Complement C5 Inhibition Blocks the Cytokine Storm and Consumptive Coagulopathy By Decreasing Lipopolysaccharide (LPS) Release in *E. coli* Sepsis

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E. coli

lipopolysaccharide (LPS)

outer membrane

peptidoglycan

periplasmic space

cytoplasmic membrane

GRAM-NEGATIVE
Inflammation

Microvascular thrombosis/DIC

LPS release

CD14-TLR4

NFκB signaling

Inflammation

Microvascular thrombosis/DIC

E. coli C3 convertase

C3b

C3a

C5a

C5b

C6-9

C5b9

TCC

C5 inhibitor

C3 inhibitor

Phagocytosis

Opsonization

Cytolytic pore

Lysis of host’s cells/tissues

Bacteriolysis

C5 convertase

C3 convertase

C5b9

C6-9

C5 inhibitor

C3 inhibitor

C3b

C3a

C5a

C5b

C6-9

C5b9 TCC

E. coli

AP

CP

LP

CD14

TLR4
Experimental Approaches

RA101295 C5 inhibitor: macrocyclic peptide; binds C5 with nanomolar affinity, and blocks C5 cleavage into C5a and C5b

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• In vitro: C5 inhibition in whole blood models of Gram negative sepsis (live E. coli or LPS)

• In vivo: C5 inhibition in a baboon model of E. coli sepsis
In vitro: C5 inhibitor (RA101295) reduced the C5b-9 and C5a formation in whole blood sepsis model.

HEK-TLR4 reporter cell assay for LPS.

Endotoxin (LPS) → TLR4 → QUANTI-Blue™ → Measure absorbance at 620-655 nm and calculate endotoxin concentration.
**In vitro:** C5 inhibitor reduced the oxidative burst but not bacterial phagocytosis in whole blood sepsis model

DHR123 oxidation test

In vitro phagocytosis test (Phagotest®)
**In vivo:** C5 inhibition in a baboon model of *E. coli* sepsis

**Experimental plan**

- **C5 inhibitor:** RA101295; 10 mg/kg SC
- **Full C5 inhibition**
- **Euthanasia**

- **T-1hr**
- **T0**
- **T+2hr**
- **T+8hr**
- **T+24hr**
- **T+36hr**
- **T+168hr**

- Vital signs/physiological parameters were recorded
- Blood biomarkers were measured

Tissue analysis
C5 inhibitor blocked C5b9 formation without affecting C3b generation thus allowing bacterial clearance by phagocytosis.
C5 inhibitor treatment decreased bacteriolysis and explosive LPS release

**LPS in plasma (HEK-TLR4 reporter cell assay)**

![Graph showing LPS levels over time](image)

**LPS Lipid A staining on blood smears**

(2hrs postchallenge)

![Images of blood smears and corresponding graphs](image)

**LPS (lipid A) staining in the lung**

![Images of lung tissue and corresponding graphs](image)

E. coli
E. coli +RA C5-I

C5 inhibitor treatment decreased bacteriolysis and explosive LPS release.
C5 inhibitor reduced sepsis-induced cytokine storm and neutrophils activation and release.
C5 inhibitor protects against consumptive coagulopathy
RAW TEXT
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