

Demystifying Medicine: “Sepsis and the NIH Clinical Center”

January 15, 2019

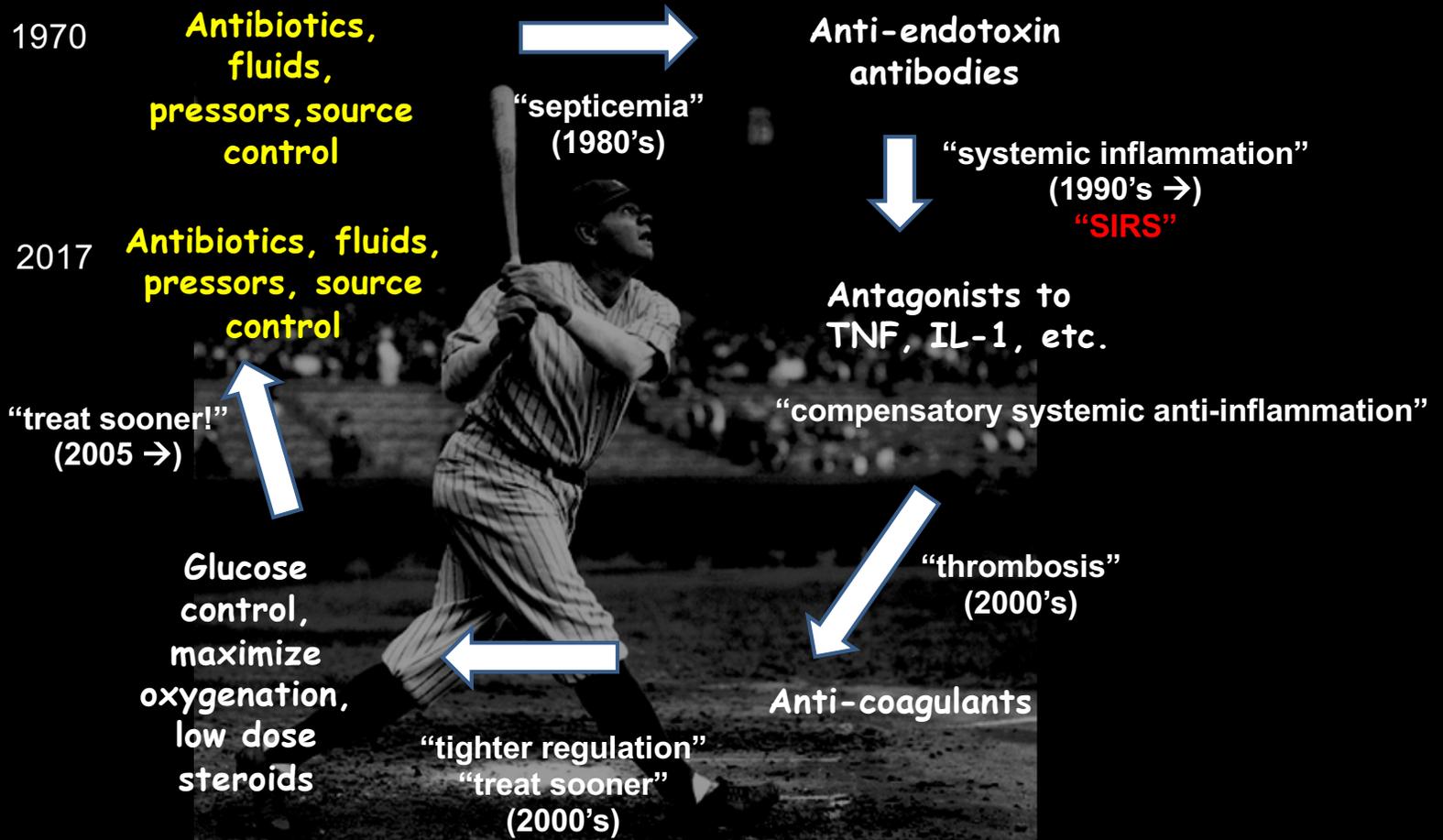
What is “Sepsis”?

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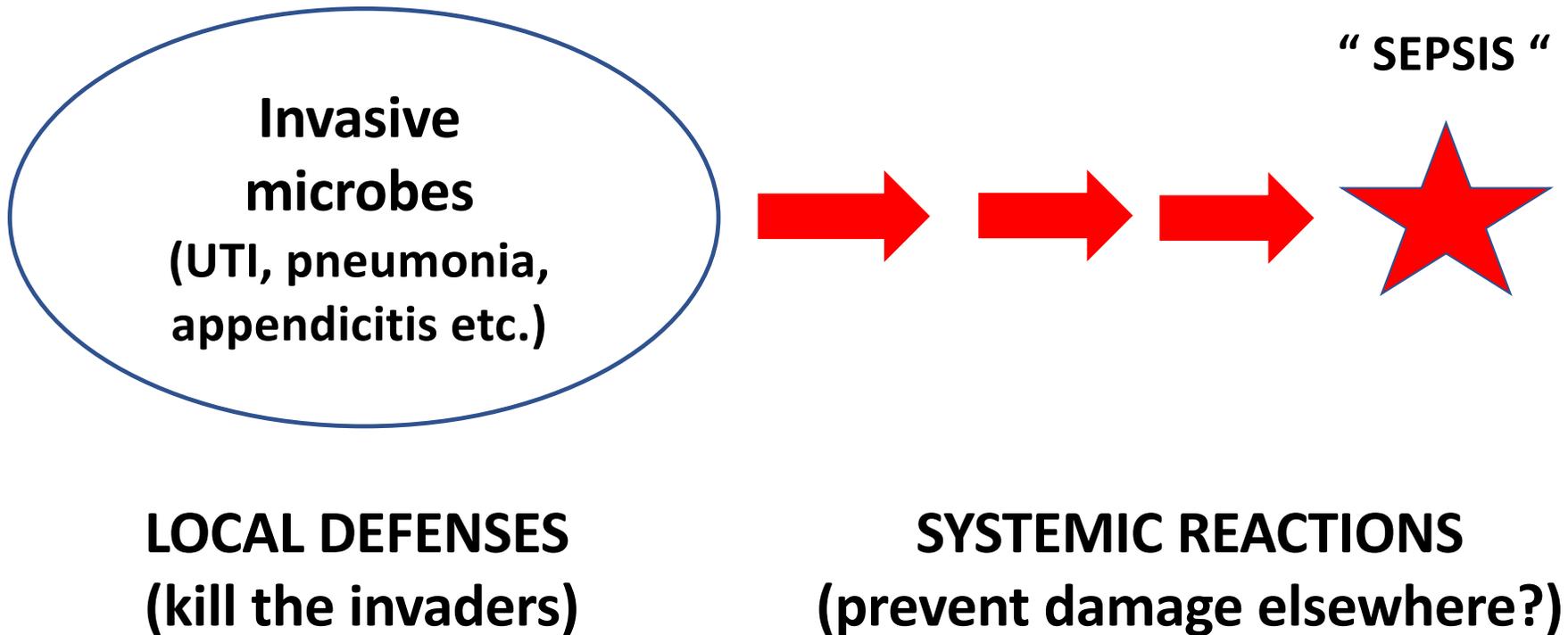
What is sepsis? Hypotheses tested 1970 - 2018



Sepsis pathogenesis: some assumptions

- Theodore Dobzhansky: **“Nothing in biology makes sense except in the light of evolution.”**
 - 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



