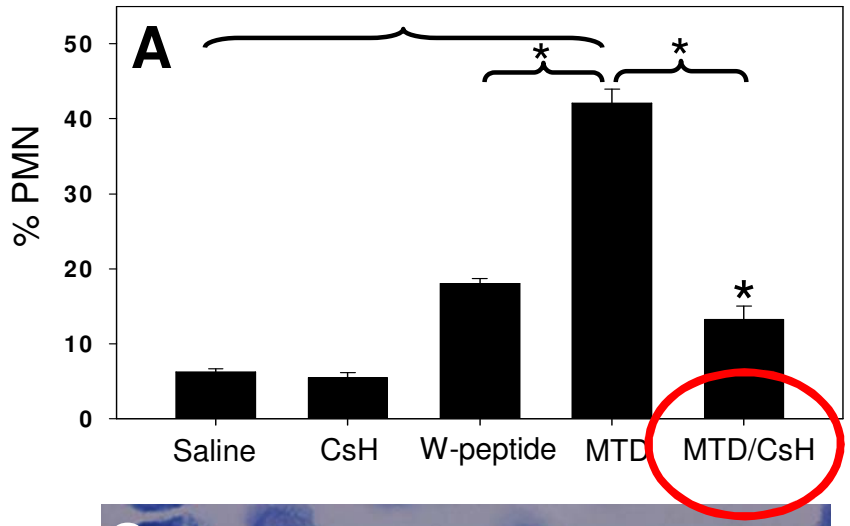


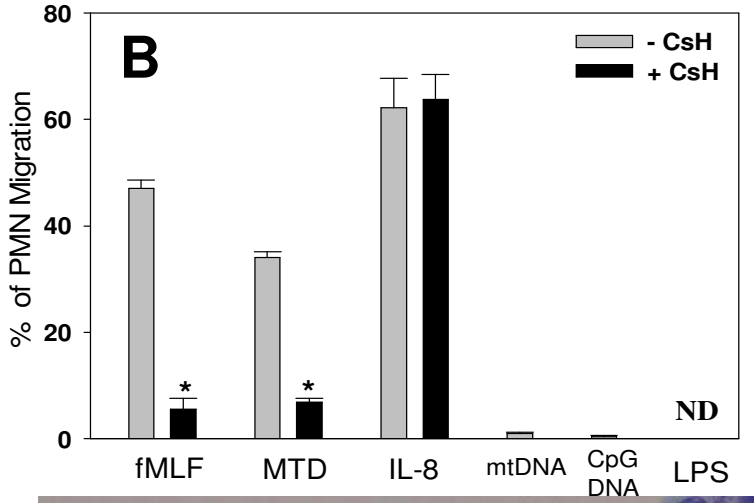


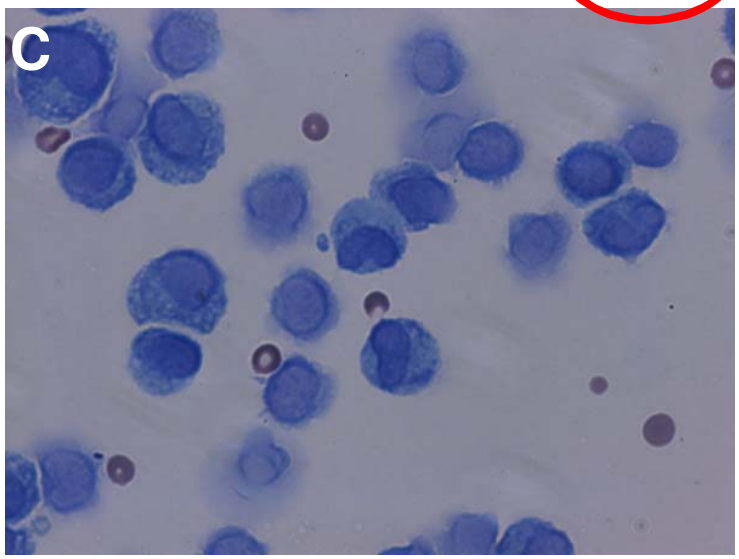


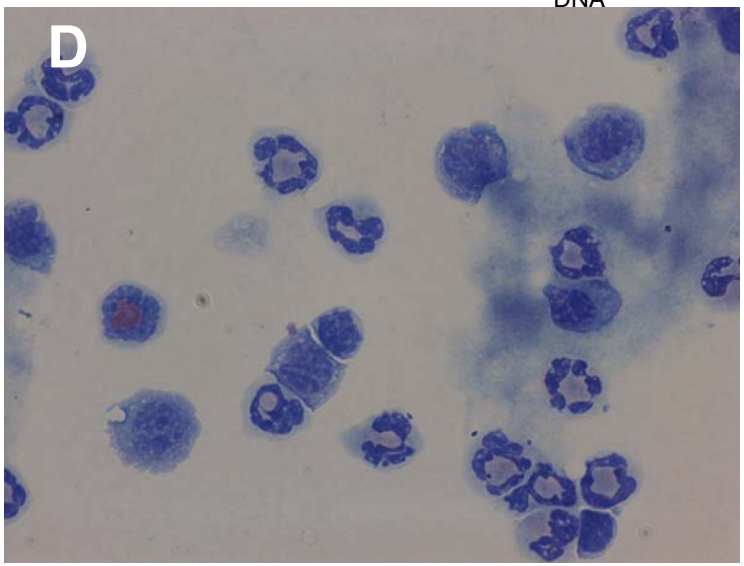


