Demystifying Medicine: “Sepsis and the NIH Clinical Center”

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What is “Sepsis”?  

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What is sepsis? Hypotheses tested 1970 - 2018

1970
Antibiotics, fluids, pressors, source control
“treat sooner!” (2005 →)

2017
Antibiotics, fluids, pressors, source control
Glucose control, maximize oxygenation, low dose steroids
“treat sooner!” (2005 →)

Anti-endotoxin antibodies
“septicemia” (1980’s)

Antagonists to TNF, IL-1, etc.
“systemic inflammation” (1990’s →)
“SIRS”

“compensatory systemic anti-inflammation”

Anti-coagulants
“thrombosis” (2000’s)

“tighter regulation” “treat sooner” (2000’s)
Sepsis pathogenesis: some assumptions

  - Vertebrates evolved responses to infection and injury that promote survival.
- Organs lose function when the body’s normal, adaptive responses are stimulated beyond, or longer than, their ability to be protective.
  - The same basic responses occur in almost everyone. They can be modified by underlying illness, age, the invading microbe, etc.
  - Any organ can be affected. BUT there is little cell death and return of baseline function is common if the patient survives.
- So we should look for normal, reversible phenomena that can decrease organ function if they are pushed too hard.
Local and systemic reactions to local infection

Invasive microbes (UTI, pneumonia, appendicitis etc.)

LOCAL DEFENSES (kill the invaders)

SYSTEMIC REACTIONS (prevent damage elsewhere?)

“SEPSIS”
Dolor
Rubor
Tumor
Calor
Bacteria

Neutrophils, macrophages, dendritic cells

Infected site

Epidermis

Dermis

Bacteria
Local (tissue) defenses

• Activation of local cells: macrophages, dendritic cells, pain fibers
  → cytokines (TNF-α, IL-1, IL-6, IL-8, etc.) & other molecules that promote
  • Pain
  • Increased local blood flow
  • Recruitment of neutrophils and monocytes to the site
  • Endothelial barrier leakage, allowing plasma into the infected tissue (antibodies, complement, etc.)
  • Local hypercoagulability
How do microbes activate our cells?

• We have a sensory system for recognizing microbial “flags” -- molecules they make that we don’t.

  • Also called “pathogen-associated molecular patterns (PAMPS)” or “microbe-associated molecular patterns (MAMPS)”
Gram-negative bacterial cell envelope

- Outer membrane
  - O-polysaccharide
  - Core polysaccharide
  - Lipid A
- Peptidoglycan
- Periplasmic space
- Inner membrane
  - Phospholipid
  - Membrane protein
- Porin
Animals sense bacterial “flag” molecules to mobilize their defenses

Dissemination unusual
Some bacterial “flag” molecules

- LPS
- Cell wall peptidoglycan, peptidoglycan fragments
- Bacterial lipoproteins
- Bacterial DNA
- Flagella

- Different receptors, signaling mechanisms, etc.
The body’s responses to infection and trauma are “compartmentalized.”

- The **local** tissue response is **pro-inflammatory** (defensive but potentially damaging).

- The dominant **systemic** response is **anti-inflammatory** (protective but potentially immunosuppressive).

J-M Cavaillon et al. (many publications re. compartments) 
Munford and Pugin 2001 Am J Respir Crit Care Med 163:316-321
Activation of the hypothalamic-pituitary-adrenal axis and the autonomic nervous system +/- fever

The brain responds...

- ACTH
- Sympathetic
- Adrenal gland
- Epinephrine cortisol
- Vagus
- IL-6, IL-1, other bloodborne mediators
- (-) Ach
- Systemic anti-inflammation
- Acute phase protein production

Systemic responses to control inflammation

1. The nervous system → epinephrine, cortisol, α-MSH, other hormones
   --Anti-inflammatory (IL-10)
   --Metabolic changes to provide glucose and lactate to tissues for fuel, amino acids for protein synthesis, etc.

2. Acute phase responses
   Proteins made by liver in response to cytokines from infected tissue (IL-6, IL-1b), cortisol
   --Anti-inflammatory (inhibitors of TNF and IL-1b)
   --Anti-infective (complement, Fe and Zn lowering proteins)
   --Pro-coagulants (PAI), protease inhibitors, etc.

