Clostridium sordellii: Clinical Settings, Diagnostic Clues and Pathogenic Mechanisms

Dennis L. Stevens, M.D., Ph.D.

Veterans Affairs Medical Center, Boise, ID and
University of Washington School of Medicine,
Seattle, WA

Introduction & Historical Perspective

- Organism 1st isolated by Alfredo Sordellii
- Named it *Bacillus oedematious sporogenes* for the marked tissue edema associated with infections
- Renamed *C. sordellii* in 1937
- Gram positive, spore forming, anaerobic rod
- Thought to be a "virulent" form of *C. bifermentans*
- The 2 species are differentiated on the basis of urease production

Case Report #1

- Day 1
  - 4 yo male; broke arm in fall
  - Vitals were normal; arm casted
- Day 2
  - Pain & marked swelling; afebrile, BP 128/82, pulse 150
  - IV cefazolin started; volar fasciotomy performed
- Day 2.5
  - ↑swelling & pain, BP 108/60, pulse 164, WBC 31,900 with left shift
  - Second trip to OR revealed necrotic muscle & fascia
  - Tissue samples grew *C. sordellii*
- Day 3
  - WBC 41,200, ↑ hypotension, ↑ tachycardia, metabolic acidosis
  - Pericardiocentesis yielded 75 mls fluid
  - Patient died

Case Report #2

- Day 1
  - 21 yo female; vaginal laceration during childbirth
- Day 5
  - Perineal pain, difficulty urinating, chills
  - At ER, temp 37.2, BP 96/57, pulse 132, WBC 67K
  - IV gentamicin and clindamycin begun
  - Fasciotomy of vulva; vancomycin added
  - Post-operatively, WBC 123.4K
  - Hypotension, metabolic acidosis, SVT (pulse 170)
  - Copius IV fluids administered
  - Cardiac arrest; patient died
  - Tissues grew *C. sordellii*

Database Search for Reported *C. sordellii* Infections

- Searched Index Medicus, PubMed and Medline over years 1925-present
- Keywords: *Clostridium sordellii, Bacillus sordellii and B. oedematious sporogenes*
- Yielded 28 reports describing 41 patients
- Two additional cases reported by us
- Males 49%; female 51%
- Age Range: 17 days – 95 years; Mean: 36 yrs

Vital Statistics

	Average Value (range)	
	Survivors N=12	Non-Survivors N=31
Age (years)	39.3 (12 - 61)	34.6 (0 - 95)
Gender	0 F/9 M/3 Unk	23 F/5 M/3 Unk
Temperature (C)	37.7 (37 - 38.6)	37.3 (35.3 - 41.1)
WBCx1000/mm^3	18.5 (9.2 - 32.3)	75.2 (19.0 – 200.0)

C. sordellii Infections

	Non-Survivors (N=31)	Survivors (N=12)
Post-partum/OB-GYN infection	9	0
MIA	5	0
Spontaneous abortion	2	0
Injection Drug Users	5	5
Trauma	4	2
Surgical Procedure	4	1
Other	2	4

Initial Symptoms of Fatal *C. sordellii* Infections	Number (of 26)* with Indicated Feature
Onset of illness 2-6 days	22
Decreased blood pressure	19
Vomiting	11
Nausea, dizziness	11
Generalized weakness	11
Diarrhea	5
Skin blue/ pale	3
Chills	2

*Only 26/31 fatal cases had enough clinical description to be included in the analysis.

Clinical Features *C. sordellii* Infections	Number (of 26) with Indicated Feature
Septic shock	23
Mild infection site pain	21
Leukemoid reaction	20
Afebrile	19
Tachycardia	19
Hemoconcentration	16
Tissue or visceral edema	16
Reduced serum protein	13
Metabolic acidosis	9
Decreased platelet count	7
RBC and WBC in urine	4