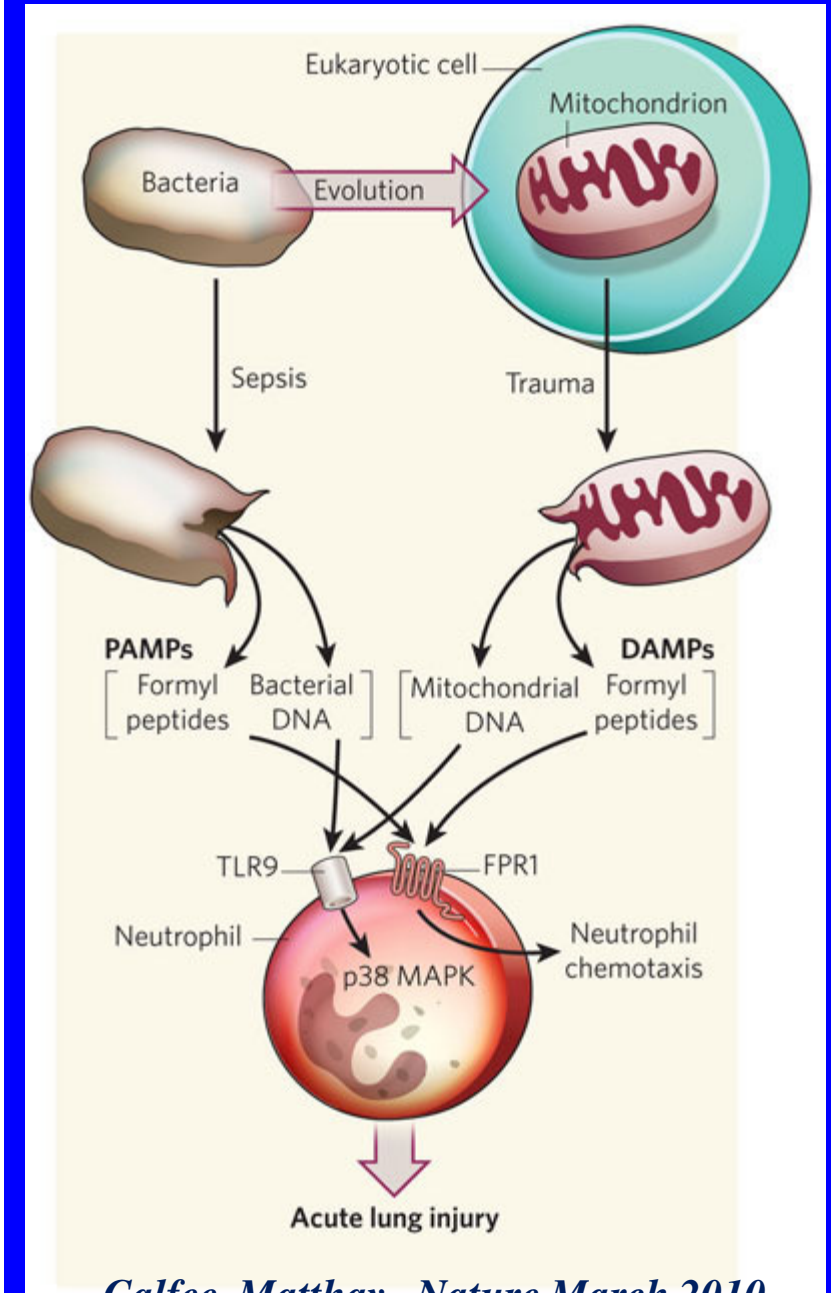
Peritonitis







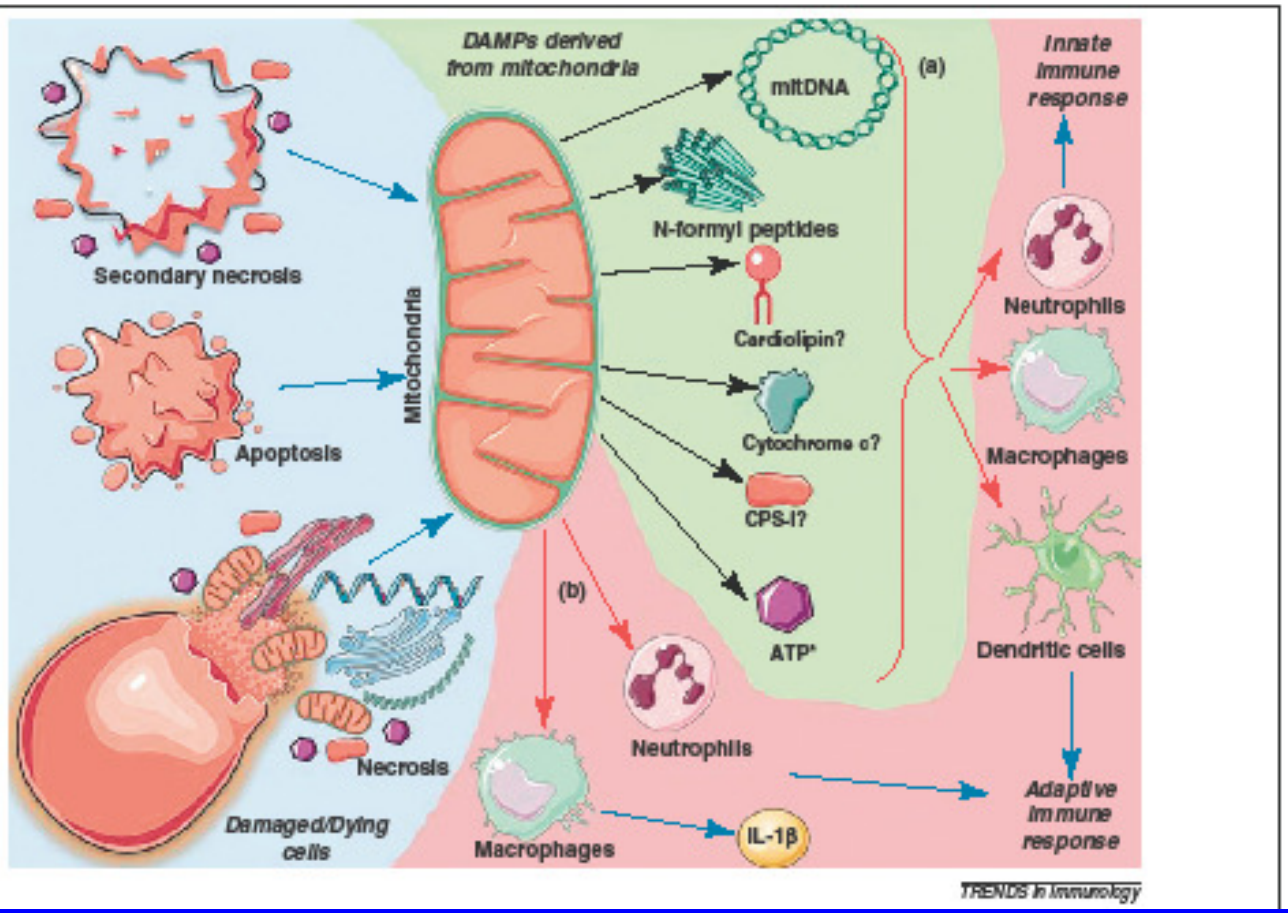




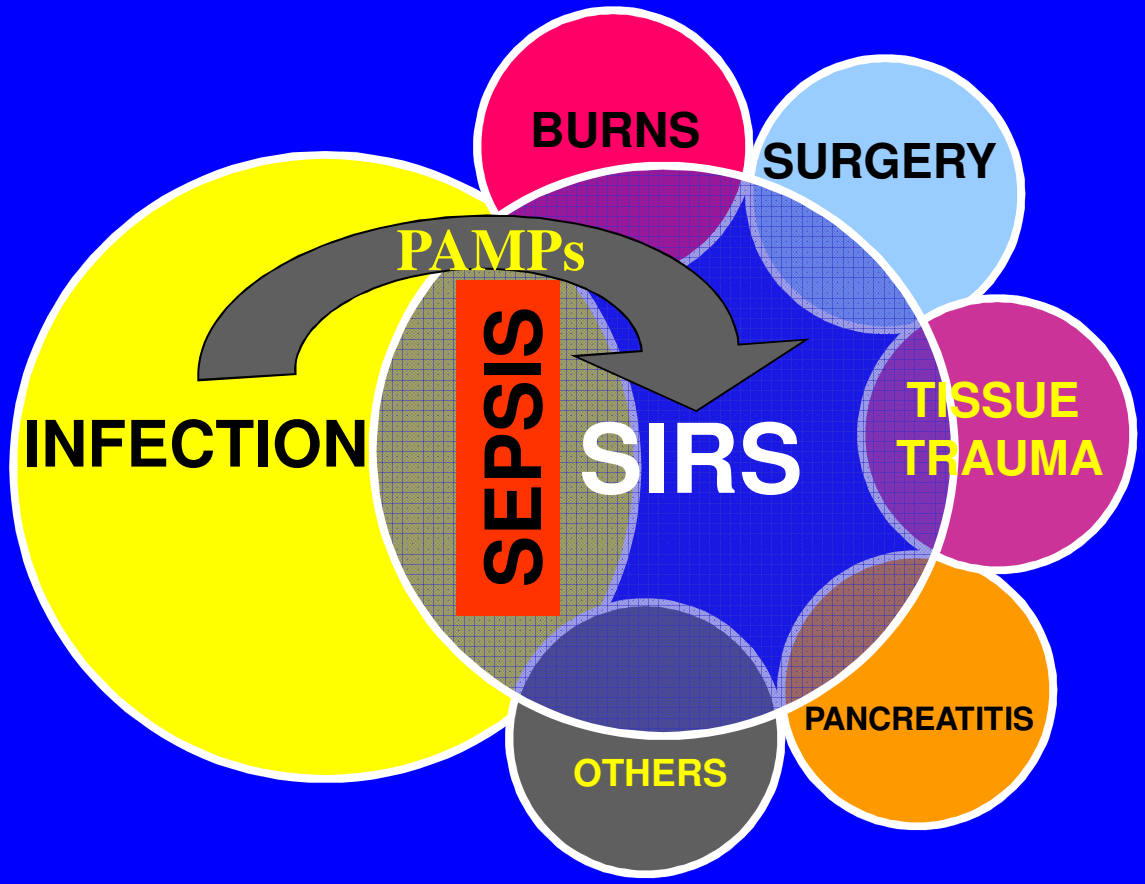
Calfee, Matthay, Nature March 2010

Evolutionary conservation ***of molecular patterns in bacteria and mitochondria contribute to the similarity of sepsis and SIRS***