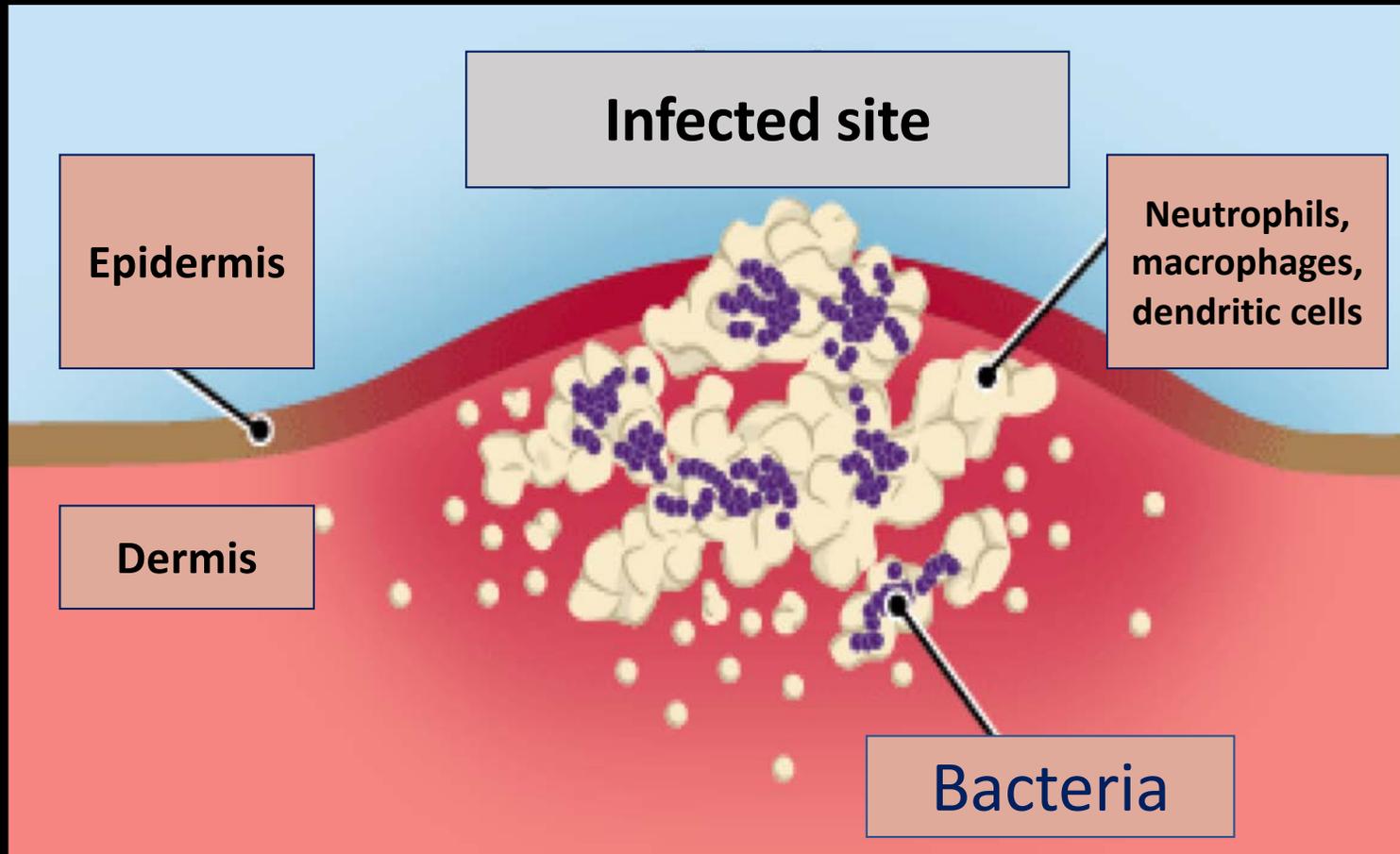


Dolor

Rubor

Tumor

Calor



Local (tissue) defenses

- **Activation of local cells: macrophages, dendritic cells, pain fibers**

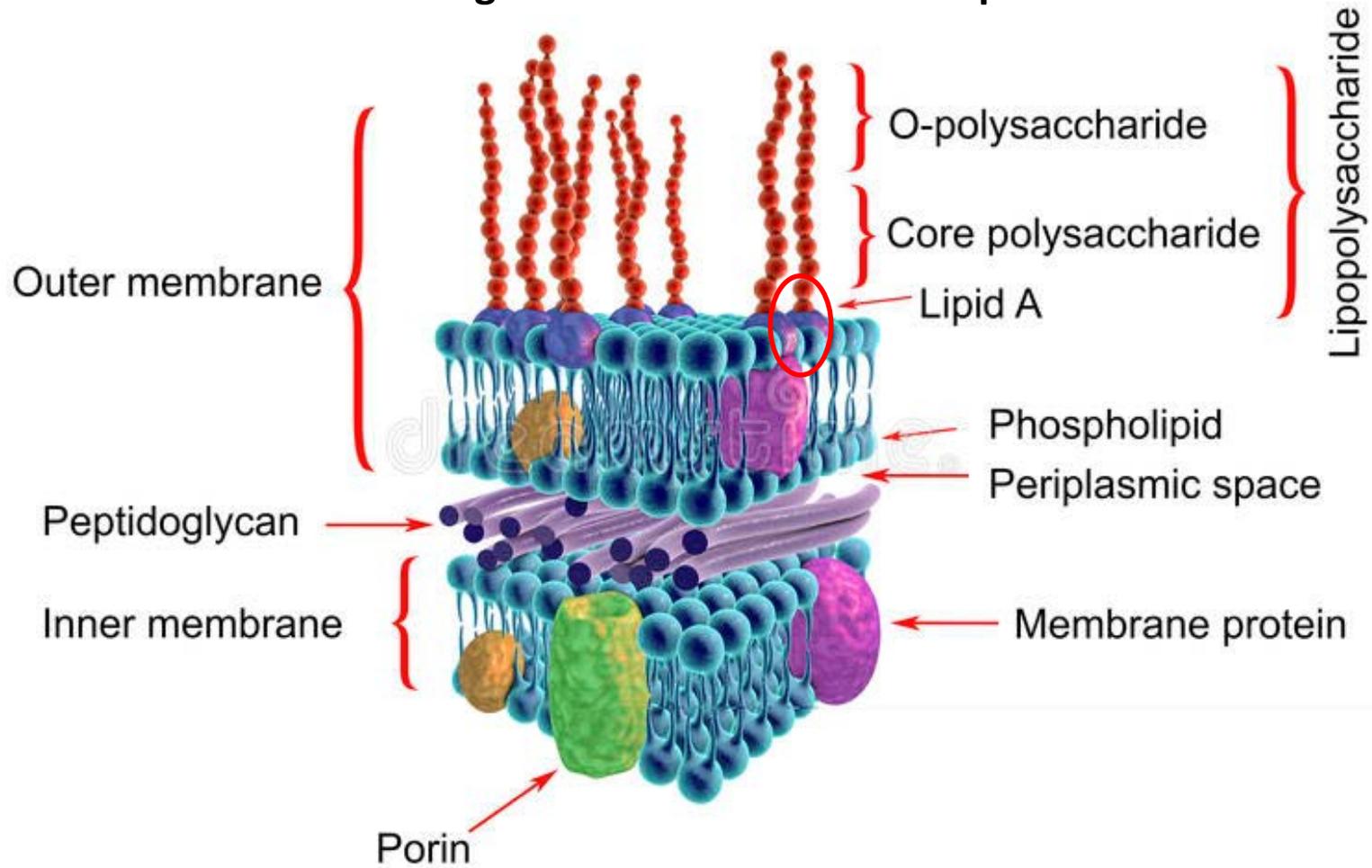
→ cytokines (TNF- α , IL-1, IL-6, IL-8, etc.) & other molecules that promote

- **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**

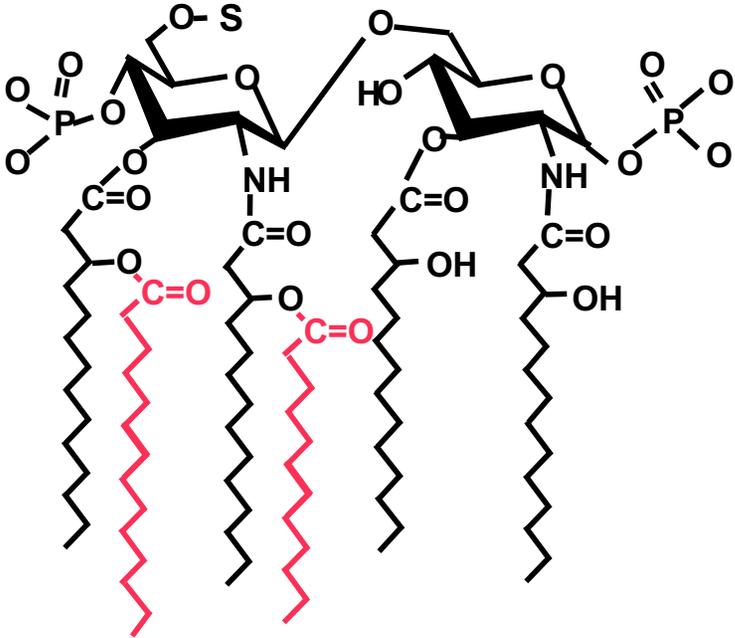
How do microbes activate our cells?

- **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



Animals sense bacterial “flag” molecules to mobilize their defenses



E. coli

Dissemination unusual

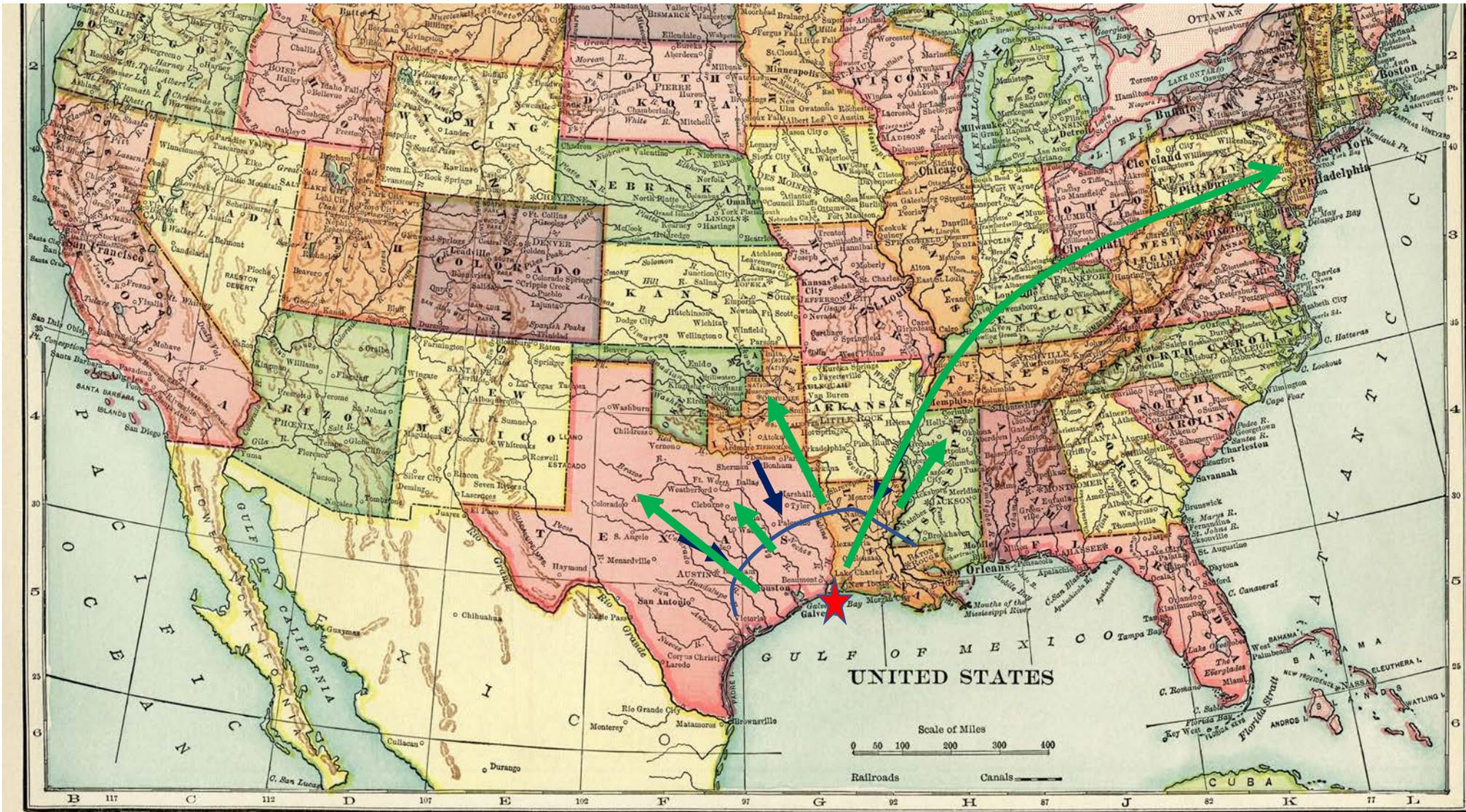
Some bacterial “flag” molecules

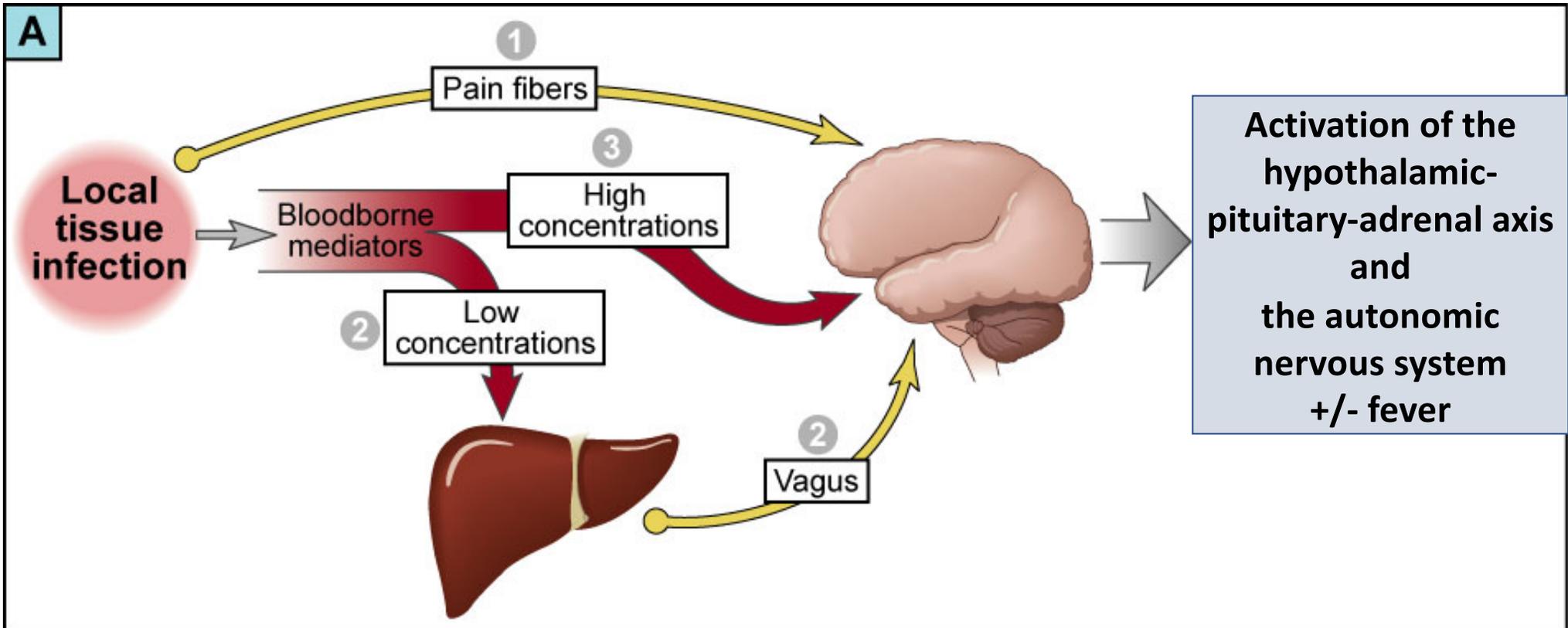
- **LPS**
 - **Cell wall peptidoglycan, peptidoglycan fragments**
 - **Bacterial lipoproteins**
 - **Bacterial DNA**
 - **Flagella**
-
- **Different receptors, signaling mechanisms, etc.**

The body's responses to infection and trauma are “compartmentalized.”