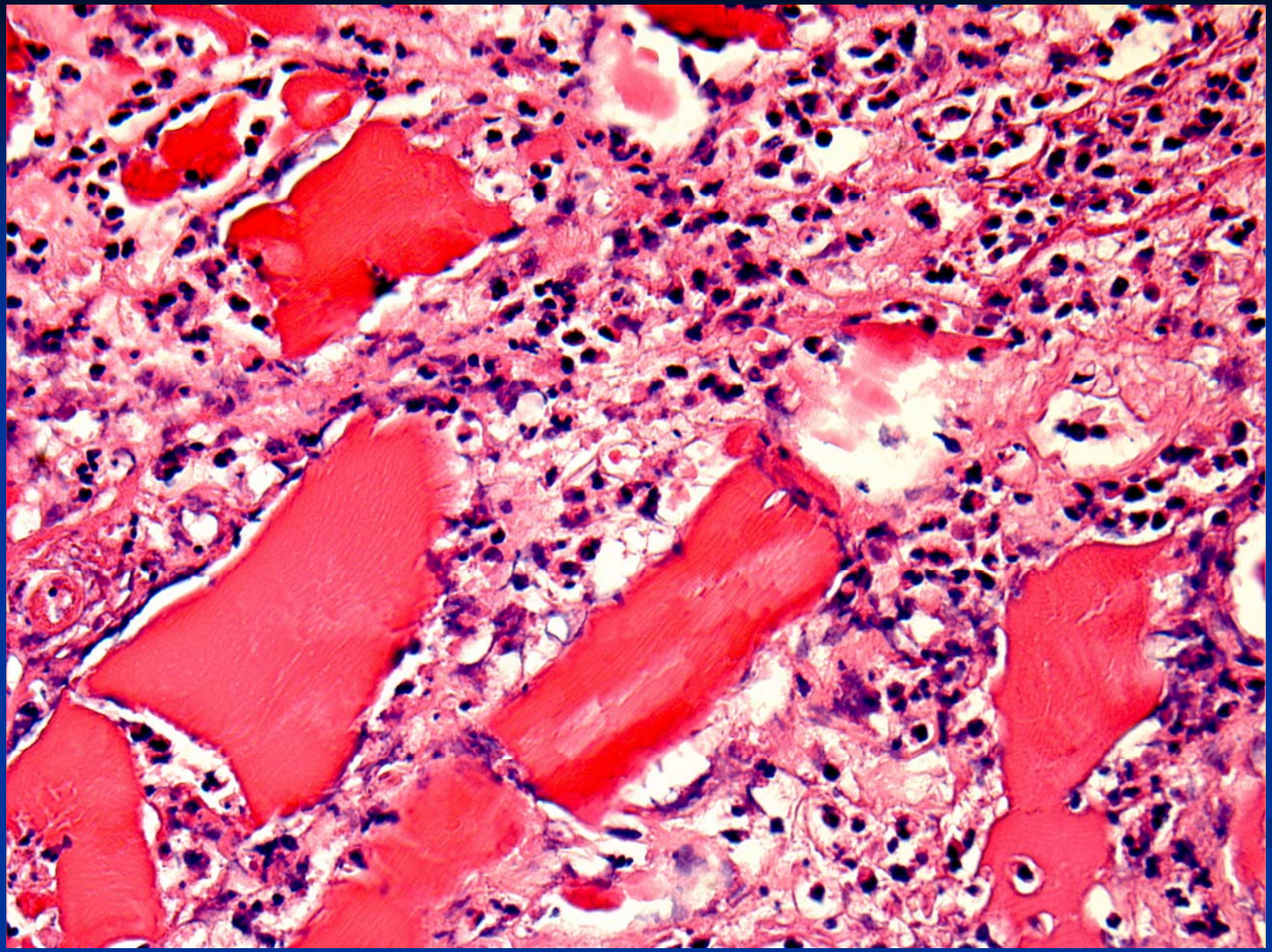
Treatments for *C. sordellii* Infections	No. (of 26) Receiving Indicated Treatment
Antibiotics	25
IV fluids and/or plasma	19
Debridement/amputation	16
Vasopressors, $NaHCO_3$	12
Steroids, morphine, atropine	11
Supplemental oxygen	5