3. Monocytes, other cells → IL-10, IL-4, HLA-DR (immunosuppression)
TNF-α, IL-1β, tissue macrophages, dendritic cells, epithelium, bacteria, neutrophils, IL-8, TNFα, IL-1β, IL-10 (others), Cortisol, Epinephrine.
Some key mediators are **anti-inflammatory in blood and pro-inflammatory in tissues:**

- Epinephrine, norepinephrine
- Prostaglandin E$_2$
- Interleukin-6
- Corticotropin releasing hormone (CRH)

**Others are (only) anti-inflammatory:**

- ACTH, alpha-MSH
- IL-10
- IL-4, IL-13
- IL-1Ra, soluble TNF receptors
Example case: appendicitis in previously healthy young persons

- The inflamed appendix is the "local" compartment.
- Perforation or necrosis of the appendix = more severe infection/inflammation, “complicated” appendicitis.
- Plasma cytokine concentrations integrate the local and "systemic" cytokine responses.
56 young patients with appendicitis in Dallas, Texas

**Plasma cytokine concentrations**

**Uncomplicated Appendicitis** vs. **Complicated Appendicitis**

The blood’s “anti-inflammatory” profile intensified as the local infection advanced.

**High:** IL-1 receptor antagonist, IL-10, IL-6

**Low:** interferon-γ, TNF, IL-1β

Systemic reactions to local infection

Invasive microbes (appendicitis, many others) → "SEPSIS"
Beneficial local responses can become harmful if they extend to other organs

- Pain
- Increased endothelial permeability
- Vasodilation
- Clotting, containment
- Metabolism shift $\rightarrow$ mediators

Harmful if systemic

- Delirium
- Diffuse vascular leakage
- Hypotension
- Disseminated coagulopathy
- Metabolic acidosis
“Sepsis”

The most recent consensus definition: “life-threatening organ dysfunction caused by a dysregulated host response to infection”.

*Singer et al. 2016  JAMA 315:801*
“Despite the relative preservation of tissue morphology, tissue function is often markedly impaired. Cardiac myocytes stop contracting normally, alveoli cease to maintain the air–liquid barrier interface in lung tissue, hepatocytes no longer secrete bilirubin, endothelial cells retract, become permeable to macromolecules and lose their anti-adhesive and anticoagulant surface characteristics, and so on.”

S. Opal, T. van der Poll, 2015 J. Internal Medicine 277:277
What makes “septic” organs slow/stop functioning?

- In “septic” organs that don’t function normally, there is
  - Very little cell death
  - Normal tissue pO₂
  - Functional recovery if the patient survives
  - ....the cells are said to be “hibernating” or “quiescent”.
Invasive microbes (complicated appendicitis) → ↑ LOCAL INFLAMMATION → Tachycardia, Tachypnea, Leukocytosis, Fever → Endothelial leak, Microcirculatory dysfunction, Cell slow-down → SEPSIS → Organ Hypofunction, Immunosuppression
What makes cells in “septic” organs hibernate?

• Likely contributors:
  • **Leaky endothelium** $\rightarrow$ increased interstitial fluid, poor drainage
  • **Abnormal microcirculation** $\rightarrow$ intermittent blood delivery, poor venous drainage
  • $\rightarrow$ cells become surrounded by *interstitial fluid* that decreases their ability to function.
A major contributor: disordered microcirculation

Abnormal capillary blood flow
Orthogonal Planar Spectometry (OPS) analysis - tongue

Heterogeneous flow
Capillary collapse, filling
Shunting

Applying nitroglycerin to the tongue restores normal microcirculation.

De Backer et al. 2002. AJRCCM 166:98-104
Adapted from Trzeciak et al. 2008 Acad Emerg Med 15:399

↓ eNOS

(interstitial fluid)
How local inflammation might become systemic

Local stimulus

NADPH oxidase(s) (neutrophil) → blood → $\text{O}_2^-$ → NO → Peroxinitrite (ONOO-) → eNOS uncoupling

Endothelial cell

$\text{BH}_4 = \text{tetrahydrobiopterin}$

$\text{eNOS} = \text{endothelial nitric oxide synthase}$

$\text{eNOS uncoupling is promoted by both peroxinitrite and VEGF (vascular endothelial growth factor).}$
NORMAL

Capillary Endothelium

Tissue cells

cytokines
Findings: extracellular acidity $\rightarrow$ cellular quiescence

- When cultured at low pH (6.8 – 7.0) for 48 – 72 hrs, murine macrophages
  - Greatly decrease usage of glucose and fatty acids
  - Increase mitochondrial mass, length
  - Decrease ROS, NO production, maintain mitochondrial inner membrane potential, low-level ATP production
  - Alter cytokine production in response to LPS
  - Retain phagocytic ability
  - Survive, regaining baseline functionality when pH is increased to 7.4.
Extracellular acidity induces many cell types to stop using glucose and fatty acids for energy and rely upon mitochondrial ATP production to survive.