Many <u>other</u> mitochondrial and cellular DAMPs waiting to be discovered

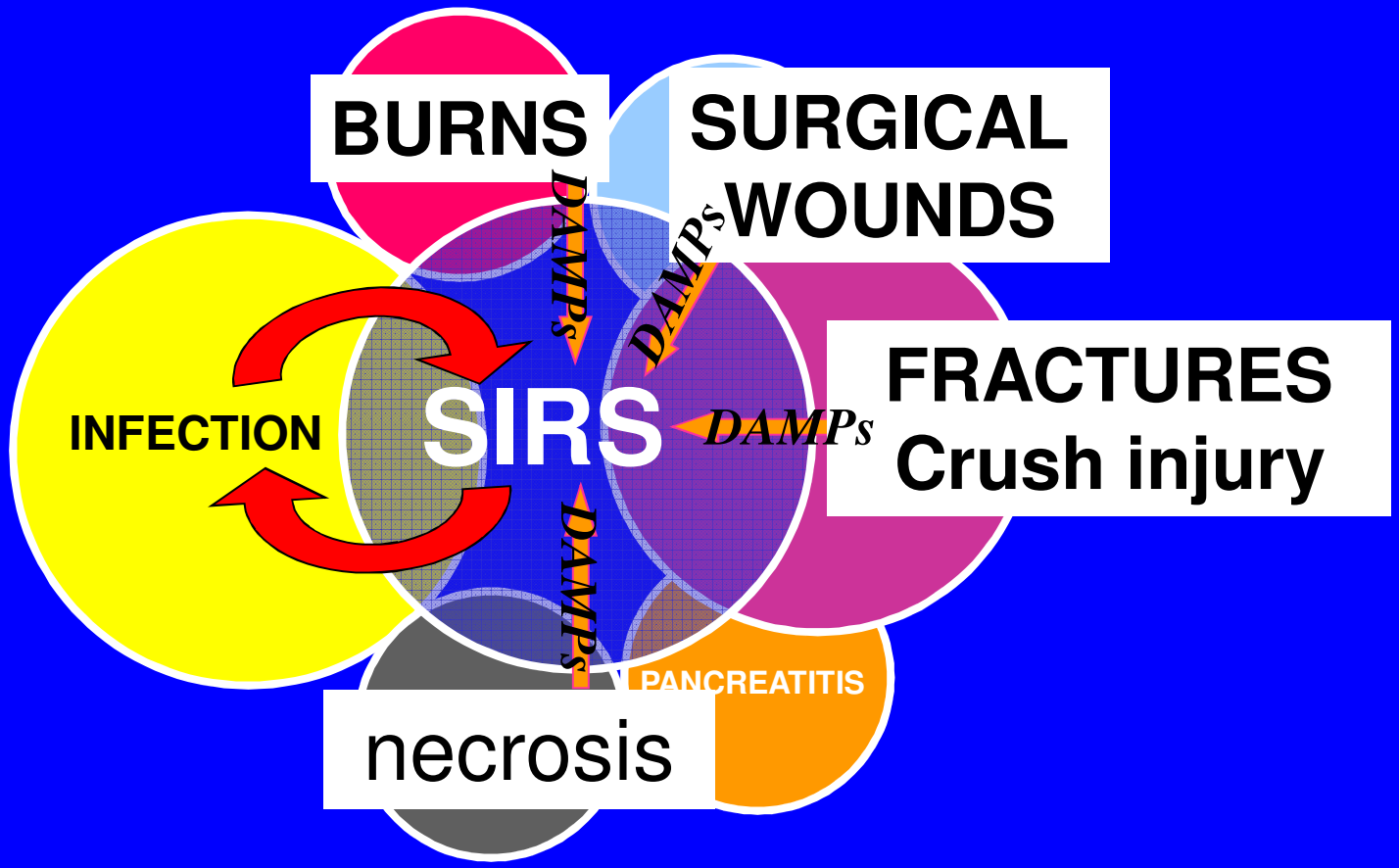


What is ‘septic’ SIRS?



PAMPs from infection cause SIRS

What is traumatic SIRS?



Sepsis ***perpetuates*** SIRS →MOF→death

Implications

<u>Profoundly</u> alters our understanding of:

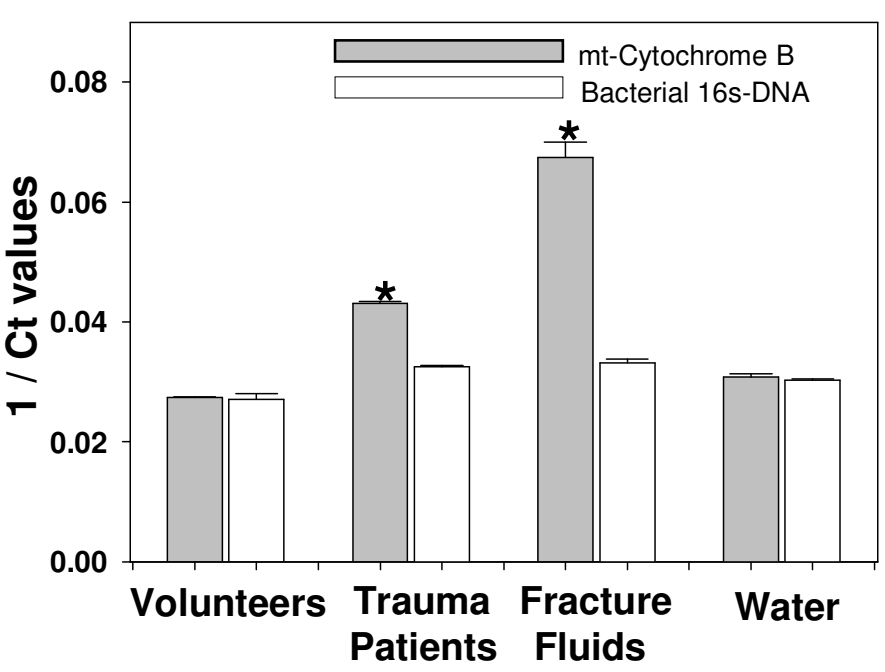
- *SIRS and MOF* after trauma
  - Lung, renal, cardiac, CNS, hepatic, metabolic
  - Fractures, crush injury, ischemia
- *SIRS* after surgery
  - Clinical febrile responses
  - Open vs 'minimally invasive' surgery
  - "Atelectasis"
- *Tumor surgery, vascular surgery*
  - Tumor lysis syndromes, febrile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