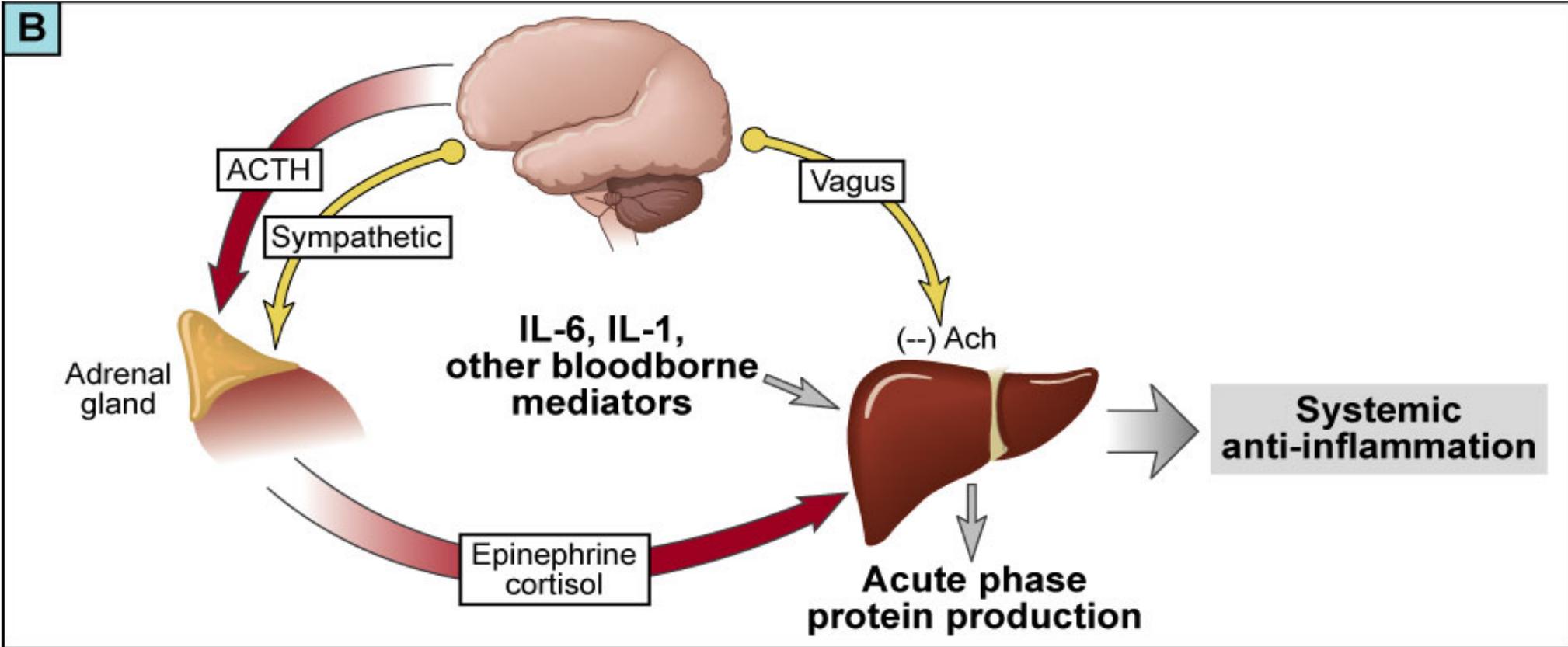
- The local tissue response is pro-inflammatory (defensive but potentially damaging).
- The dominant systemic response is anti-inflammatory (protective but potentially immunosuppressive).

J-M Cavaillon et al. (many publications re. compartments)
Munford and Pugin 2001 Am J Respir Crit Care Med 163:316-321





The brain responds...



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

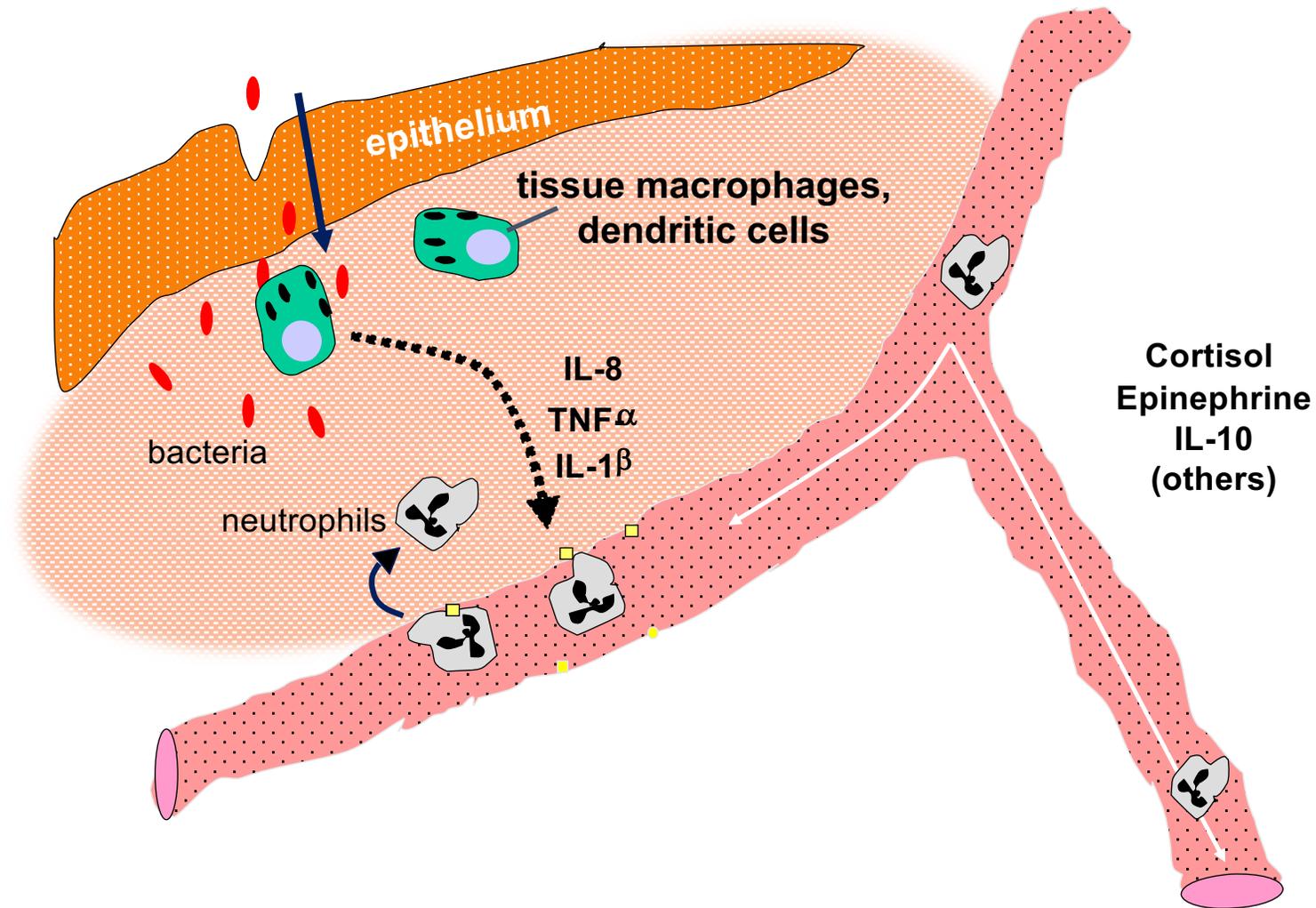
Proteins made by liver in response to cytokines from infected tissue (IL-6, IL-1b), cortisol

--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)



Some key mediators are anti-inflammatory in blood and pro-inflammatory in tissues:

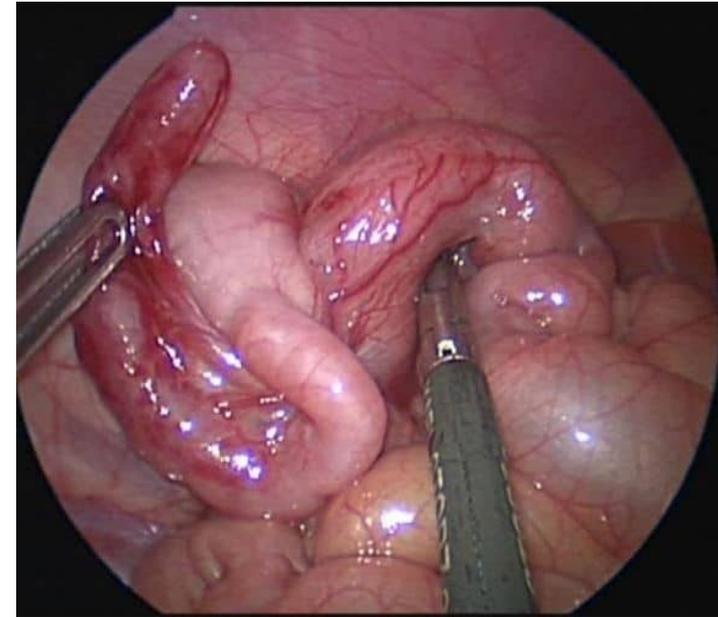
- Epinephrine, norepinephrine
- Prostaglandin E₂
- Interleukin-6
- Corticotropin releasing hormone (CRH)

Others are (only) anti-inflammatory:

