IMMUNOREGULATORY ROLE OF TISSUE MICROENVIRONMENT IN KIDNEY INJURY

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Ischemia-Reperfusion Injury
Controlling Inflammation in the Microenvironment

- Acute Tubular Injury
- Apoptosis
- Necrosis
- Necroptosis
- Ferroptosis
- Pyroptosis
- Tubular Obstruction
- Backleak

- Microvascular Injury
- Vasoconstriction
- Leukocyte Adhesion
  ↑ Permeability
- Microvascular Congestion

- Innate Immunity
- DAMPS
- Immune Cells
- Cytokines
- Inflammation

- Acute Tubular Injury
- Apoptosis
- Necrosis
- Necroptosis
- Ferroptosis
- Pyroptosis
- Tubular Obstruction
- Backleak

- Systemic Response
- Hemodynamic
- Neural
- Immunological
- Oxidative Stress

- Controlling Inflammation in the Microenvironment
Purinergic signaling – in the Microenvironment

Modified, Lu, AJP-Cell Physiology, 2014
Renal Interstitial Microenvironment

Mononuclear Phagocytes- contiguous network in kidney

T J Soos et al. Kidney Int. 2006 70:591–596

L. Li et al. Kidney Int. 2008 74:1526-37

Green = CX3CR1-GFP
Red = MHC Class II
Red = F4/80
Dendritic Cell Microenvironment

- Autophagy
- Cell to Cell Contact
- PAMPS
- DAMPs
- Humoral mediators (adenosine)
- Filtered Antigens
- Tissue Resident

Tolerance
- Immature DC
- T Reg Cell

Immunity
- Mature DC
- Effector T cell
CD73 Is a 5’-Nucleotidase Generates Extracellular Adenosine

B. Kaissling, M. Le Hir *Histochem Cell Biol.* 2008
CD73 Expression in PT Cells and Mesangial Cells

Ischemia-Reperfusion Injury: Methods

Creatinine is a Marker of Kidney Function

Equilibration  Ischemia  Reperfusion
22-26 min  24 hr

\[ \text{Creatinine} \rightarrow \text{Kidney Injury} \]

Plasma Creatinine, mg/dl

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>IRI</th>
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<tbody>
<tr>
<td>Creatinine</td>
<td>0.5</td>
<td>1.5</td>
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Graph showing an increase in creatinine levels in the IRI group compared to the Sham group.
Absence of CD73 Sensitizes Mice to IRI


CD73 KO mice was a gift from Linda Thompson
Non-hematopoietic cell CD73 protects kidneys from mild IRI

Cre-LoxP System

 Conditional KO mice

Floxed CD73 mouse

CD11c Cre mouse

PEPCK Cre mouse

CD11c Cre CD73<sup>fl/fl</sup> "DC" CD73 KO

PEPCK Cre CD73<sup>fl/fl</sup> Proximal Tubule CD73 KO

PEPCK Cre Gift of Volker Haase, Vanderbilt University
CD73<sup>fl/fl</sup> Gift of Dr. Jurgen Schrader, Heinrich-Heine-Universität Düsseldorf
Proximal Tubule Deficient CD73 (PEPCK Cre CD73$^{fl/fl}$)

PEPCK Cre gift of Volker Haase, Vanderbilt
Proximal Tubule CD73 Is Necessary To Protect From IRI

CD73 inhibition increases IRI susceptibility in WT mice

CD73 Protection Due To Its Enzymatic Activity

5’ Nucleotidase or A2a agonists Rescue CD73 KO Mice

A

Plasma Creatinine (mg/dL)

Sham, Vehicle, AMP-CH2-P

Mean

E

Plasma Creatinine (mg/dL)

PCre73FL/Veh-S, PCre73FL/Veh, PCre73FL/5NT, PCre73FL/AI, Veh-S, Veh, 5NT, ATL

F

% Tubular Damage

PCre73FL 73/-

Chronic Inflammation and Fibrosis: *Microenvironment*


Li Li et al 2012 JCI
Perivascular cell-specific CD73 KO (FoxD1CreCD73^{fl/fl}) mouse model

Foxd1Cre CD73^{fl/fl} and littermate control CD73^{fl/fl} mice

Sung et. al, JASN 2017
Kidney function and collagen deposition in FoxD1CreCD73^{fl/fl} mice are increased in 14-day unilateral IRI-fibrosis model.***p≤0.001, 2-way ANOVA, t-test
Plasma creatinine and collagen deposition in *FoxD1CreCD73^{fl/fl}* mice are increased in 14-day unilateral IRI-fibrosis model

Male, 8-12 weeks old, 18-25 g

***p≤0.001, 2-way ANOVA, t-test
Lack of CD73 in perivascular cells leads to more myofibroblast (PDGFRβ+ αSMA+) transformation

N. Goerldt, J. Schrader, M. Okusa et al. unpublished observations 2018
Isolated pericytes are PDGFRβ-positive

N. Goerldt, J. Schrader, M. Okusa et al. unpublished observations 2018
CD73 deficient pericytes/fibroblasts proliferate faster and express more α-smooth muscle actin.

N. Goerldt, J. Schrader, M. Okusa et al. unpublished observations 2018

**p≤0.01, ****p≤0.0001, 2-way ANOVA
Summary/Conclusions

Acute Kidney Injury
- The kidneys of PT CD73 deficient mice are more susceptible to IRI.
- Reconstitution of CD73 KO mice with 5’NT rescues mice to minimize injury.

Kidney Fibrosis
- Presence of CD73 in perivascular cells protects the kidney from progression to fibrosis after unilateral IRI
- CD73 deficient pericytes/fibroblasts are more prone to transform into myofibroblasts

CD73 in the kidney microenvironment controls inflammation and protects kidneys from AKI and Progressive Fibrosis
Pannexin1 is an ATP release channel

- Pannexin 1 (PANX1), a **transmembrane protein** belongs to a family of proteins exhibiting a structural homology to gap junction-forming invertebrate innexins.
- Large, non-selective transmembrane channels that **efficiently release ATP** to the extracellular space upon activation.
- Pannexin 1 is the most **ubiquitously expressed** of the three pannexins.

Penuela, BBA-Biomembranes, 2013
ATP release through Pannexin 1 channels leads to protection and cell metabolism regulation. ATP activates P2X, P2Y, and AR receptors.

Panx1 knockout models show differences in plasma creatinine levels between sham and IRI conditions, with specific knockout models indicating potential protection and regulation.

Microenvironment in AKI

Immune Cell Migration Amplify Tissue Injury

PMN Recruitment

Adenosine Receptors A1, A2A, A2B, A3

ATP/ADP

CD39

AMP

CD73

IL-12

IFN-γ

IL-17

IL-23

Kinsey et al 2012 JASN

Li Li et al 2012 JCI

Lappas et al 2006 JEM

Li Li et al 2010 JCI

Li Li et al 2007 JI
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Damp

Pannexin 1

ATP

Retained ATP

Innate Immunity/Inflammation

ATP → ADP → AMP → Adenosine

P2X

Cell Death

P2Y

CD 73

Cell Survival

AR