Together, Taking on Cancer’s Toughest Challenges

2019 Scientific Retreat
FRIDAY, APRIL 26, 2019
DISCOVERY WORLD PAVILION

REPROGRAMMING NEUTROPHILS TO CONTROL TISSUE INJURY

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NEUTROPHILS SEARCH FOR INJURY AND INFECTION

Damage-Associated Stimuli:
- Signs of Injury
- Mitochondrial DNA
- Mitochondrial Peptides (Formylated)
- Purinergic stimuli (ATP, etc.)

Bacterial Stimuli:
- Lipopolysaccharide (Gram Negatives)
- Lipopeptides
- Peptidoglycans
Damage-Associated Stimuli:
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INTRAVITAL IMAGING OF VENTILATED LUNGS
SURGICAL PREPARATION FOR LUNG IMAGING
INTRAVITAL IMAGING OF VENTILATED LUNGS

- Neutrophils crawling in normal lung
- 5 minutes real time or ~600 breaths

40 second projection

Stabilized

Unaligned

Albumin
Neutrophil

100µm
IMAGING WITH CELLULAR RESOLUTION

- Neutrophils adhere to inflamed capillaries
- 5 minutes real time or ~600 breaths

Neutrophil crawling velocity (µm/min)

Hours after Aerosol LPS

- p < 0.000001
- p < 0.001

Septal Thickness (µm)

Unstim LPS

- p < 0.02
- p < 0.05

Albumin

Stabilized

Unaligned
NITRIC OXIDE SYNTHASE 1 PROMOTES SEPTIC LETHALITY AND LUNG INJURY

NOS1 (Neuronal NOS)

Percent survival

Hours after LPS

WT
(n=42)

NOS1−/− (Neuronal NOS)
(n=31)

NOS2−/− (Inducible NOS)
(n=40)

NOS3−/− (Endothelial NOS)
(n=32)

NH2 PDZ L-Arg Dimer/Zn2+ Heme/BH4 OR-Hinge FMN R-Hinge FAD/NADPH COOH

P<0.0005
NITRIC OXIDE SYNTHASE 1 PROMOTES SEPTIC LETHALITY AND LUNG INJURY

**WT NOS1-/−**

### Lung Filtration Coefficient

- **WT**
- **NOS1−/−**

### Percent Survival

- WT
- NOS1−/− (Neuronal NOS) *(n=31)*
- NOS2−/− (Inducible NOS) *(n=40)*
- NOS3−/− (Endothelial NOS) *(n=32)*

### Hours after LPS

- 0
- 24
- 48
- 72
- 96

### Lung Filtration Coefficient

**LPS: − + − +**

- **WT**
- **NOS1−/−**
NITRIC OXIDE SYNTHASE 1 PROMOTES SEPTIC LETHALITY AND LUNG INJURY

NOS1 (Neuronal NOS)

Oxygenase

\[ \text{NH}_2 \text{PDZ} \rightarrow \text{L-Arg Dimer/Zn}^{2+} \text{Heme/BH}_4 \rightarrow \text{OR-Hinge} \rightarrow \text{FMN} \rightarrow \text{R-Hinge} \rightarrow \text{FAD/NADPH} \rightarrow \text{COOH} \]

Reductase

**WT NOS1-/- Untreated LPS, 8h**

Lung Filtration Coefficient

\[ \text{ml/min/cm H}_2 \text{O/g} \]

LPS: 0.01 0.02 0.03 0.04 0.05 0.06 0.07

WT

Unintreated

NOS1-/-

LPS, 8h

NITRIC OXIDE SYNTHASE 1 PROMOTES SEPTIC LETHALITY AND LUNG INJURY

NOS1 (Neuronal NOS)

nNOS

\( n=42 \)

P<0.0005

NOS1-/- (Neuronal NOS)

\( n=31 \)

NOS2-/- (Inducible NOS)

\( n=40 \)

NOS3-/- (Endothelial NOS)

\( n=32 \)

Percent survival

Hours after LPS

0 24 48 72 96

Lung Filtration Coefficient

\[ \text{ml/min/cm H}_2 \text{O/g} \]

LPS:

- +

INACTIVATION OF NOS1 LEADS TO NON-INJURIOUS SWARMING AND BLOCKS INFLAMMATORY VASCULAR INJURY

- 6 hours after LPS
- 30mg/kg, i.p.
- 5 minute time lapse

WT                  NOS1^-/-

Albumin
Neutrophli

CANCER COLLABORATIVE
NOS1 DRIVES INJURY, NOT NEUTROPHIL RECRUITMENT

Endothelial Neutrophil Albumin
NO S1 IS NOT REQUIRED FOR NEUTROPHIL SENTINEL FUNCTION OR PHAGOCYTOSIS

Endothelial Neutrophil Albumin Latex Beads 5 min. real time
REPROGRAMMING NEUTROPHILS IN TUMORS?

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- Peptidoglycans
If you want to go fast, go alone.

If you want to go far, go together.

– African Proverb

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