- ACTH, alpha-MSH
- IL-10
- IL-4, IL-13
- IL-1Ra, soluble TNF receptors

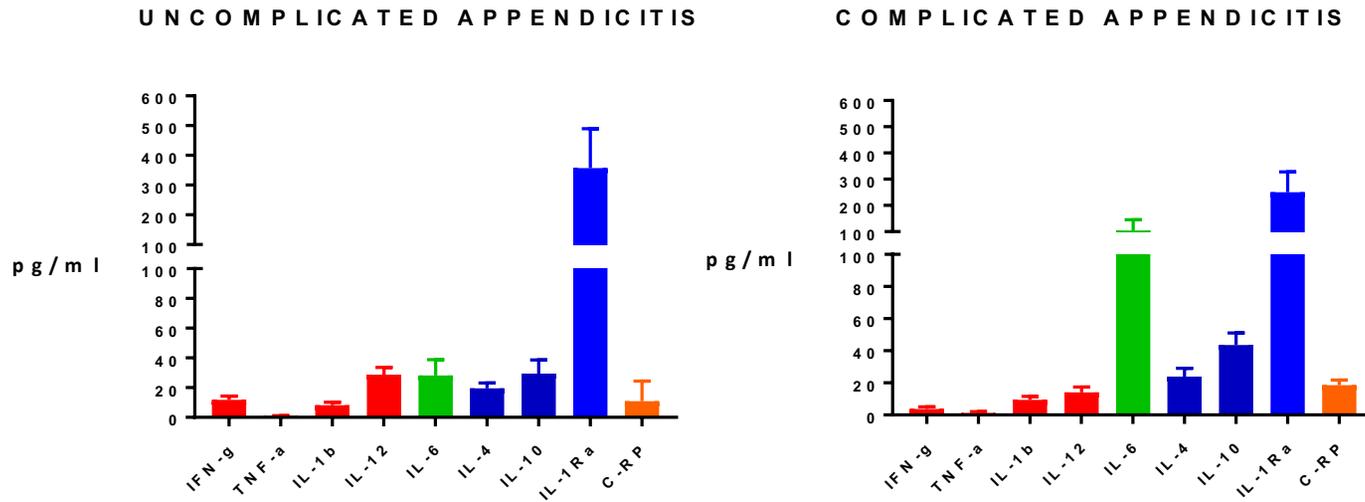
**Example case:
appendicitis in previously healthy
young persons**

- **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

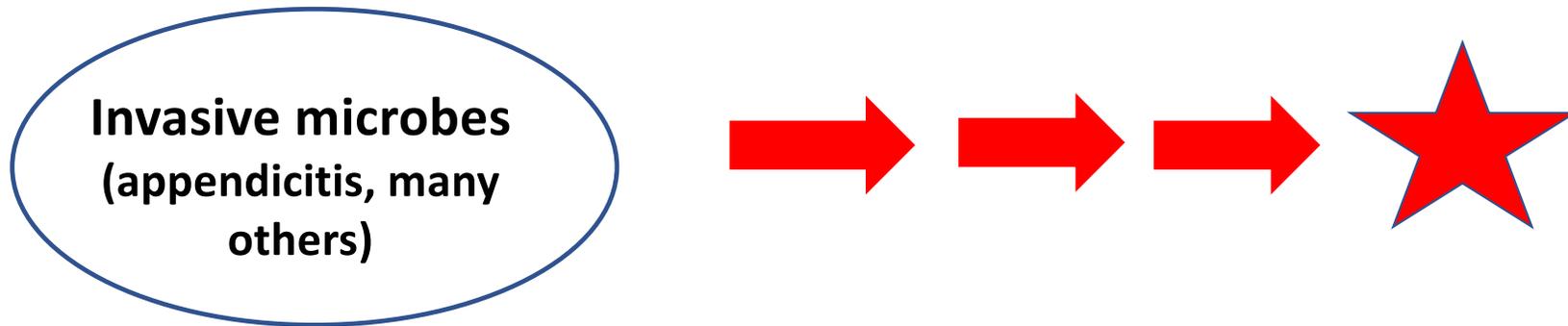
Plasma cytokine concentrations



High: IL-1 receptor antagonist, IL-10, IL-6
Low: interferon- γ , TNF, IL-1 β

The blood's "anti-inflammatory" profile intensified as the local infection advanced.

Systemic reactions to local infection



Beneficial local responses can become harmful if they extend to other organs

Beneficial locally



Harmful if systemic

- Pain
- Increased endothelial permeability
- Vasodilation
- Clotting, containment
- Metabolism shift → mediators

- 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

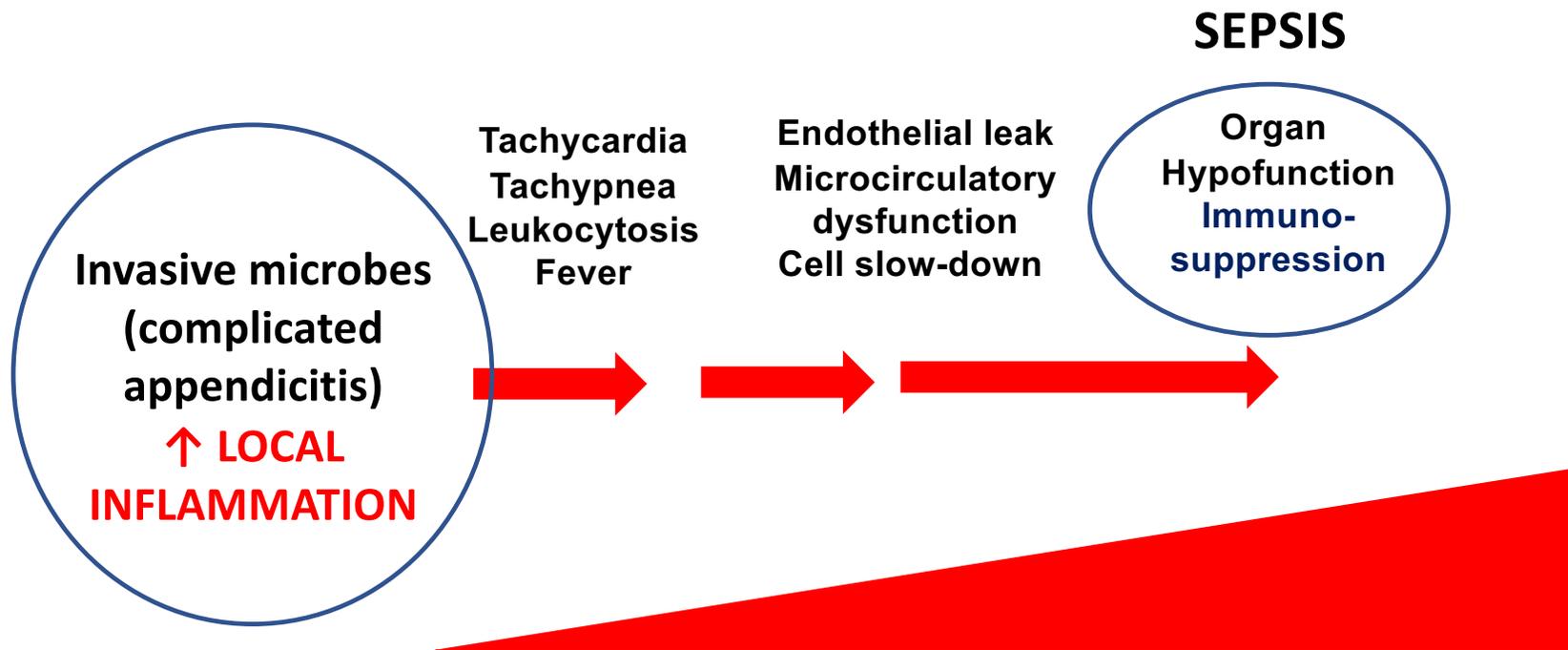
“Despite the relative preservation of tissue morphology, tissue function is often markedly impaired. Cardiac myocytes stop contracting normally, alveoli cease to maintain the air–liquid barrier interface in lung tissue, hepatocytes no longer secrete bilirubin, endothelial cells retract, become permeable 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 pO_2
 - Functional recovery if the patient survives
- ...the cells are said to be “hibernating” or “quiescent”.

Systemic reactions to infection



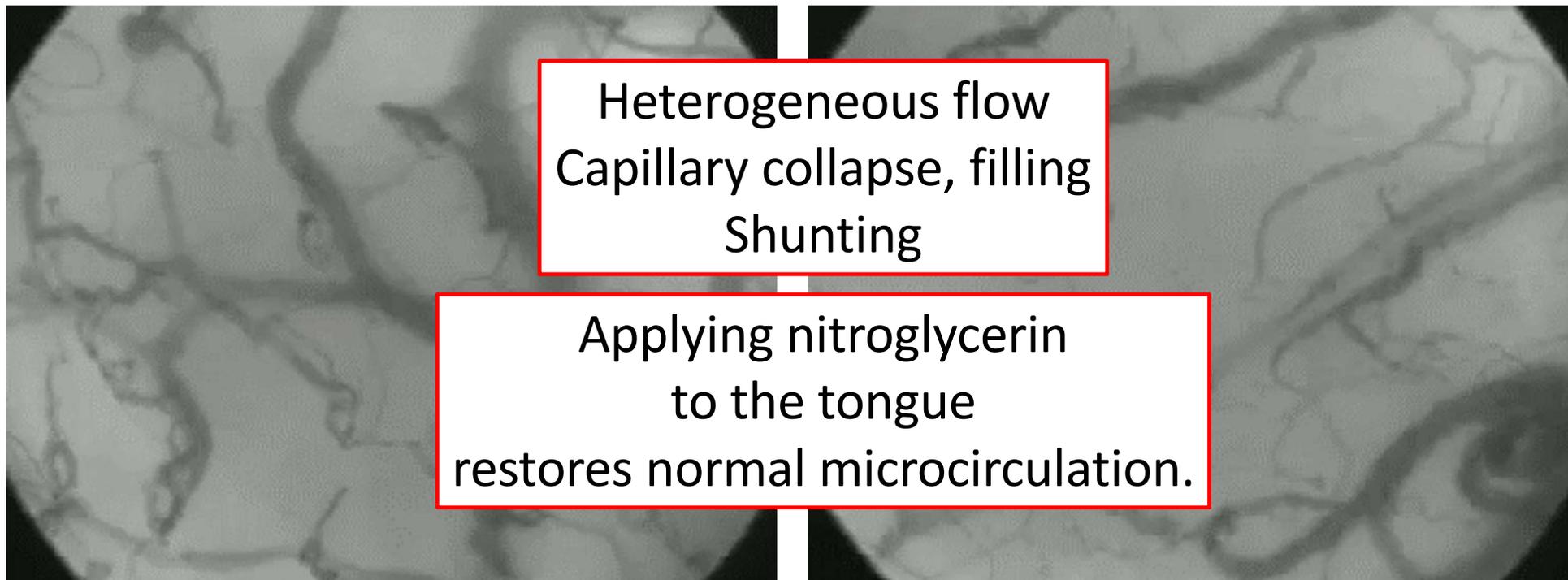
What makes cells in “septic” organs hibernate?

- **Likely contributors:**
 - Leaky endothelium → increased interstitial fluid, poor drainage
 - Abnormal microcirculation → intermittent blood delivery, poor venous drainage
 - → cells become surrounded by *interstitial fluid* that decreases their ability to function.