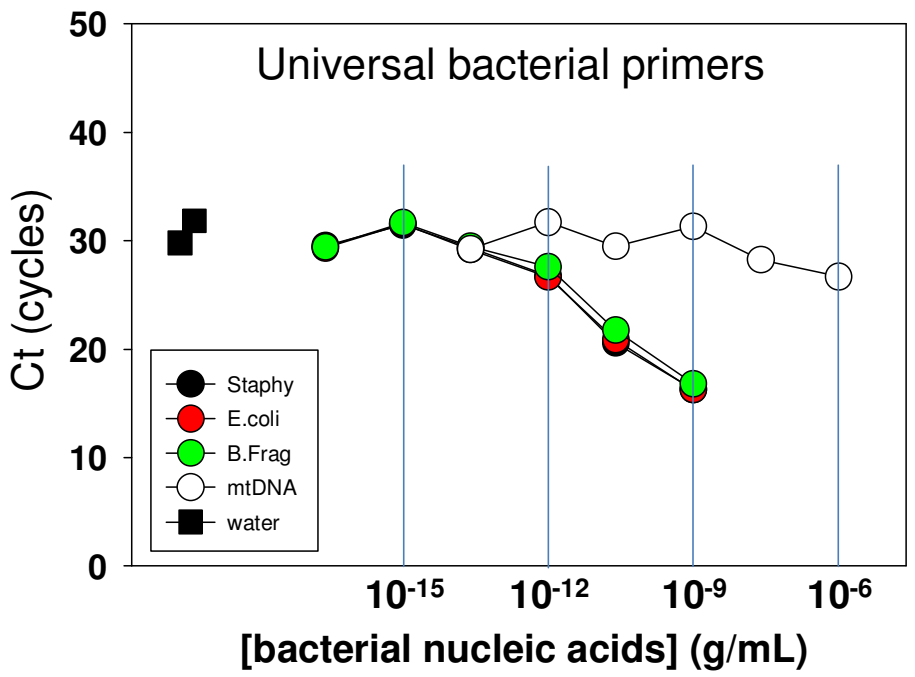
Applications

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

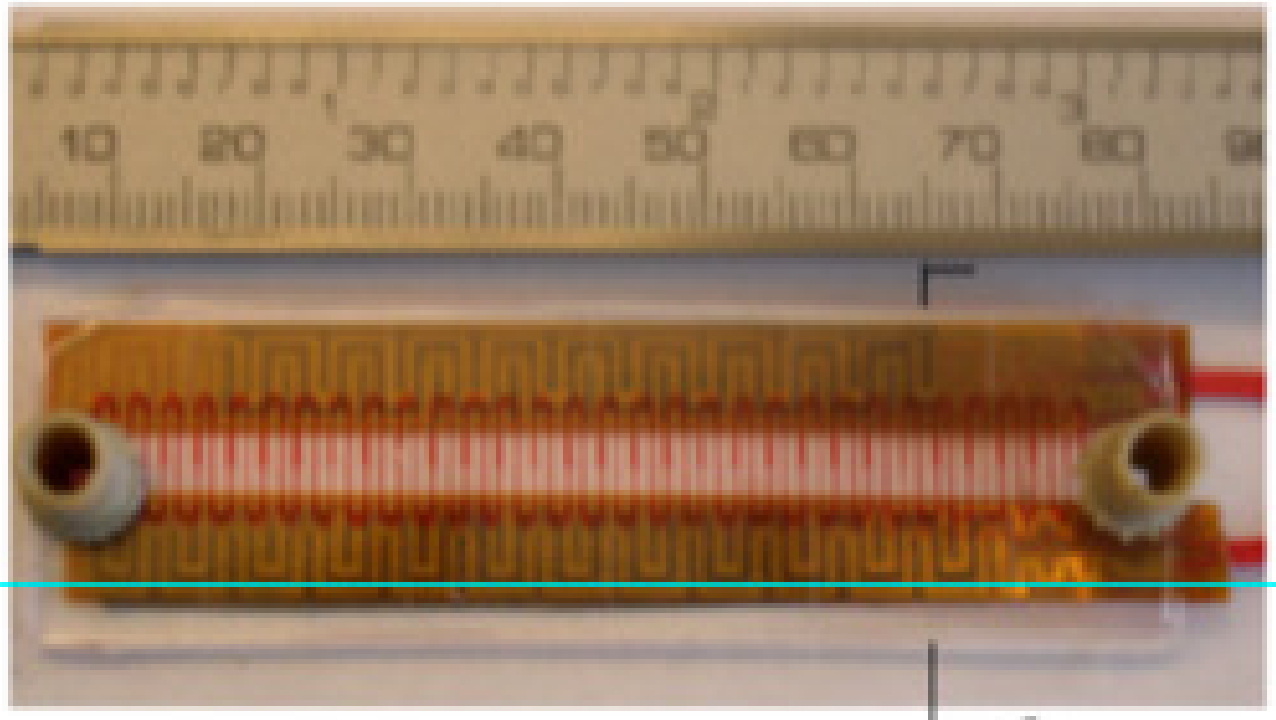




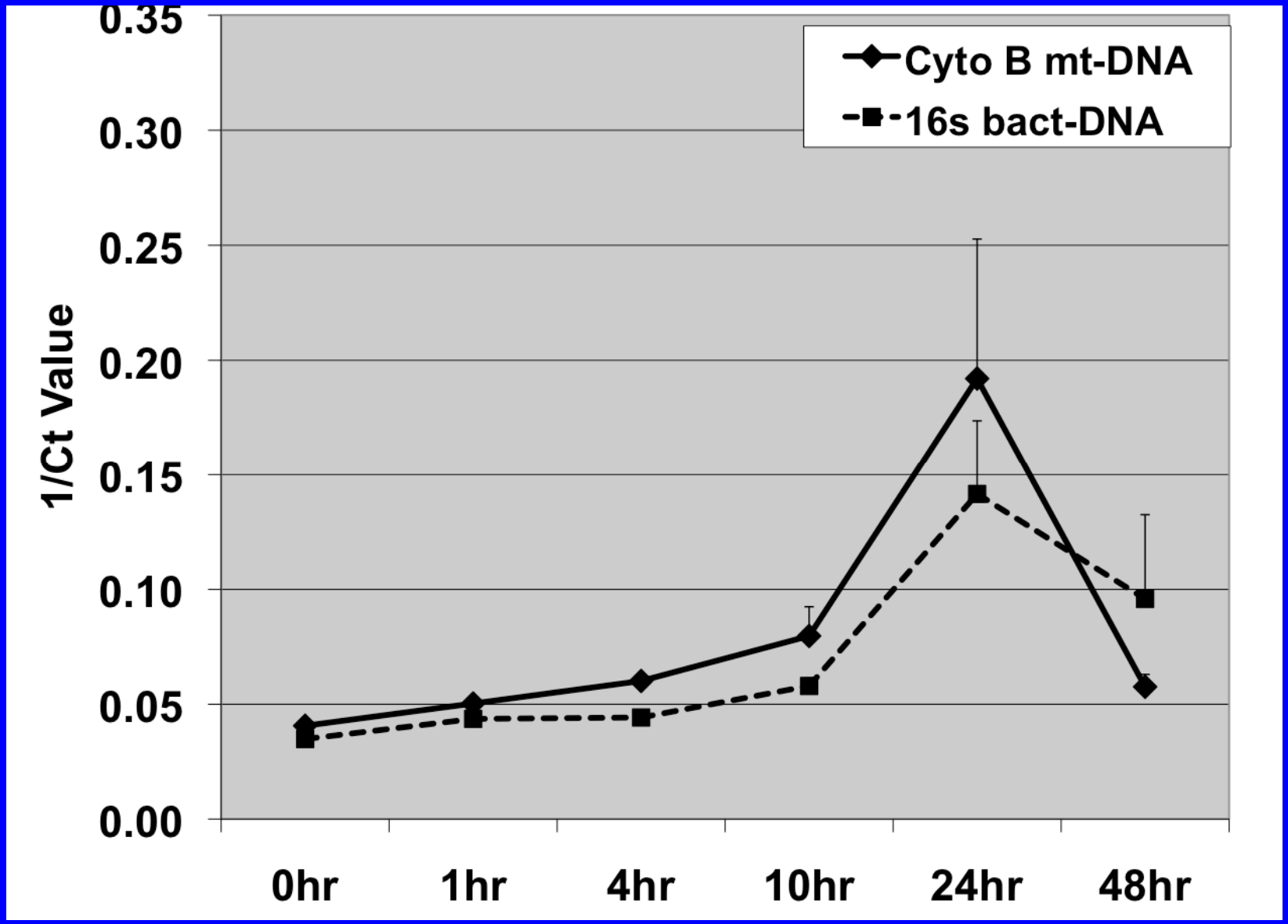
‘Chip-based’ PCRs

a)