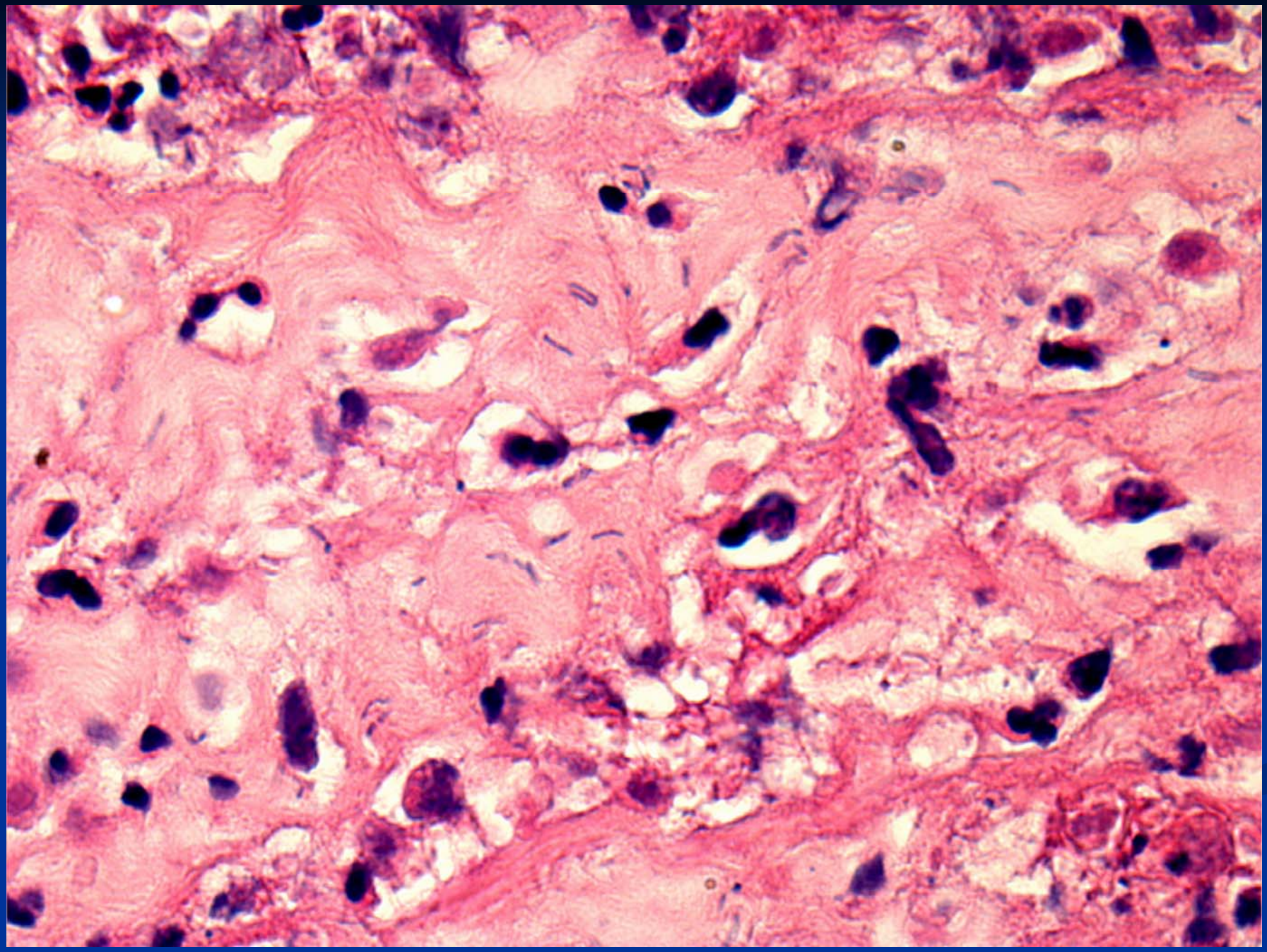
Microbiology of Fatal *C. sordellii* Infections	No. (of 26) with Indicated Feature
C. sordellii at site of infection	26
Other organisms at infection site	19
C. sordellii in blood	6
Clostridial antigens in localized blood vessels	4