Extracellular acidity “protects cells from hypoxia.”

Early response: conserves energy fuels (glucose, FA) → too much, too long → organs slow → death?
Septic organ hypofunction: hypothesis

**Endothelial leakage** and **microcirculatory derangement** interact so that tissue cells are surrounded by **interstitial fluid** that inhibits their ability to carry out many specialized functions yet preserves their viability.
What about lactate?

• The blood lactate level is the most consistently reported predictor of outcome in septic patients—both high and prolonged levels predict death.

• Major conceptual change:
  • **High blood lactate levels are not caused principally by tissue hypoxia.**
  • Lactate is produced by the actions of epinephrine in many tissues, most prominently LUNG and muscle.
  • It is used by many tissues as fuel (\(\rightarrow\) pyruvate \(\rightarrow\) TCA, etc.)
  • Garcia-Alvarez et al.: “the characteristics of lactate production best fit the notion of an **adaptive survival response** that grows in intensity as disease severity increases.”

  Crit Care 2014 18:503
Survival of patients with serious Gram-negative bacterial infections

Many deaths occur late in the course.
- Second infections
- Other complications

*Immunoparalysis, immunosuppression*

Invasive microbes (complicated appendicitis, many others.)

- Tachycardia
- Tachypnea
- Leukocytosis
- Fever

Endothelial leak
Microcirculatory dysfunction
Cell slow-down

Organ Hypofunction
Immunosuppression

Systemic reactions to infection lead to Death
What makes systemic responses last so long?

--- and how do they cause immunosuppression?
Prolonged immunosuppression

• Can last days/weeks after the infection has been treated.
• IL-10 is a major influence (and bad prognostic sign).
• Decreased monocyte surface HLA-DR, monocyte “tolerance” are common.
• T cell apoptosis

• The driving force(s) are very poorly understood, yet understanding them may be the key to effective treatment.
Findings at autopsy in 235 surgical ICU patients who died from severe sepsis/septic shock

“In spite of the fact that immediate removal of the septic focus combined with antibiosis is the cornerstone of sepsis therapy, it appeared impossible to control the focus in the vast majority of our study patients, and this seems to have been the main cause of death.”

Infected animals can react to microbial molecules long after the microbes that made them are dead:

- *Streptococcus pyogenes*: “erysipelas”.
  - Bacterial enzymes, flags → inflammatory reaction → lymphatic engorgement, hyperemia, RASH

- Many bacteria, viruses:
  → inflammation → “innate immune tolerance”

  Microbes don’t destroy their own “flags”.
Some animal “flag burners”

- Acyloxyacyl hydrolase – LPS
- Chitinase – fungal chitin
- Lysozyme – bacterial cell wall peptidoglycan
- Peptidoglycan binding proteins
- Bactericidal/permeability-increasing protein (LPS)
- Alkaline phosphatase (?) – LPS
- DNAses
- RNAses
- Several lipases – bacterial lipopeptides
- Molecules that may sequester, excrete flags
Host enzymes can destroy many microbial flags.

ACYLOXYACYL HYDROLASE (AOAH)
(monocytes, dendritic cells, NK cells, microglia, neutrophils, renal tubule cells)
Persistent microbial flags

• After the initial response to a microbial molecule, a second exposure often triggers an anti-inflammatory response from macrophages, dendritic cells, and others.
• This is usually thought to “prevent friendly fire” – to allow recovery from inflammation. It usually lasts a few days.
• BUT this anti-inflammatory response, often called “tolerance”, can last a long time if the microbe’s flag(s) remain intact.
• An “innate immune paradox”: after the initial “active defense” response to microbes is over, continuing to sense their flags can be immunosuppressive.
Flag destruction prevents long-term “innate immune tolerance”

AOAH inactivates LPS – after “tolerance” is induced

Macrophages, dendritic cells, others

“innate immune (or endotoxin) tolerance”
“Cellular reprogramming”

Lu et al. 2008 Cell Host Microbe 4:293
Cavaillon and Adib-Conquy 2006 Crit Care 10:233
In septic patients, circulating monocytes often are “tolerant” or “immunoparalyzed” – combining features of “tolerance” and “acid-induced” metabolic changes.