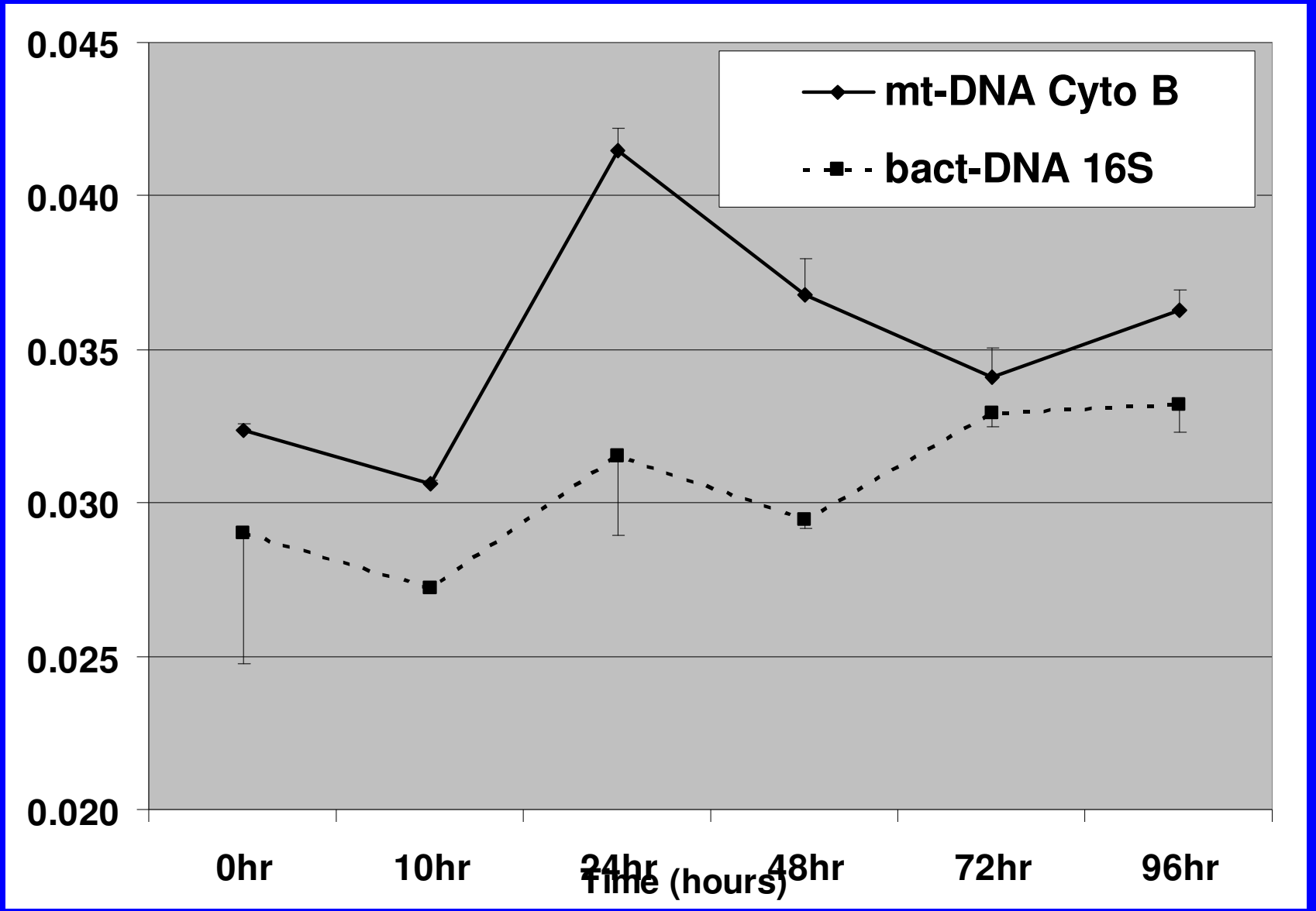
b)



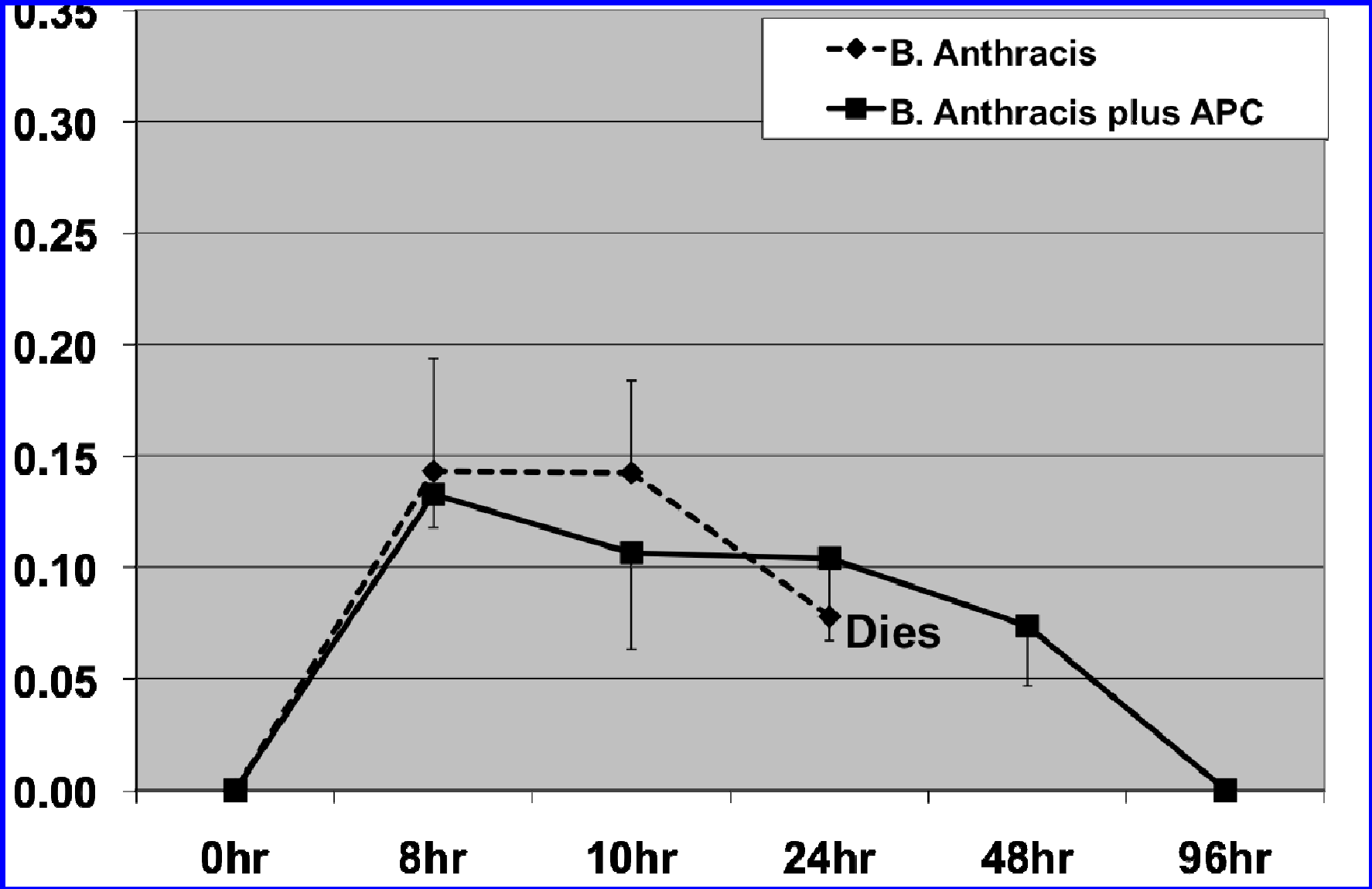
Sublethal E.coli sepsis (baboon)