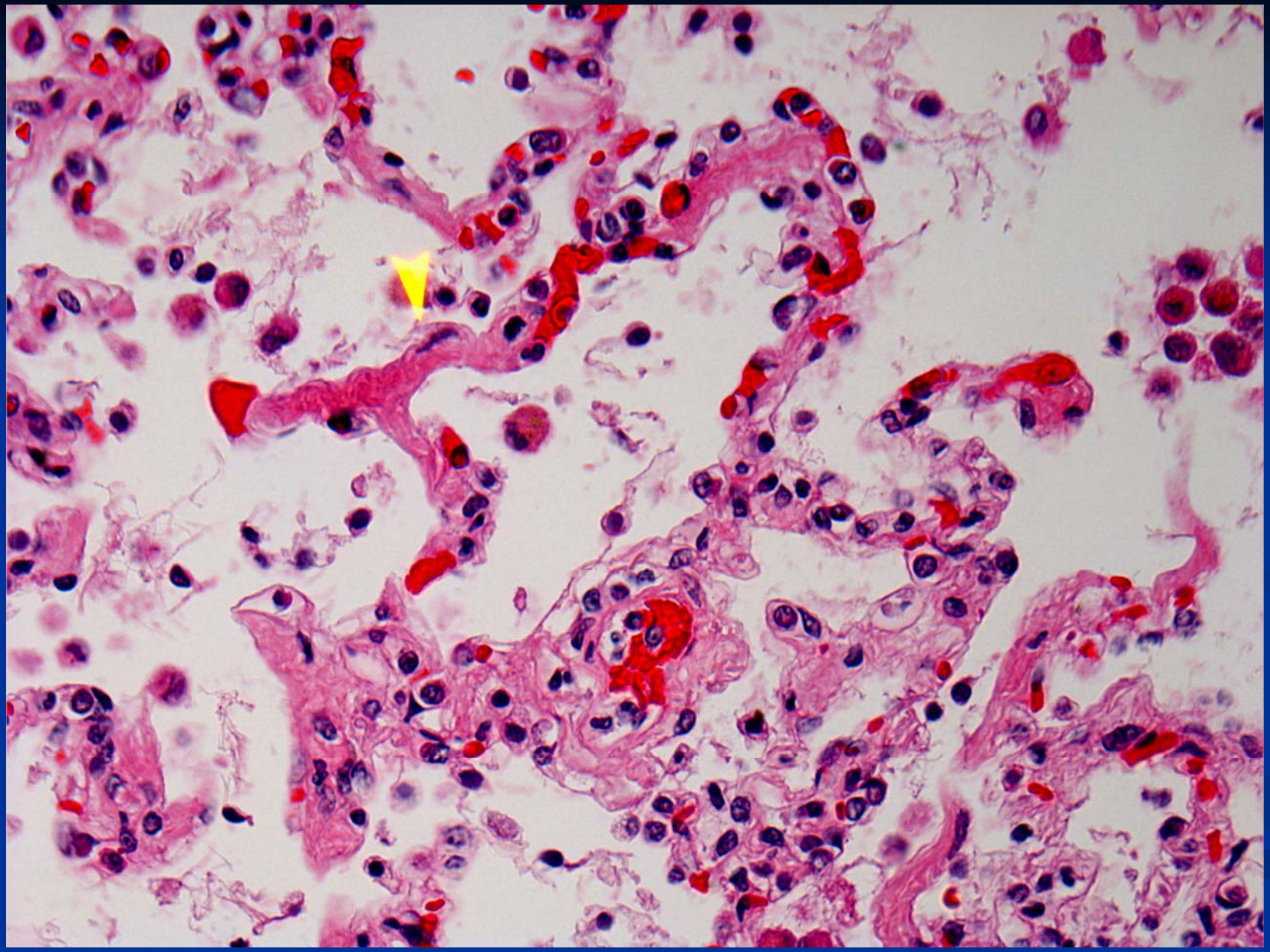
Organisms Isolated from Fatal *C. sordellii* Infections (N=31)

- *C. sordellii* (31)
- *C. perfringens* (6)
- *Escherichia coli* (5)
- *Staphylococcus spp.* (4)
- *Bacteroides spp.* (3)
- Group D streptococci (3)
- *Staphylococcus aureus* (3)
- α hemolytic streptococci (2)
- *Bacillus* spp. (2)
- *Enterococcus spp.* (2)
- Group G streptococci (2)
- *Peptostreptococcus spp.* (2)
- *Candida albicans* (1)
- *Clostridium beijerinckii* (1)
- Coryneform bacteria (1)
- *Enterobacter cloacae* (1)
- Group C streptococci (1)
- *Klebsiella pneumoniae* (1)
- Lactobacillus spp. (1)
- *Peptococcus prevotti* (1)
- *Prevotella loeschii* (1)
- Viridans streptococcus (1)

Autopsy Findings	No. (of 26) with Indicated Feature
Local necrosis, acute inflammation	20
Marked tissue and/or visceral edema	19
Pericardial, pleural and/or peritoneal effusion	19
Thrombosis of localized blood vessels	9
Neutrophil degeneration at margins of necrotic tissue	6







Pathogenesis

- Attributable to potent exotoxins
- Lethal toxin (LT) and Hemorrhagic Toxin (HT) have received most attention
- LT and HT are members of a family of Large Clostridial Cytotoxins (LCC) having molecular weights of > 200 kDa.
- Members are highly homologous
- Modify host proteins, such as signaling molecules Rho, Rac, CDC42, by attaching a glycosyl moiety
- The roles of other toxins have not been investigated