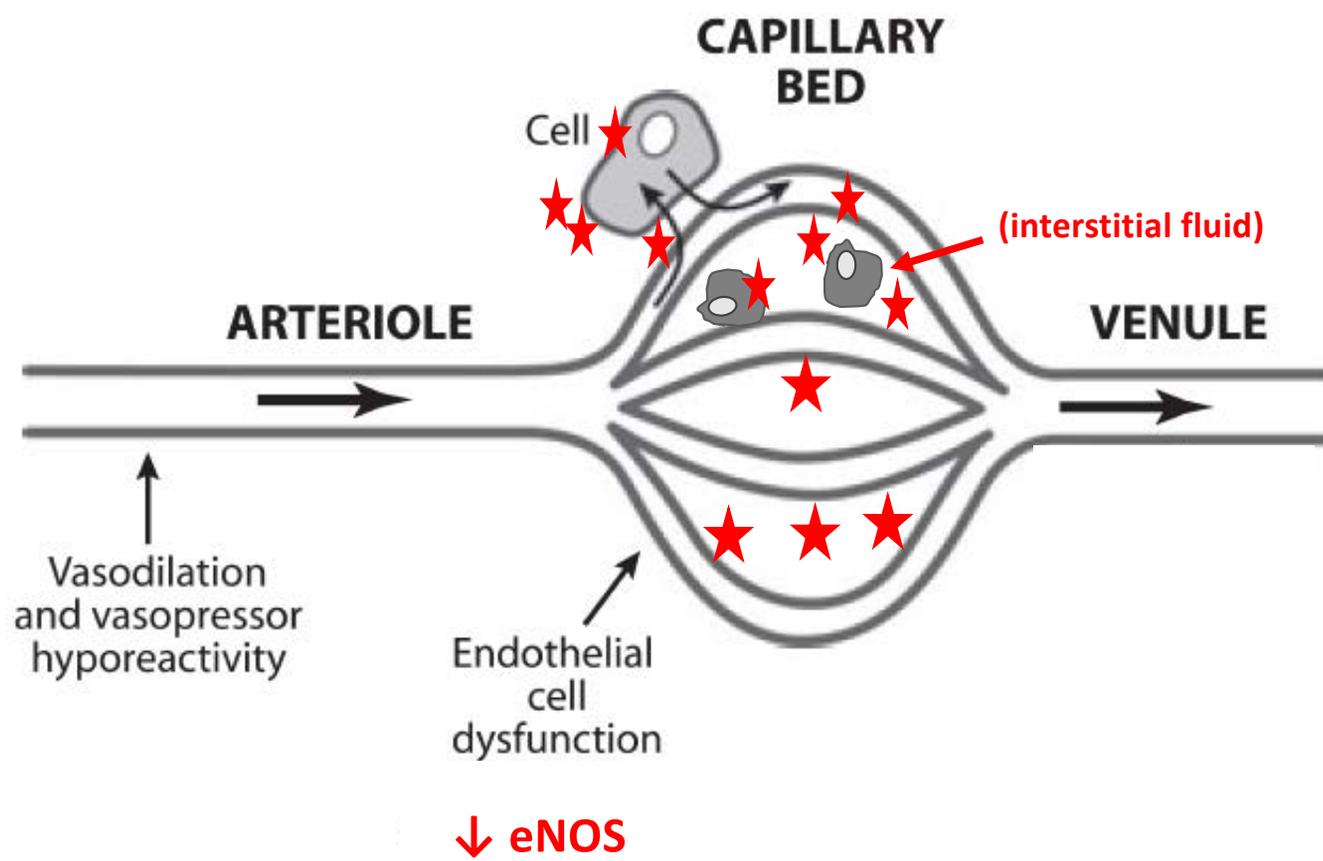
A major contributor: disordered microcirculation

Abnormal capillary blood flow

Orthogonal Planar Spectrometry (OPS) analysis - tongue

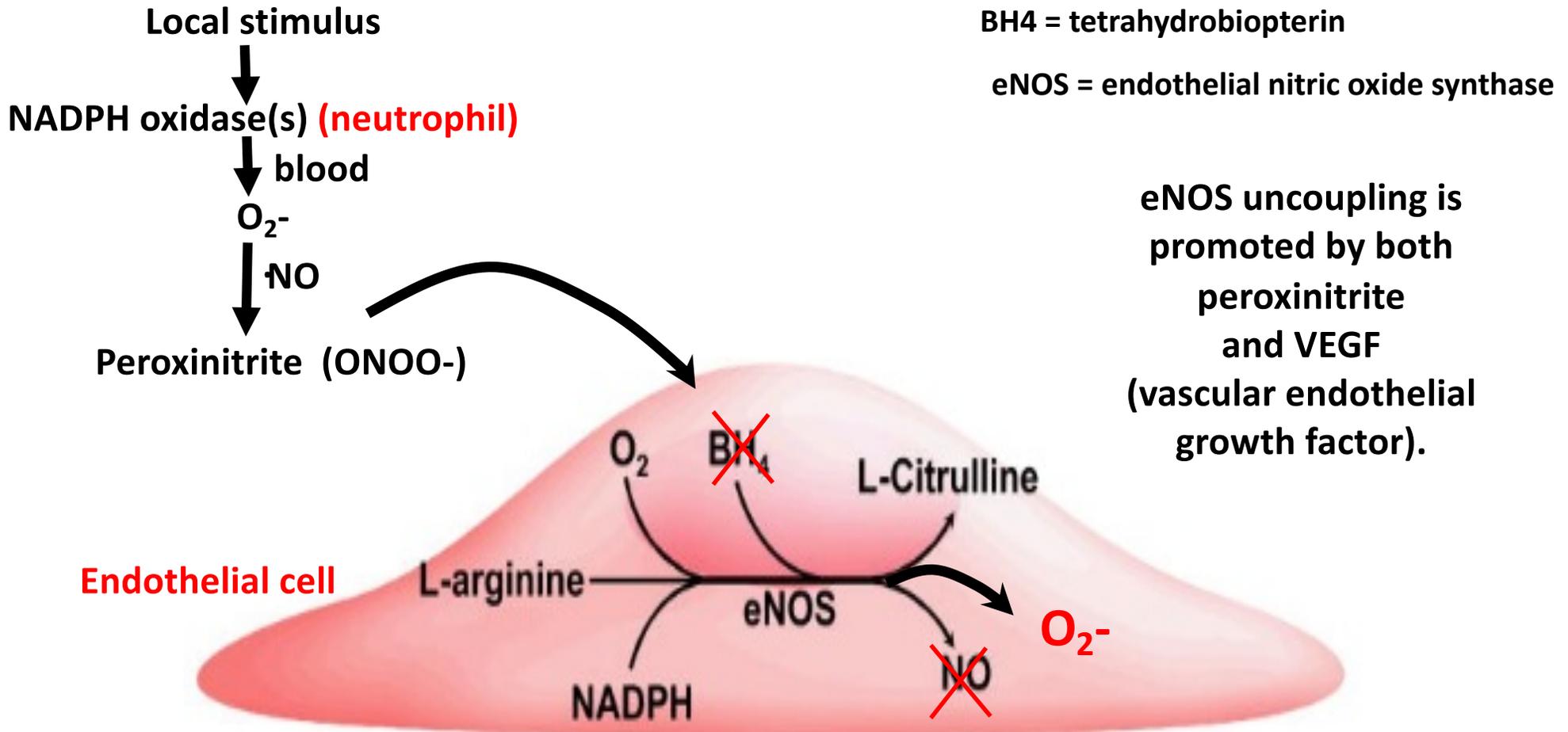


De Backer et al. 2002. AJRCCM 166:98-104



Adapted from Trzeciak et al. 2008 Acad Emerg Med 15:399

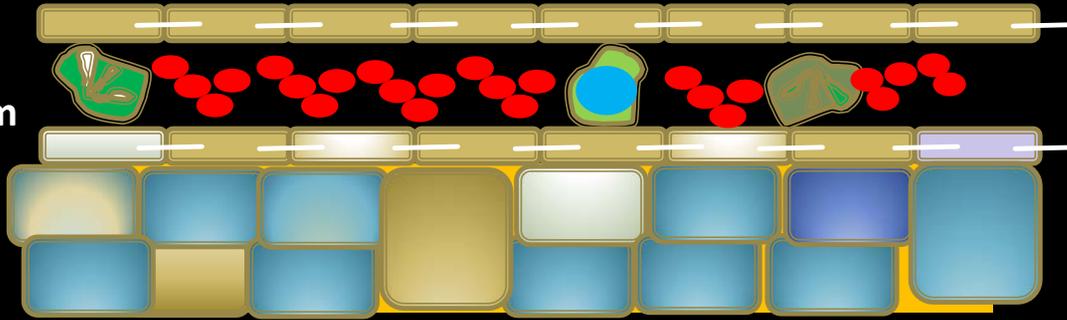
How local inflammation might become systemic



NORMAL

Capillary
Endothelium

Tissue cells



cytokines

Findings: extracellular acidity → cellular quiescence

- **When cultured at low pHe (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 pHe 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

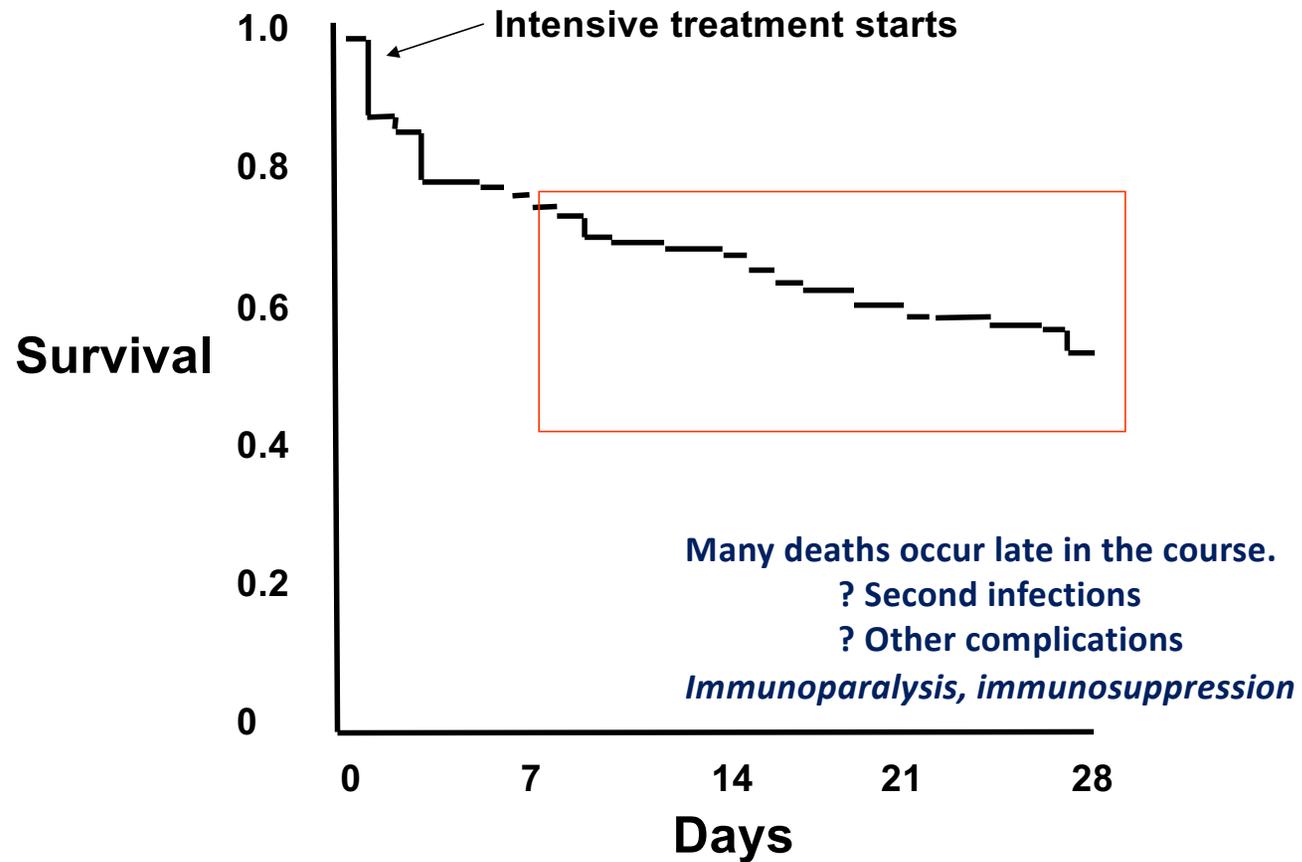
Endothelial leakage and microcirculatory derangement interact so that tissue cells are surrounded by interstitial fluid that inhibits their ability to carry out many specialized functions yet preserves their viability.