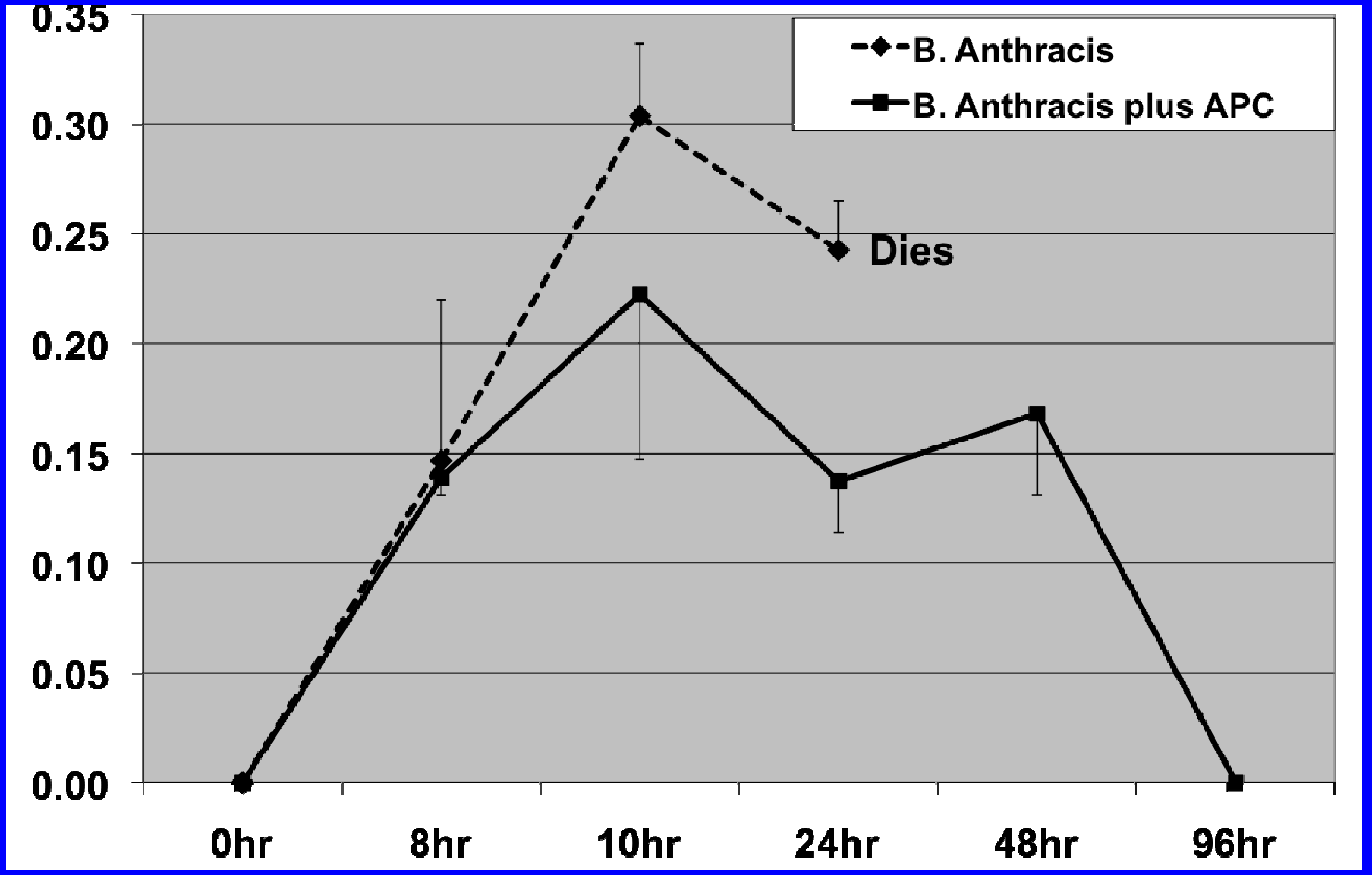
Shiga-toxin infusion (baboon)



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-inflamation
- Wait until PAMP ***biomarkers abate***