Species	Toxins Produced	Activity
Clostridium sordellii	Lethal Toxin *	Inhibits signaling G-proteins Rac, Cdc42, Ras and Rap.
	Hemorrhagic Toxin *	Inhibits signaling G-proteins Rho, Rac, and Cdc42.
	Hemolysin	Cholesterol-dependent hemolysin.
	Neuraminidase	Cleaves sialic acid residues from sialoglycoconjugates.
	PLC	Hydrolyzes lecithin.
	DNAse	Potential destruction of host cell nuclei.
	Hyaluronidase	Splits hyaluronic acid, increasing connective tissue permeability.
	Collagenase	Hydrolyzes collagen and gelatin.
Clostridium difficile	Toxin A *	Inhibits signaling G-proteins Rho, Rac, and Cdc42
	Toxin B *	Inhibits signaling G-proteins Rho, Rac, and Cdc42
	Binary Toxin	Actin-specific ADP-ribosyltransferase
	Hyaluronidase	Splits hyaluronic acid, increases tissue permeability
	Collagenase	Hydrolyzes collagen and gelatin.
Clostridium novyi		
Type A and B	α- Toxin *	Inhibits signaling G-proteins Rho, Rac, and Cdc42.
Type A	γ-Phospholipase C	Hydrolyzes lecithin.
Type A	Hemolysin	Cholesterol-dependent hemolysin.
Type B and D	Tropomyosinase	Cleaves tropomyosin and myosin.
Type B and D	β-Phospholipase C	Hydrolyzes lecithin; human RBC hemolysin.
Type D	Lipase	Hydrolyzes fats into glycerol and fatty acids.

* Indicates large clostridial cytotoxin.

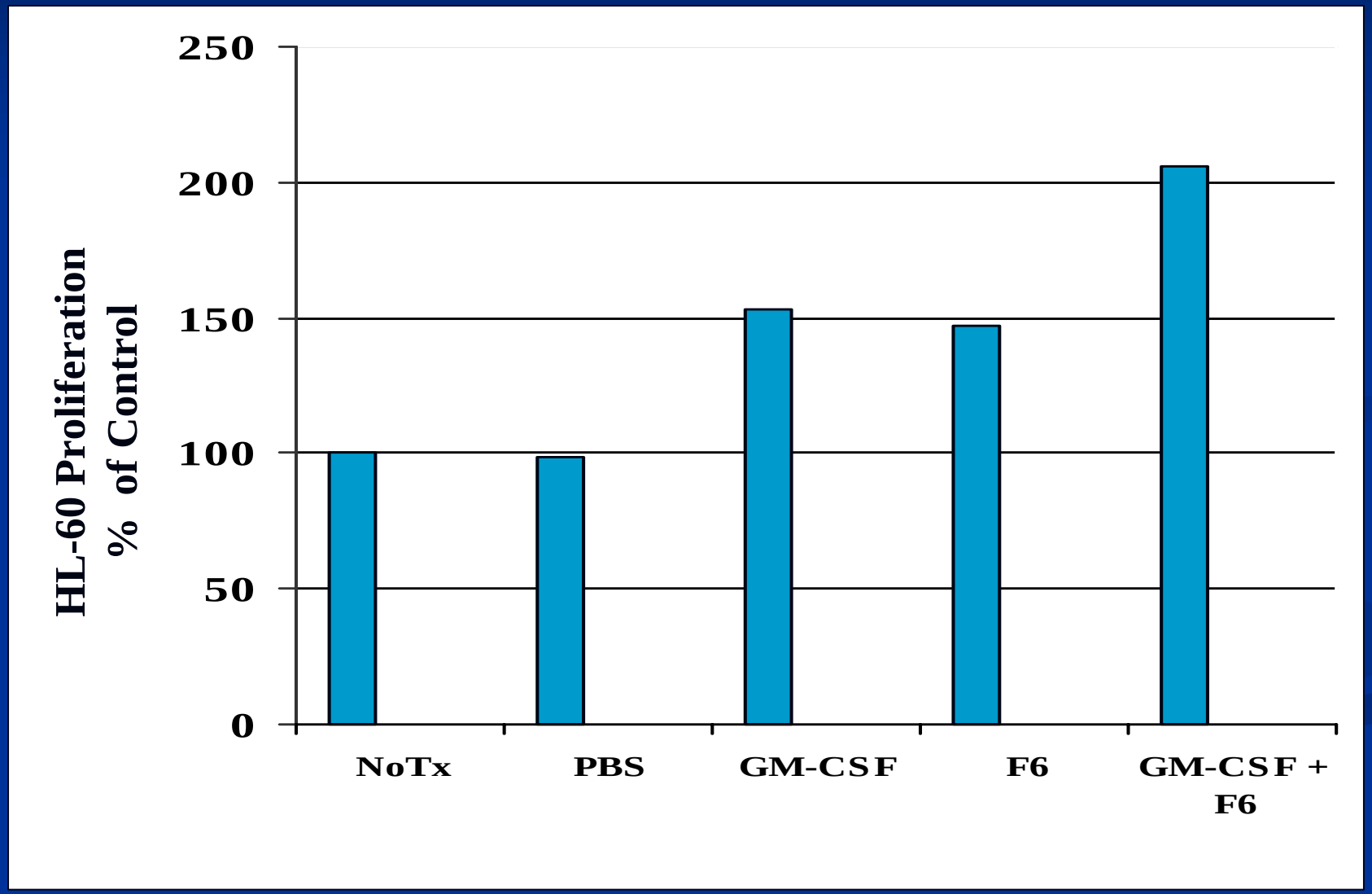
Our Investigations into the Mechanisms of Capillary Leak and Leukemoid Reaction