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 (→ pyruvate → 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.”

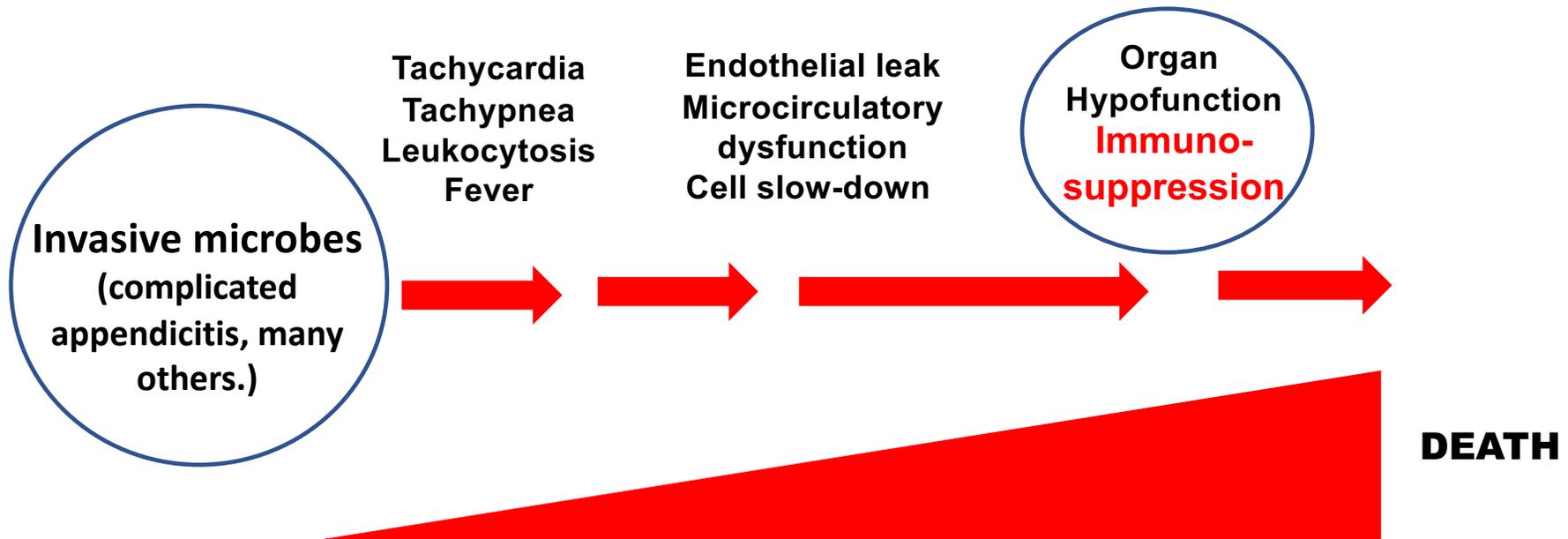
Crit Care 2014 18:503

Survival of patients with serious Gram-negative bacterial infections



Ziegler et al. 1991. New Eng. J. Med. 324:429.

Systemic reactions to infection



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 antibiotics 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.”

Torgersen et al. 2009. Anesthesia-analgesia 108:1841-1847

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

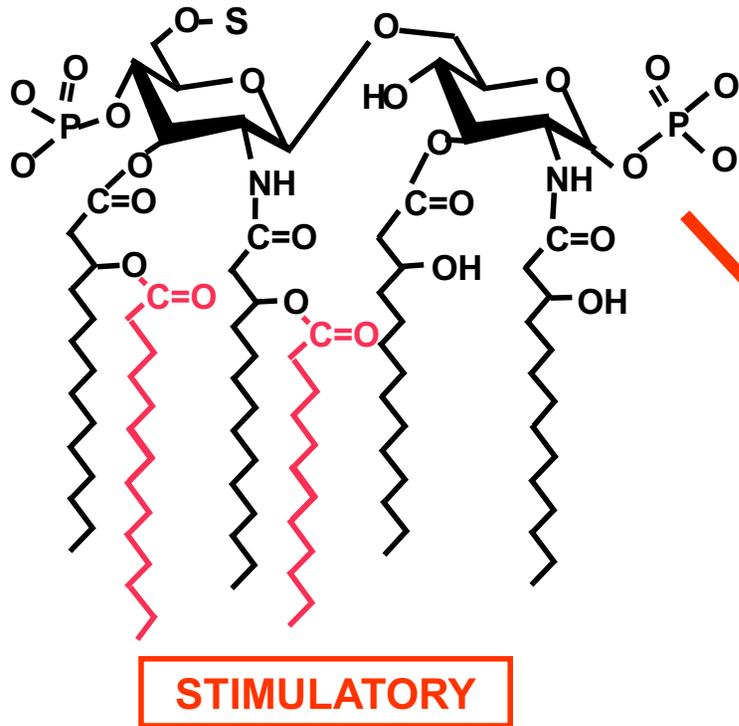
- *Streptococcus pyogenes*: “erysipelas”.
 - Bacterial enzymes, flags → inflammatory reaction → lymphatic engorgement, hyperemia, RASH
- Many bacteria, viruses:
 - inflammation → “innate immune tolerance”

Microbes don't destroy their own “flags”.

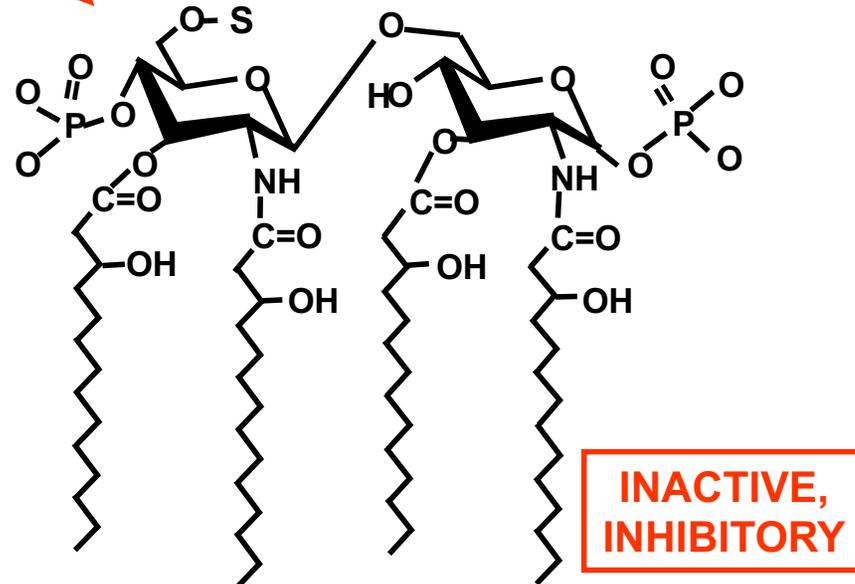
Some animal “flag burners”

- **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.



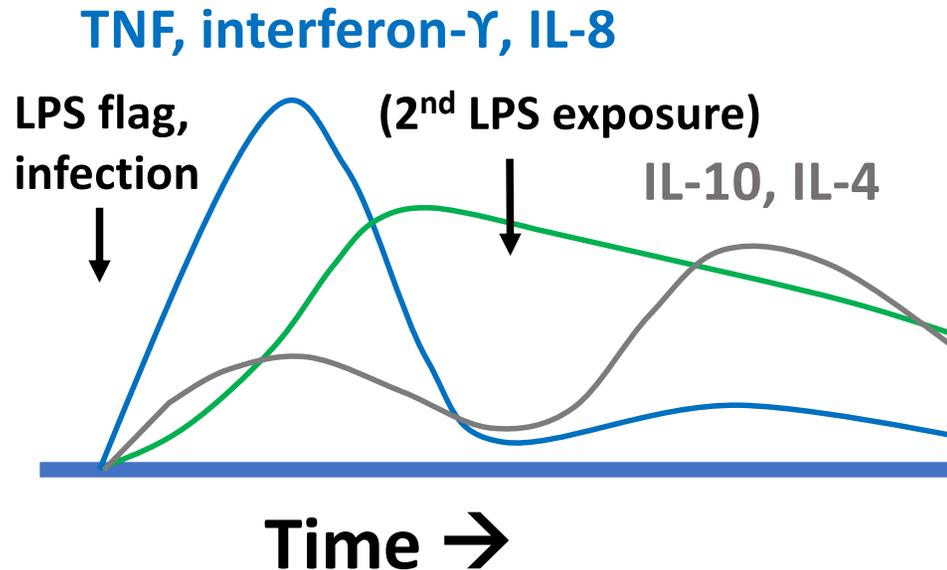
**ACYLOXYACYL HYDROLASE
(AOAH)**
(monocytes, dendritic cells, NK cells,
microglia, neutrophils, renal tubule cells)



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”



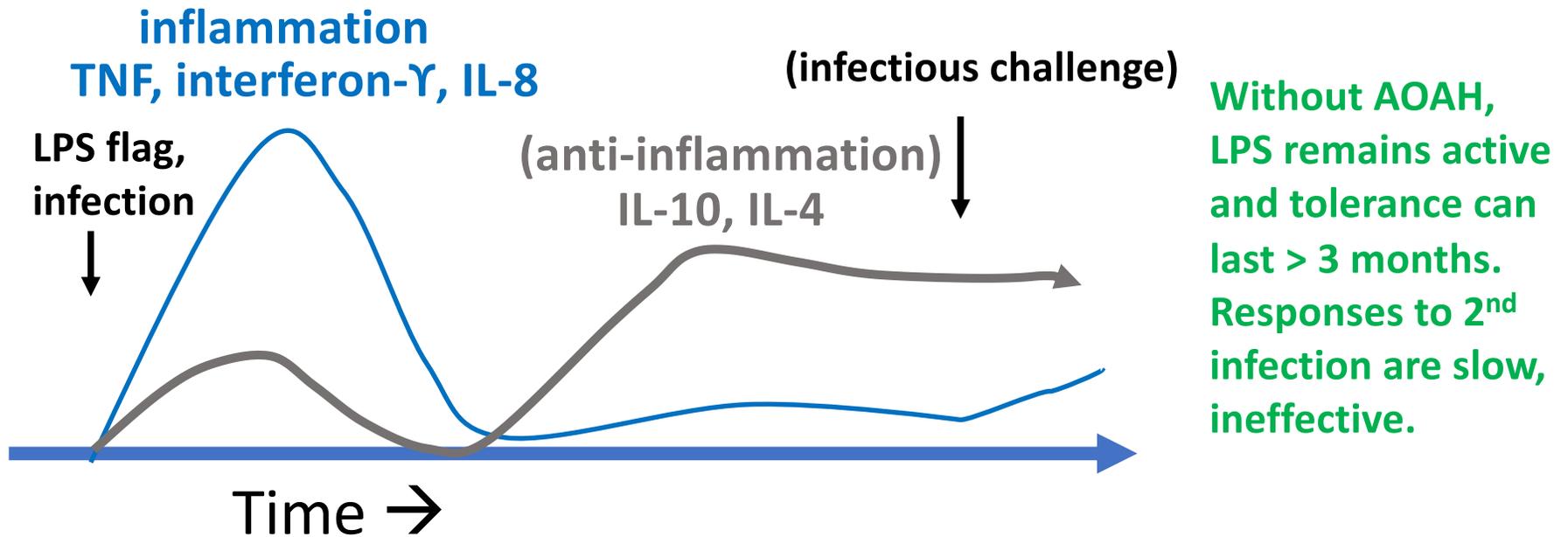
Macrophages, dendritic cells, others

AOAH
inactivates
LPS – after
“tolerance”
is induced

“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

Flag persistence may prolong immunosuppression.



