Targeted deletion of DJ-1 attenuates morbidity and mortality in experimental sepsis through improved bacterial clearance

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Background

- Multi-organ dysfunction syndrome (MODS) is a hallmark of severe sepsis and septic shock.

- Mortality from sepsis: 30 – 50%. Rising incidence: >18 million cases of severe sepsis worldwide annually.

- Management of sepsis is largely supportive.

- Mesenchymal stem cell (MSC) administration reduced morbidity and mortality in experimental model of sepsis. Our lab exploited microarray analysis to understand the transcriptional effects of MSC administration on the host.
Background

Network Analysis Showing Mitochondrial Related Pathways Up-regulated after MSC treatment
Background

- originally identified as a gene mutated in familial Parkinson’s Disease (PARK7).
- majority of the papers – nervous systems (others: carcinomas, diabetes)
- functions as a scavenger of ROS; oxidized DJ-1 dimerizes and localizes to mitochondria; protects neurons against oxidative stress.
- widely expressed in tissues
- role of DJ-1 in sepsis – unknown.
Objectives

- To determine role of DJ-1/PARK7 in an experimental model of sepsis

- To understand the mechanisms by which DJ-1 contributes in the course of sepsis.

- To identify the clinical relevance of DJ-1 in septic patients.
Methods

1) In vivo:
Cecal Ligation and Perforation Model
18 gauge; through and through puncture
Fluid Resuscitation + Pain Management (Buprenorphine)
7 day study only – Antibiotics (Imipenem-Cilastatin)
DJ-1 Knockouts (Dr. Tak Mak)

2) In vitro:
Primary Bone Marrow Derived Macrophages (BMMs) & Peritoneal Macrophages (PMs)
LPS stimulation

3) Clinical:
Serum samples from Healthy, ICU control and Sepsis Patients
Polymorphonuclear Cells from Healthy and Sepsis Patients
DJ-1 knockout mice have significantly reduced mortality and morbidity from CLP-induced sepsis

A)  

B)  

C)  

D)
DJ-1 knockout mice have a marked increased in pro-inflammatory markers in CLP-induced sepsis.

A)

24 hours

- BALF Total Cell Count: WT > DJ-1 KO
- Total BALF Protein: WT < DJ-1 KO
- BALF IL-1β: WT < DJ-1 KO

48 hours

- BALF Total Cell Count: WT > DJ-1 KO
- Total BALF Protein: WT < DJ-1 KO
- BALF IL-1β: WT < DJ-1 KO

B)

LUNGS

- 24 hours: Fold Change over sham controls
- 48 hours: Fold Change over sham controls

SERUM

- 24 hours: Fold Change over sham controls
- 48 hours: Fold Change over sham controls

Legend:
- WT
- DJ-1 KO
DJ-1 knockout mice have a dramatically decreased bacterial load at 12 and 24 hours post sepsis.
DJ-1 knockout bone marrow derived macrophages (BMMs) have increased phagocytosis of *E. coli* and *S. aureus* particles.
**DJ-1 knockout bone marrow derived macrophages (BMMs) have increased expression of NADPH oxidase complex subunits and increased NADPH oxidase activity**

### A)

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### B)

- **Non-stimulated BMMs**
  - Nox2 activity (RLU/μg protein)
  - Time (mins)

- **LPS pre-stimulated BMMs**
  - Nox2 activity (RLU/μg protein)
  - Time (mins)

### C)

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Increased DJ-1 in serum is associated with worse outcome in sepsis patients.
Summary

**Bioinformatics**

- Gene Set Enrichment Analysis & Ingenuity Pathway Analysis: Importance of mitochondria related pathways
- Identification of DJ-1

**In vivo validation**

- DJ-1 KOs have improved survival after CLP
- Enhanced pro-inflammatory response in DJ-1 KOs.
- More Effective bacterial clearance in DJ-1 KOs.

**In vitro assessment**

- Evaluation of mechanism in isolated primary macrophages
- Parallel findings of effective phagocytic response due to enhanced NADPH oxidase activation

**Clinical relevance**

- Increased DJ-1 in serum of septic patients compared with controls
- Higher levels of DJ-1 correlate with increased mortality and MODS

**Evaluation as therapeutic target?**

- Delivery of anti-DJ-1 or DJ-1 knockdown therapies in experimental model of sepsis?
Acknowledgements

Collaborators:
Dr. Shirley Mei
Dr. Jack Haitsma
Dr. Srinivas Murthy
Dr. Pingzhao Hu
Dr. Golam Kabir
Dr. Jennifer Tsang

Dos Santos Lab
Dr. Yuexin Shan
Dun Yuan Zhou
Patricia Gali
Louis Zhou
Nima Jaberi

Committee:
Dr. Haibo Zhang
Dr. Howard Leong-Poi
Dr. Kim Connelly
Dr. Mingyao Liu

Collaborators:
Dr. Conrad Liles
Dr. Duncan Stewart
Dr. Mary Ellen Harper
Dr. Phil Marsden
Dr. Tak Mak
Dr. John Marshall

Early Research Award
Ministry of Economic Development and Innovation
Ontario

Canadian Intensive Care Foundation
CIHR IRSC
Ontario Genomics Institute