Amelioration of Sepsis by TIE2 Activation-Induced Vascular Protection

Jenn Hou
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Sepsis

- Globally, more than 19 million people are affected each year
- Causes mortality in half of sepsis patients
- Unregulated immune response to bacterial and viral infections
- Hallmarks: massive inflammation, blood vessel dilation, vascular leakage, multiple-organ failure (heart, kidney, lungs and/or liver)

- Risk Factors:
  - Immuno-compromised (age, chemotherapy, HIV)
  - ICU hospitalization
  - Bodily injury and wounds
  - Catheterization

Image from: https://my.vanderbilt.edu/sepsismonitor/files/2013/10/SIRS.jpg
Image from: http://www.westvalleytrauma.com/2015/03/sepsis-in-the-trauma-patient/
http://global-sepsis-alliance.org/global-burden/
http://www.mayoclinic.org/diseases-conditions/sepsis/symptoms-causes/dxc-20169787
The Pathophysiology of Sepsis


ANG2-Binding and TIE2-Activating Antibody (ABTAA) Associates with ANG2 to Promote TIE2 Activation

Can ABTAA bind to ANG2 to trigger TIE2 phosphorylation in cells?

\[ K_D = 0.2 \text{nM} \quad K_D = 78 \text{pM} \]

ABTAA binding to ANG2 promotes TIE2 activation and cell-cell localization in vitro.

ABTAA + ANG2 Promote Downstream TIE2 Signaling

How does ABTAA + ANG2 binding affect cell signaling?

Confluent, serum starved HUVEC

TIE2 Knockdown Abrogates ABTAA Mediated AKT and ERK Phosphorylation

How does the loss of function of TIE2 affect AKT and ERK signaling?

TIE2 function is critical for downstream signaling.

ABTAA Complexes with ANG2 and TIE2

Is ANG2 required for ABTAA and TIE2 interactions?

Size Exclusion Chromatography

- A2D: ANG2’s fibrinogen-like domain
- T2E: TIE2’s extracellular domain

A2D and T2E associate 1:1

Non-Reducing SDS-PAGE

Multi-Angle Light Scattering

Enzyme Linked Immunosorbent Assay

ABTAA Promotes ANG2 Oligomerization

Hypothesis: ABTAA can interact with multiple ANG2 to enhance its oligomerization.

How Does ABTAA Affect Survival in a Murine Sepsis Model?

High-Grade Cecal Ligation and Puncture (CLP)

Hypothesis: ABTAA will improve the survival of septic mice compared to ABA.

• Treat mice with Fc, ABA, or ABTAA 6 to 18 hours post-CLP.
• Track progress of mice in blind study.
ABTAA Improves the Survival of Septic Mice Post-CLP

How does co-administration of ABTAA and broad spectrum antibiotics impact survival outcomes?

Imipenem: 20% survival
ABTAA + Imipenem: 70% survival

Pre-Administration of ABTAA Prior to CLP Provides Protective Effects in Mice

Does pretreatment with ABTAA improve survival in septic mice?

ABTAA pre-treat: 50% survival  
ABA pre-treat: 17% survival

Higher doses of ABA does not improve septic mouse survival.

ABTAA Rescues Mice from Endotoxemia and Bacteremia

How general is the protective effect of ABTAA against two alternative mouse models of sepsis?

Structure of Lipopolysaccharide

ABTAA: 63% survival
ABA: 33% survival
Fc: 28% survival

ABTAA: 55% survival
ABA: 9% survival
Fc: 8% survival

Image from: https://www.researchgate.net/figure/233962820_fig1_Figure-1-Lipopolysaccharide-LPS-A-Schematic-diagram-of-the-structure-of-LPS-B
How Does Loss of Function of ABTAA’s Binding Partners Affect Its Efficacy Against Sepsis?

Loss of ANG1 accelerates death.

ANG2 antagonizes TIE2.

ABTAA association with TIE2 is required for survival.

ABTAA Protects Against Acute Lung Injury by Decreasing Leakage

Experimental Set-Up

ABTAA Protects Against Acute Lung Injury by Maintaining Pericyte Coverage

- CLP was performed, and mice were treated 6 and 18 hours afterwards.
- Pericyte coverage = NG2 area / CD31 area

ABTAA activates TIE2 to prevent EC-pericyte dissociation.

ABTAA Protects Against Sepsis by Retaining Endothelial Glycocalyx Integrity

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ABTAA reduces the expression of heparanase to maintain blood vessel wall integrity.

ABTAA Protects Against TNF-α Mediated Vascular Leakage

ABTAA Reduces Proinflammatory Cytokine and ANG2 Levels

Proposed Model of ABTAA Mediated Amelioration of Sepsis

Conclusions

- ABTAA binds to ANG2 to activate TIE2 receptors and trigger downstream phosphorylation.
- ABTAA rescues mice in three separate sepsis models.
- Co-administration of ABTAA with antibiotics further improves septic mouse survival.
- ABTAA alleviates septic severity by protecting against vascular leakage, reducing lung edema, maintaining glycocalyx integrity, and decreasing proinflammatory signals.
- ABTAA could act as a viable new therapeutic candidate for the prophylaxis of and treatment of sepsis.