- Fractionation of *C. sordellii* exotoxins
- Endothelial cell permeability assays
- HL-60 proliferation assays
- Immune Recognition and Cytokine Induction

Cell Proliferation Assay

- Cells: HL-60 – a promyelocytic cell line
- *C. sordellii* toxins
  - Stationary phase culture of clinical isolate
  - Ammonium sulfate ppt; isoelectric focusing
  - Fractions over pI range 3 – 10 collected
- HL-60 exposed to fractionated toxins; GM-CSF served as positive proliferation control
- Cells quantitated by flow cytometry at 5 days

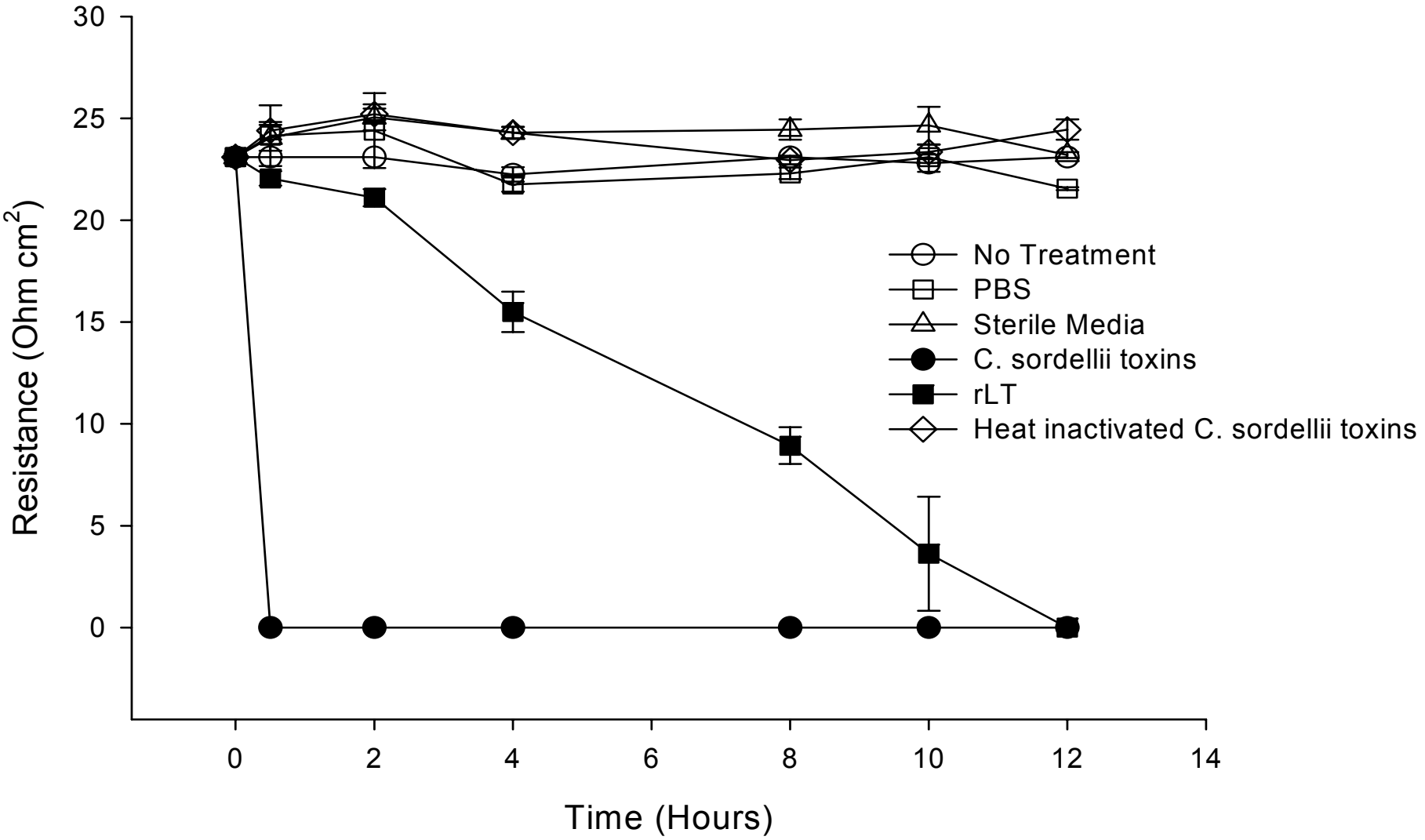
C. sordellii toxin-induced Myeloid Proliferation