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)**

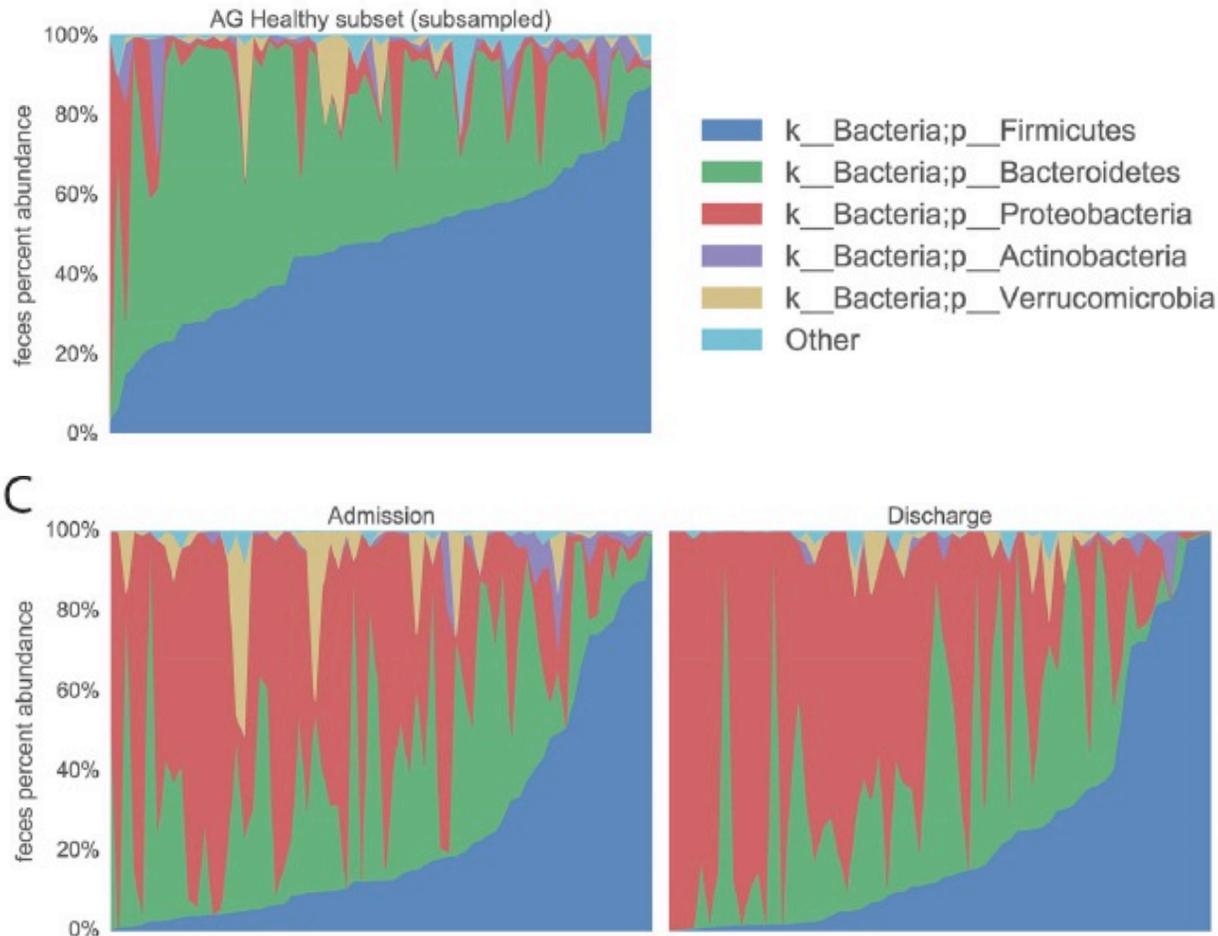


**Destroy
microbes
and their
flags**

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

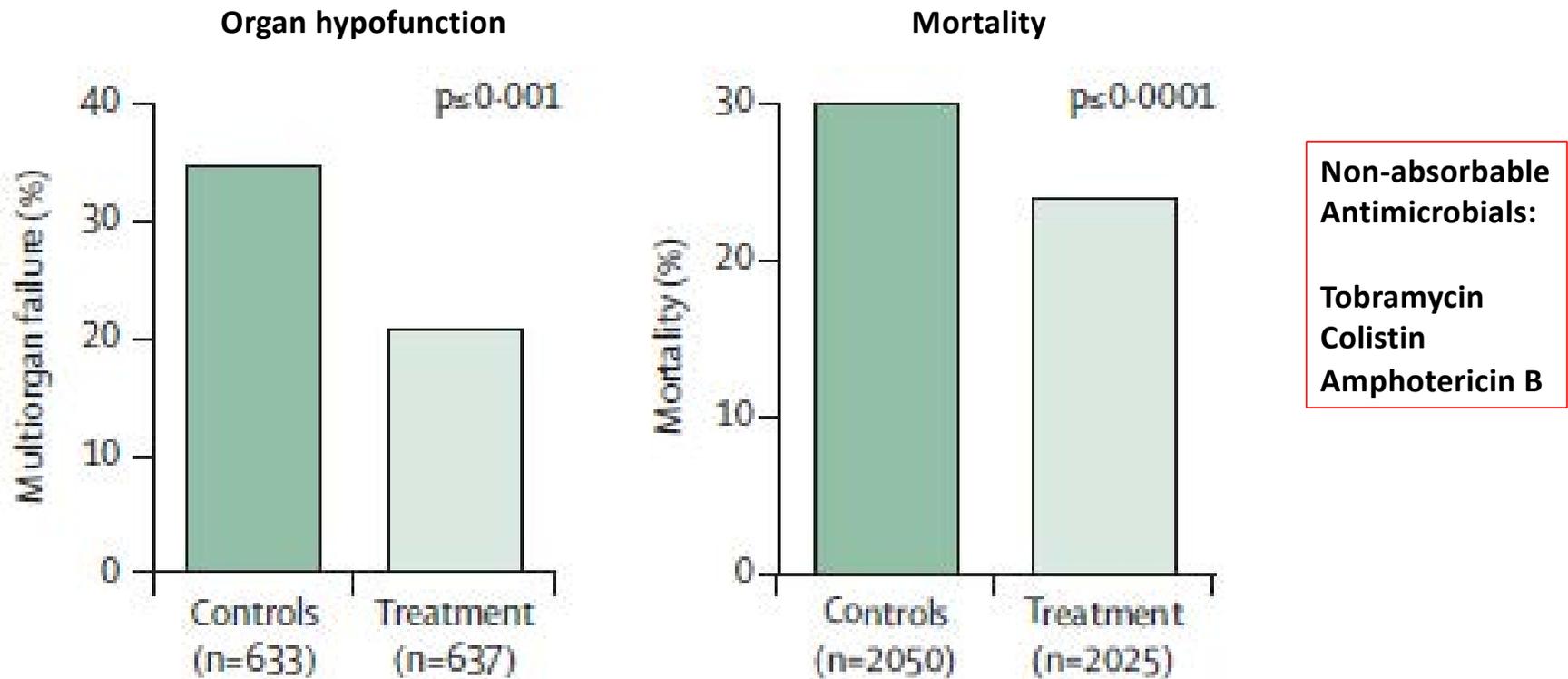


McDonald et al.
mSphere 1: e00199-16

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



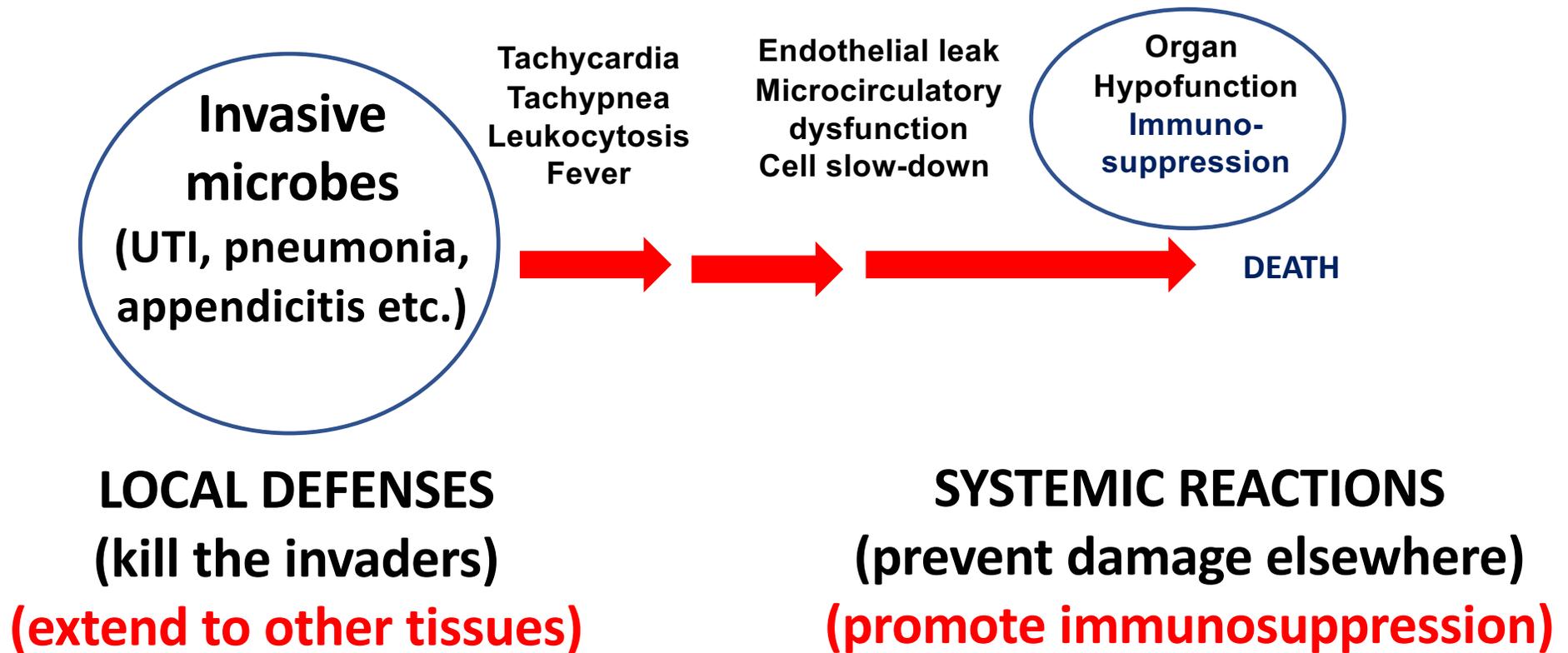
LPS translocation from intestine to blood

- **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 AOA
- **LPS-lipoprotein complexes** can be non-stimulatory, tolerance-inducing, or possibly stimulatory.

Prolonging immunosuppression: Alarmins?