After the battle is won…

dis-arm
(avoid friendly fire)

then

re-load
(to fight again)

Destroy microbes and their flags
Prolonging sepsis/immunosuppression: possible sources of persistent “flags”

• Same microbe
  • The initial site of infection -- ? abscess, local spread
  • Other tissues (local extension or bacteremia)

• Different microbe(s):
  • Nosocomial infection (bacterial, fungal, viral)
  • The GI tract (Gram-negative bacteria)
    • LPSs (etc.) translocate into blood, ?? stimulate cells in many organs
  • Unknown
Microbiota: human feces – on ICU admission and discharge

Note increase in Proteobacteria, decrease in Firmicutes and Bacteroidetes in septic patients, even on admission to ICU.
Manipulation of the microbiome may prevent sepsis and death

“Selective decontamination of the digestive tract” – clinical trials

Organ hypofunction

Mortality

Non-absorbable Antimicrobials:
- Tobramycin
- Colistin
- Amphotericin B

LPS translocation from intestine to blood

- **Upper intestine** – along with triglycerides → chylomicron-LPS complexes. Mesenteric lymphatics → thoracic duct → blood

- **Lower intestine** – LPS probably can take two routes:
  - Lymphatics
  - Portal vein → liver, probable inactivation by Kupffer cell AOAH

- **LPS-lipoprotein complexes** can be non-stimulatory, tolerance-inducing, or possibly stimulatory.
Prolonging immunosuppression: Alarmins?

• Alarmins are **host** molecules that are released from cells in response to “danger” or cell death –
  • **HMGB-1** is the alarmin most likely to prolong immunosuppression
  • Pro- and anti-inflammatory activities
  • Anti-HMGB-1 antibodies can rescue animals from sepsis
Host reactions to infection

Invasive microbes (UTI, pneumonia, appendicitis etc.)

LOCAL DEFENSES (kill the invaders) (extend to other tissues)

Tachycardia Tachypnea Leukocytosis Fever

Endothelial leak Microcirculatory dysfunction Cell slow-down

SYSTEMIC REACTIONS (prevent damage elsewhere) (promote immunosuppression)

Organ Hypofunction Immunosuppression

DEATH
The “ideal” (simple) case is not common!

• Different pathogens have different ways to cause damage – toxins, enzymes, complement resistance, etc.

• Bacteremia may spread infection to other tissues
  • *Staphylococcus aureus* → abscesses in many tissues
  • *E. coli* → low level bacteremia, doesn’t usually infect distant tissues

• Most patients who experience sepsis are elderly.
• Most also have significant pre-existing disease.
Patients with severe sepsis in the U.S. (1990's)

Case-fatality rate, %

Age, years

No co-morbidity

Staphylococci, streptococci, pneumococci, meningococci.


Patients with severe sepsis in the U.S. (1990's)

- E. coli, Pseudomonas, enterococci, fungi, etc.
- Pre-existing co-morbidity
- No co-morbidity
- Patients in clinical trials

Case-fatality rate, % vs. Age, years
Augmenting the early systemic anti-inflammatory response may be harmful!

Baseline predicted 28 day mortality (APACHE)

Predicted, placebo

Anti-inflammatory drug
-- IL-1Ra, anti-TNF, APC

Eichacker et al., 2002. Am J Respir Crit Care Med 166:1197
questions, comments?

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Therapeutic opportunities?

- Decrease accumulation of interstitial fluid in critical organs
  - Reduce fluid administration – prevent edema
- Decrease lactate production?
  - Dichloroacetate – increases conversion of pyruvate to acetylCoA
- Improve microcirculatory function
  - Several agents succeeded transiently but didn’t last
- Improve endothelial barrier
  - Anti-VEGF failed; others planned, in progress
- Identify and neutralize microbial “flags”
  - Improve methods for detecting local infectious foci
  - Tailor intervention to microbe – e.g., anti-LPS therapy only for Gram-negative bacterial infection that persists. Bactericidal/permeability-increasing protein?
- Treating multiple targets may be necessary.