Endothelial Cell Permeability Assay

- Primary human umbilical vein endothelial cells
- Cultured on membrane-lined insert to confluency
- Permeability measured by electrical resistance across membrane
- Toxins added and resistance measured over 12 hrs

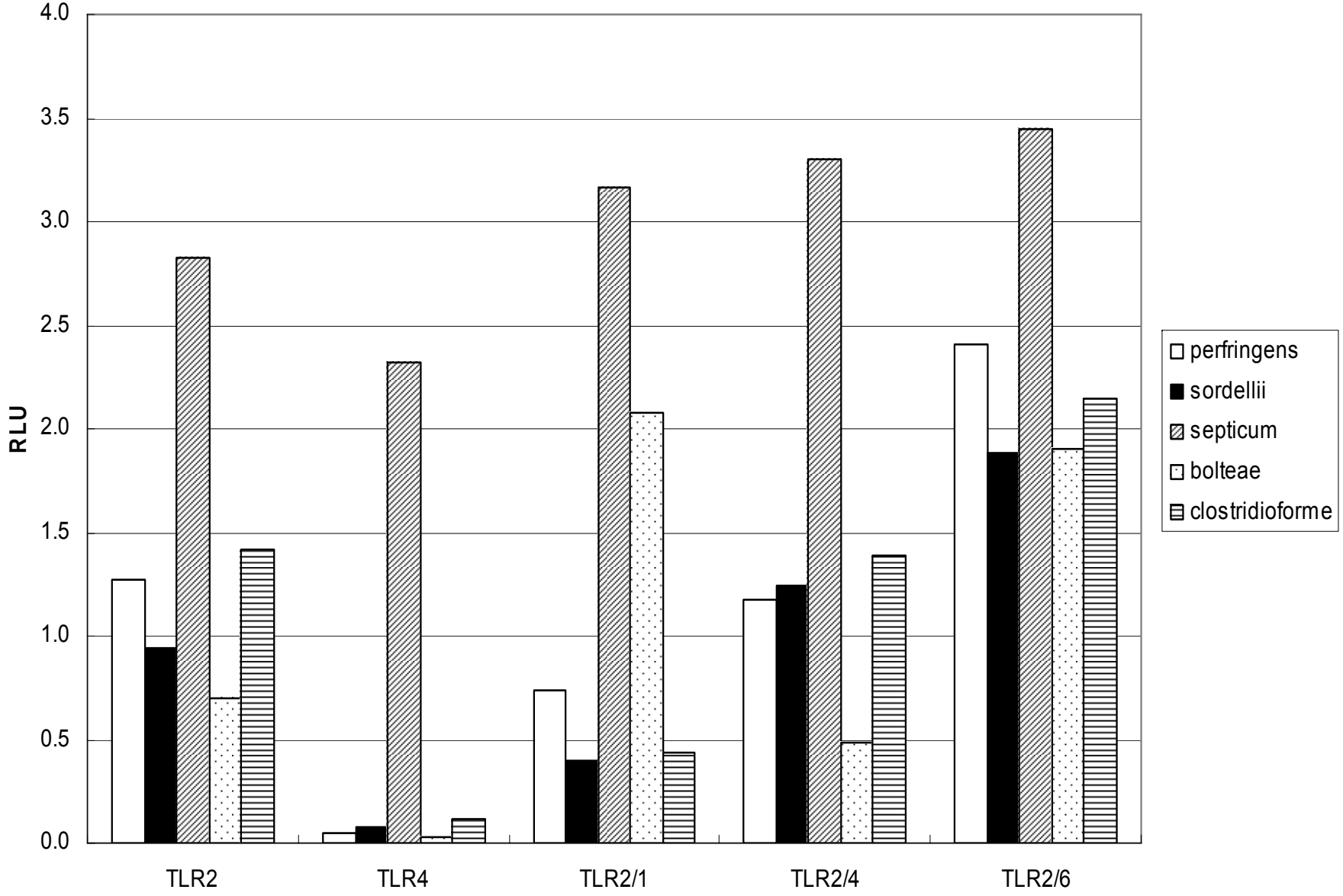
C. sordellii Exotoxin-Induced Endothelial Permeability