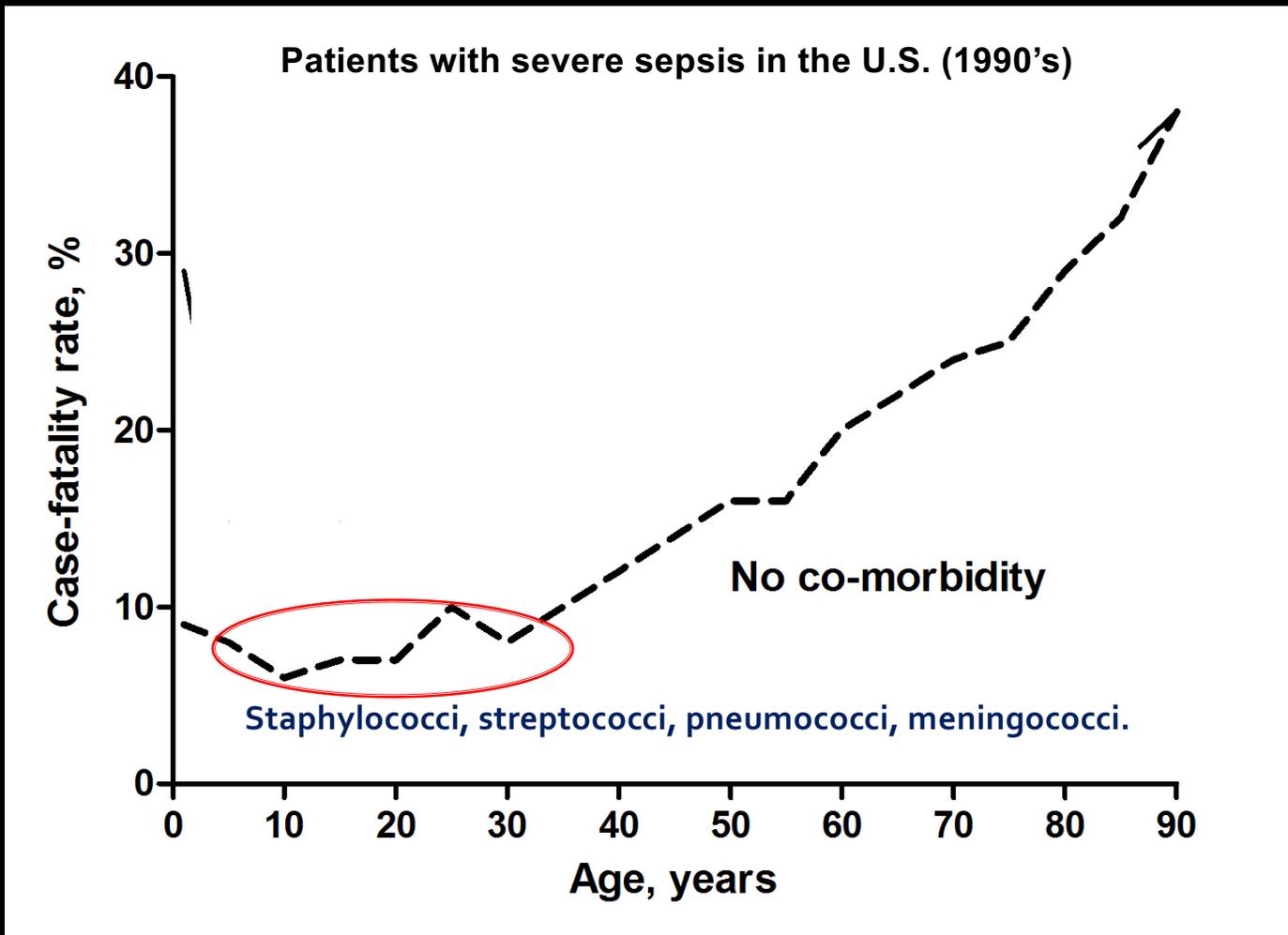
- Alarmins are **host** molecules that are released from cells in response to “danger” or cell death –
 - **HMGB-1** is the alarmin most likely to prolong immunosuppression
 - Pro- and anti-inflammatory activities
 - Anti-HMGB-1 antibodies can rescue animals from sepsis

Host reactions to infection

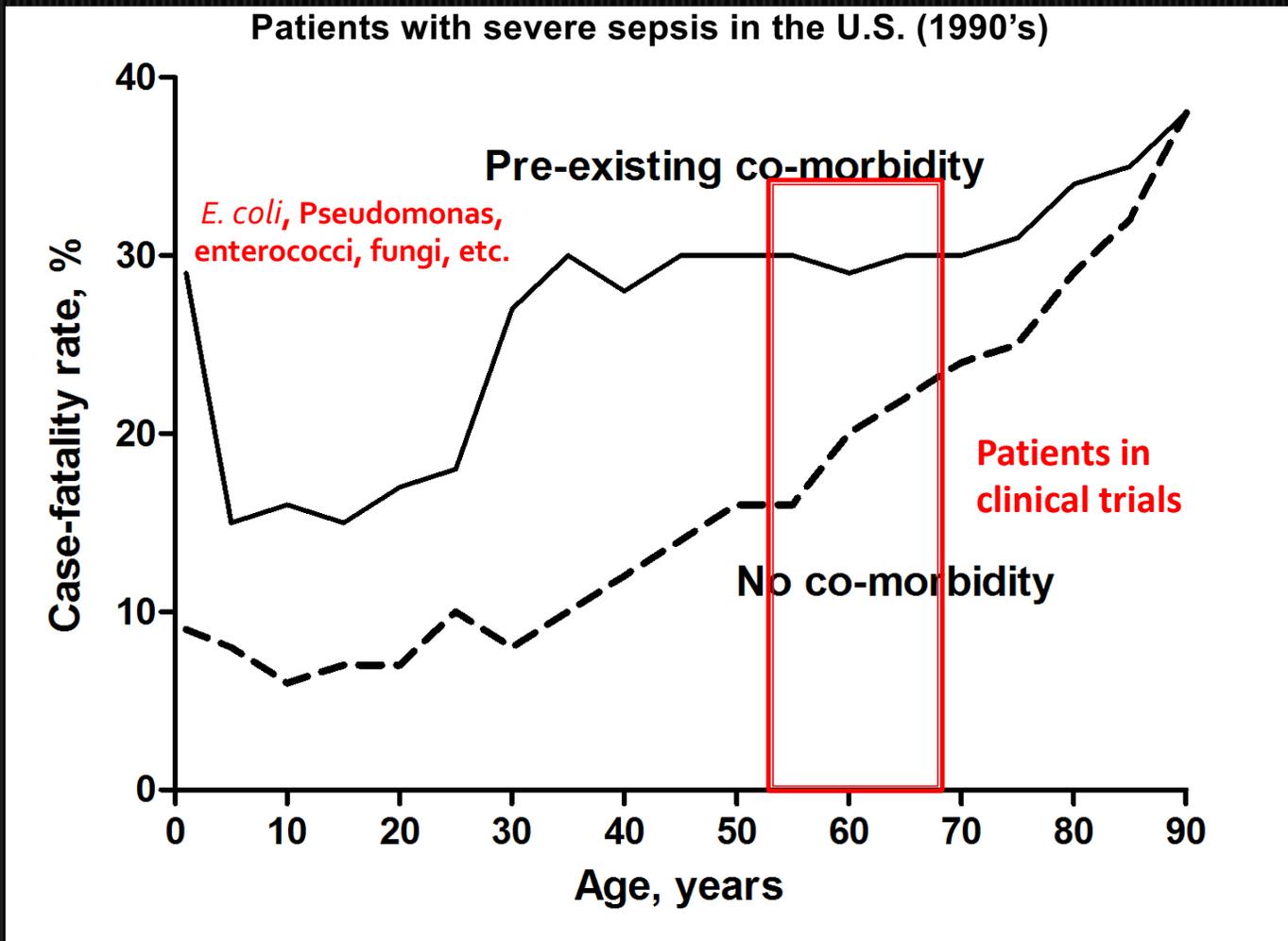


The “ideal” (simple) case is not common!

- 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.

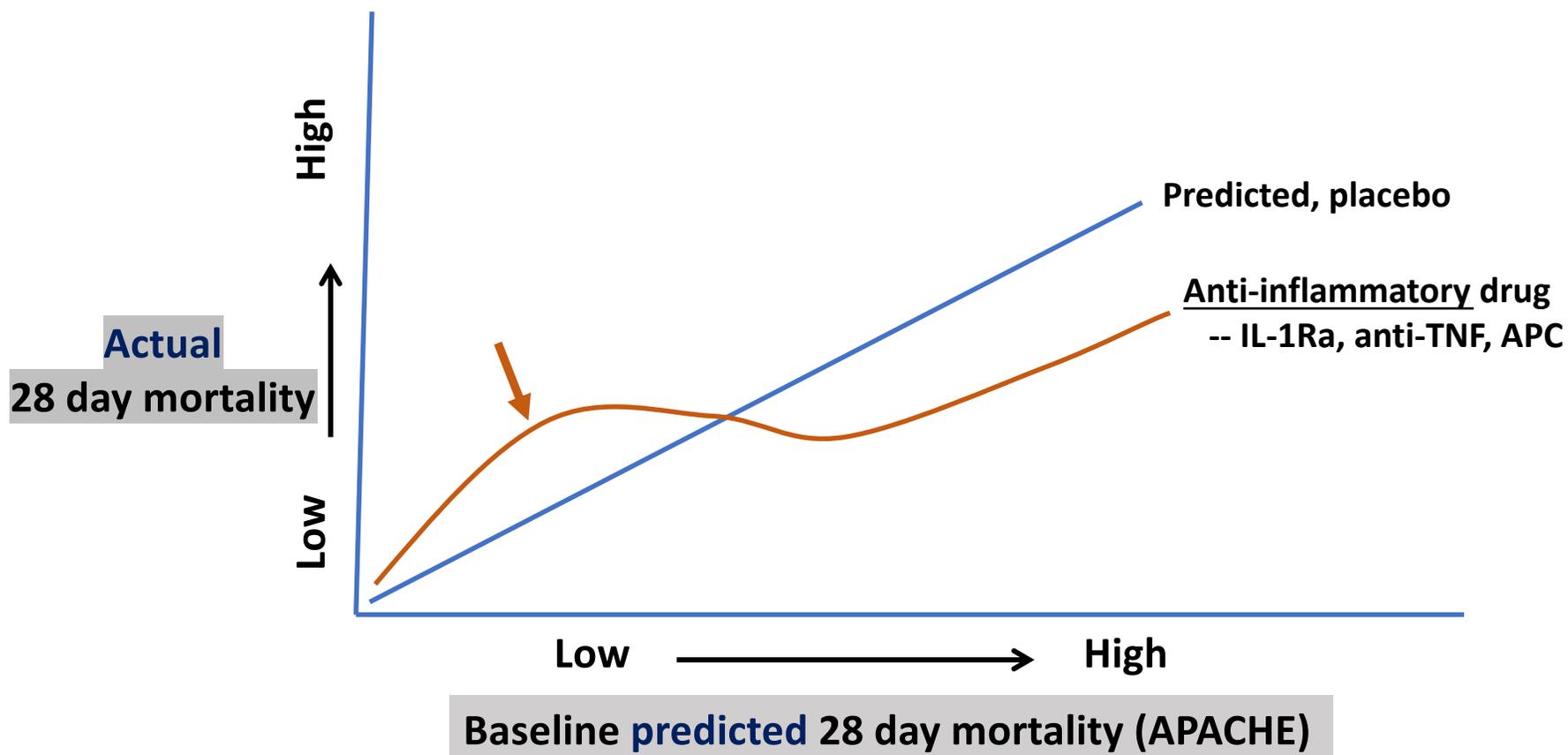


Angus et al. 2001. Crit Care Med 29:1303



Angus et al. 2001. Crit Care Med 29:1303

Augmenting the early systemic anti-inflammatory response may be harmful!



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.**