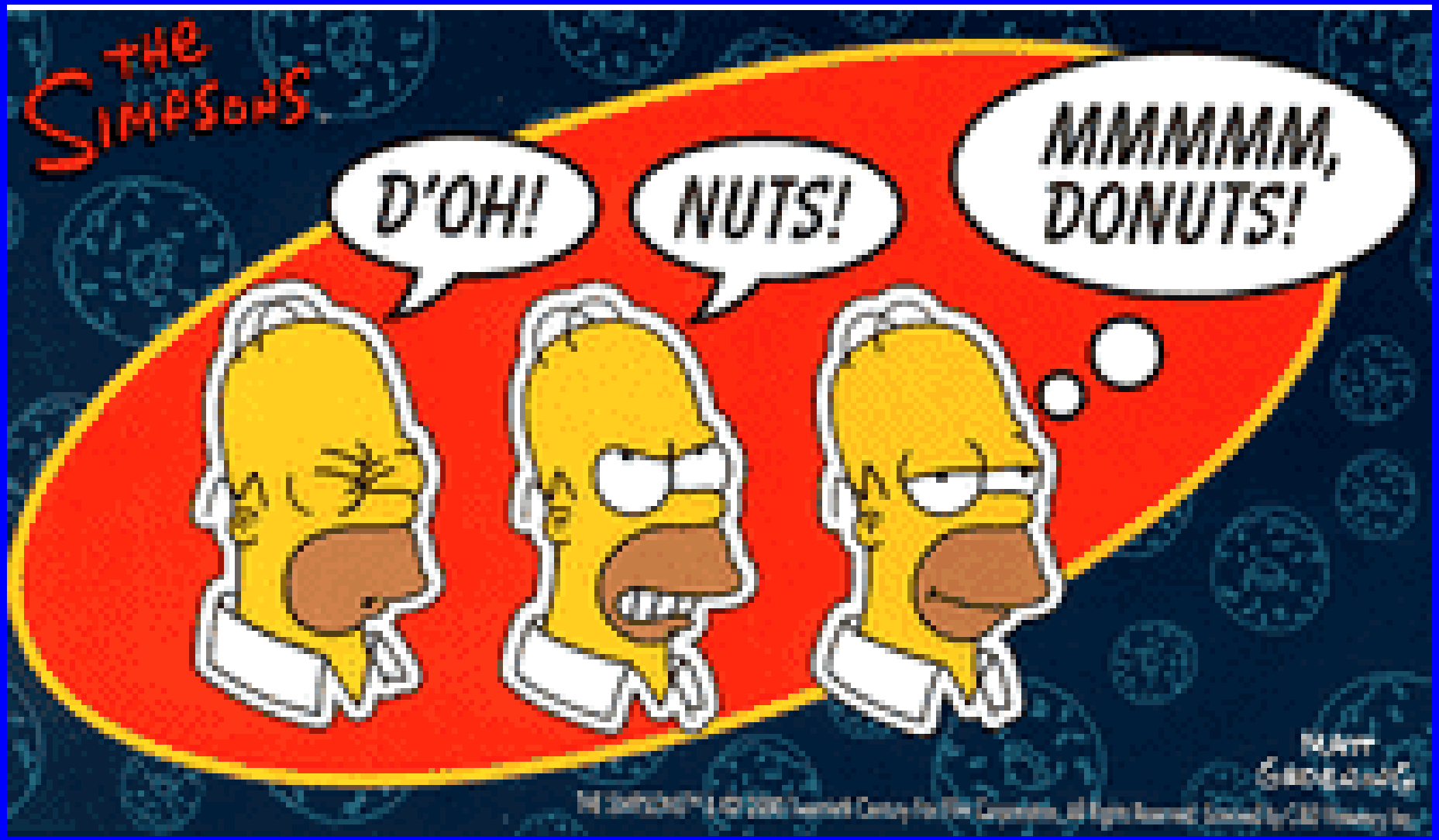
Treatment of ***traumatic SIRS***

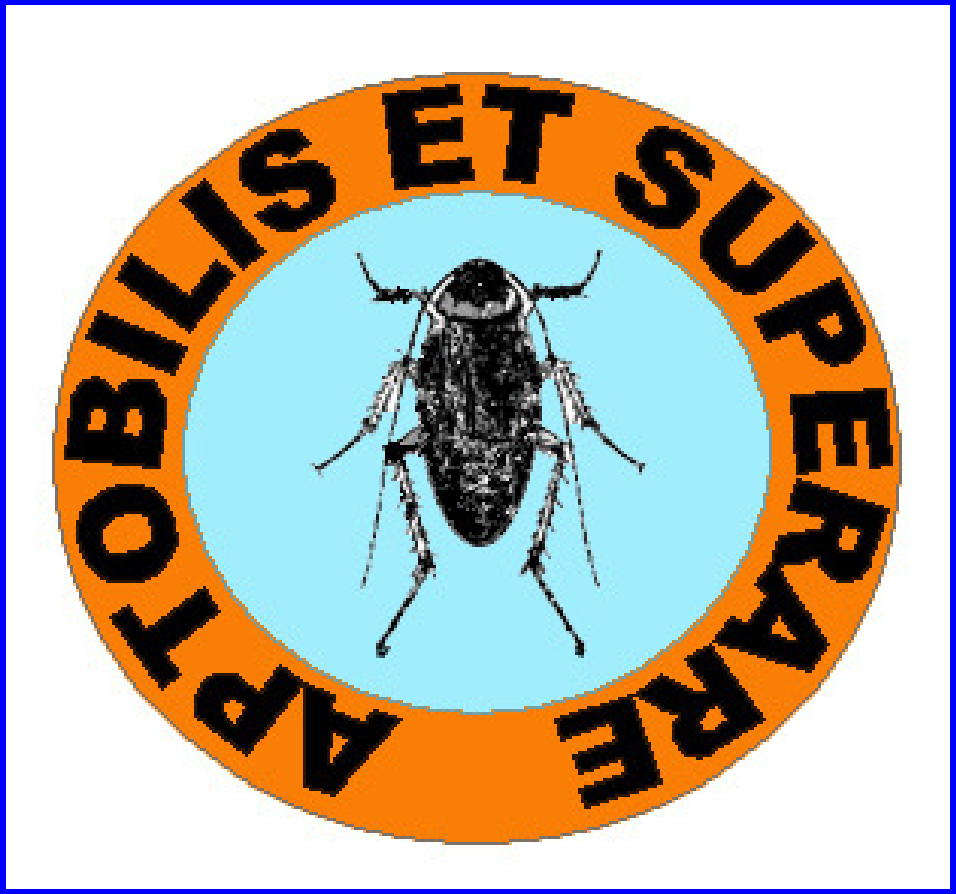
*1) Remove source of **DAMPs***

- ***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***


THE SIMPSONS
D'OH!
NUTS!
MMMMMM, DONUTS!


APTOBILIS ET SUPERARE