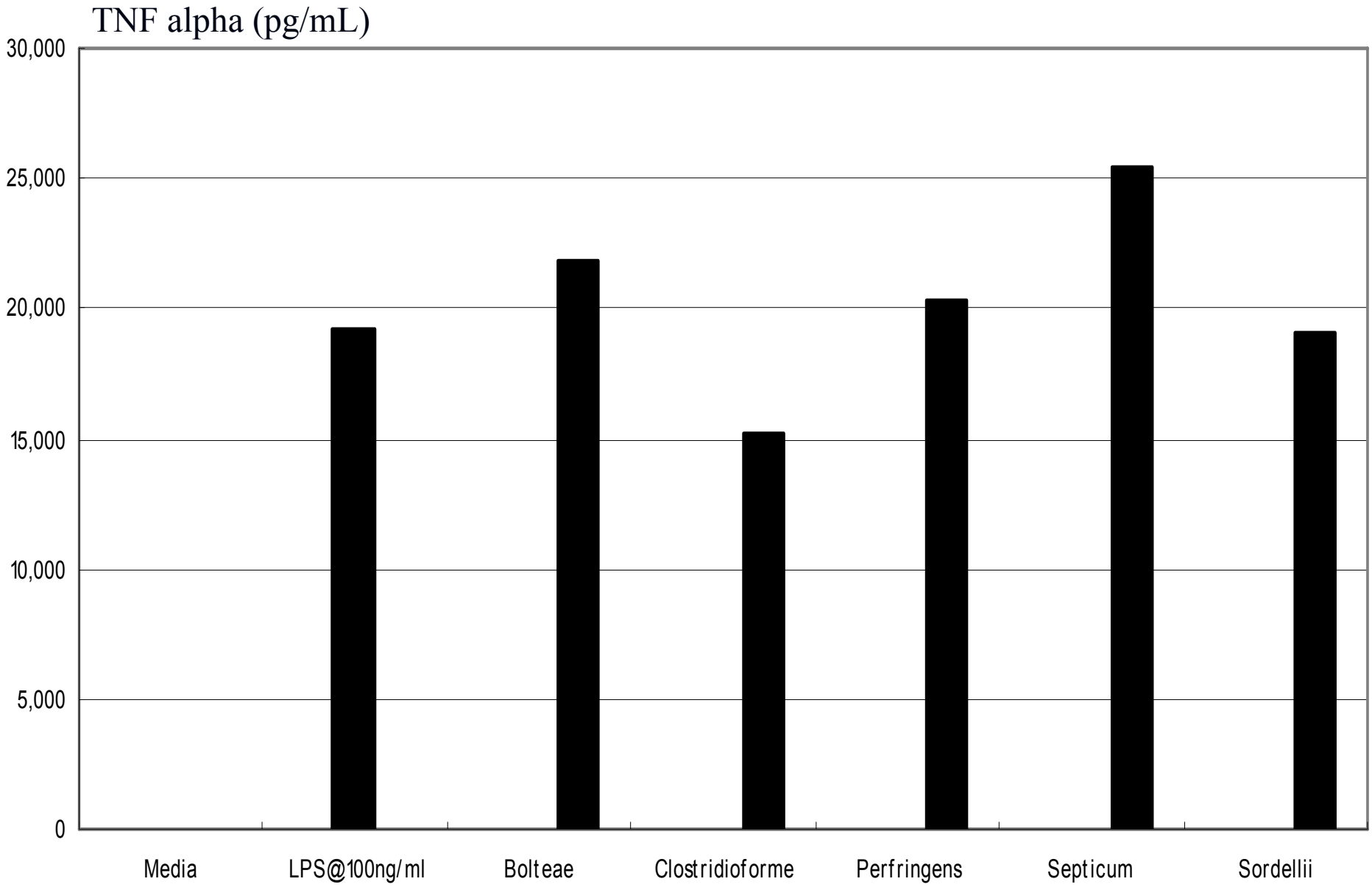
Innate Immune Recognition and Response to *C. sordellii*

- HEK-293 cells transfected with genes for TLR1, TLR2, TLR4, & TLR6, alone and in combination
- Cells also transfected with MD2, CD14, and ELAM-1-dependent luciferase reporter system
- Washed, log phase, heat-killed, LPS-free organisms added at an optical density at 650 nm of 0.3
- LPS added as a positive TLR4 agonist
- Luciferase activity measured at 4 hr
- Cytokines from PBMC stimulated in parallel were measured by ELISA

Innate Immune Recognition of the Clostridia



Innate Immune Response to the Clostridia



C. sordellii toxin-induced Cytokine Production

- PBMC isolated from healthy donors
- Stationary phase toxin preparation added for 6 hr
- Cytokines in overlying medium measured by protein microarray

C. sordellii toxin-induced Cytokine Production

	No Toxin	*C. sordellii* Toxins	rLT
TNFα	-	+++	+++
IL-1β	+/-	++	-
IL-6	-	+++	+++
IL-10	-	+	++
GM-CSF	-	+	-