Myelopoiesis following myocardial ischemia involves activation of the Nlrp3 inflammasome by neutrophil-derived S100A8/A9

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Title: Myelopoiesis Following Myocardial Ischemia Involves Activation of the Nlrp3 Inflammasome by Neutrophil-Derived S100a8/a9

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Monocytosis, Neutrophilia and Myocardial Ischemia

After acute MI, immune cells play a central role in protecting viable myocardium from ischemic damage and promoting repair of the infarcted tissue.

Patients with a proinflammatory response (monocytosis) during the acute phase of ischemia show impaired recovery of the left ventricular function (JACC 2002) and have poor functional outcomes (AHJ, 2012).

Neutrophilia is associated with adverse functional outcomes in STEMI patients (AJC, 2004). Neutrophil/lymphocyte ratio (NLR) is the strongest predictor of death/MI in high risk CAD patients (JACC 2005).

Severe proinflammatory response (enhanced myelopoiesis) is not good for the injured heart!!
Myelopoiesis during myocardial ischemia (MI)

- HSPC
- CMP
- CLP
- MEP
- GMP
- B Cells
- T Cells
- Erythrocytes
- Platelets
- Granulocytes
- Monocyte

BONE MARROW

Spleen

Ly6-C^(hi)
Ly6-C^(lo)
Neutrophils

Injured Heart

BLOOD

Block in Artery
Muscle Damage
Time course of monocyte and neutrophil recruitment to the injured heart
What drives myelopoiesis?

- HSPC
- CMP
- CLP
- MEP
- GMP
- Erythrocytes
- Platelets
- Monocyte
- Granulocytes
- B Cells
- T Cells
- Neutrophils
- Ly6-Chi
- Ly6-Clo

BONE MARROW

Block in Artery
Muscle Damage

Injured Heart

BLOOD
MI by ligation of LAD coronary artery

C57BL6/J

Sham

Day 1

Day 3

Day 5

Day 16

Monocyte, Neutrophil, HSPC and Myeloid progenitor cell profile-
Blood
Bone marrow
Spleen
Heart

n = 8-11
MI induces monocytosis and neutrophilia via proliferation of hematopoietic stem and progenitor cells in the BM
Neutrophils and Ly6-C^{hi} monocytes are rapidly recruited to the injured heart
So, what drives myelopoiesis?
DAMPs (Damage Associated Molecular Pattern Molecules)

Endogenous factors sequestered intracellularly (physiological conditions) but released into the extracellular environment by necrotic cells (cellular stress/injury)

DAMPs can be construed as signals of a potential danger to the host, trigger inflammation under sterile conditions

Important DAMPs: S100A8, S100A9 & HMGB1 and receptors include RAGE & TLR2/4 and CD36

S100A8 needs to form a hetero-tetramer complex with its binding partner S100A9 to be functionally active
Role of Damage Associated Molecular Pattern Molecules (DAMPs) in myelopoiesis

**Type 1 Diabetes**

- Blood Glucose → ROS → S100A8/A9 → RAGE → Myelopoiesis → Monocytes & Neutrophils

**Type 2 Diabetes/Obesity**

- Adiposity → Necrosis → TLR4/Nlrc3 → S100A8/A9 → ATM → IL-1β → IL-1R → Myelopoiesis → Monocytes & Neutrophils

Nagareddy and Murphy et al, Cell Metabolism 2013

Nagareddy et al, Cell Metabolism 2014
Does S100A8/A9 -Nlrp3 inflammasome pathway promote myelopoiesis in MI?
MI induces S100a8/a9 and Tlr4 in ischemic hearts

DAMPs

- S100a8
- S100a9
- Hmgb1

DAMP Receptors

- Tlr4
- Rage
- Cd36
MI induces Nlrp3 and Pro IL-1β in ischemic hearts.
What is the cellular source of S100a8/a9 and Nlrp3 inflammasome in ischemic hearts?
Isolation of neutrophils and fibroblasts from mouse hearts

Sham or MI (LAD, 24 Hrs)

Cardiomyocyte Fraction

Fibroblast Fraction

Magnetic Beads

CD11b+, Gr1+ Monocytes/neutrophils

CD11b-, Gr1-, CD45-, CD31- Fibroblasts

RT-PCR
S100a8/a9 is expressed primarily in CD11b+Gr1+ neutrophils infiltrating the ischemic hearts.
S100a8/a9 localize mainly with Gr1+ neutrophils
Nlrp3 and Pro IL-1β are mainly induced in infiltrating neutrophils.
Does depletion of neutrophils lead to a decrease in Nlrp3 inflammasome and IL-1β in the heart?
Depletion of neutrophils in the blood and heart reduces Nlrp3 and Pro-IL1β expression in the ischemic heart.
Does deletion of S100A8/A9 prevent MI-induced myelopoiesis?
S100a9⁻/⁻ mice showed reduced expression of Nlrp3 and IL-1β in neutrophils following MI.
S100a9−/− mice were protected from MI-induced myelopoiesis.
Summary and Conclusions

MI-induced myelopoiesis peaks within 24 hours of coronary artery ligation

Myelopoiesis is due to expansion and proliferation of stem and myeloid progenitor cells in the BM and spleen

Myelopoiesis is mediated by S100A8/A9 -Nlrp3 inflammasome pathway

It is likely that S100A8/A9 released from necrotic fibroblasts/cardiomyocytes attract neutrophils followed by induction of Nlrp3 inflammasome and Pro IL-1β via TLR4/Myd88 pathway

The released IL-1β in conjunction with other CSFs interact with their receptors in bone marrow and spleen to promote myelopoiesis
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Medullary and Extra Medullary Hematopoiesis

Bone Marrow

Spleen
Absence of neutrophils fails to induce Nlrp3 and Pro-IL1β expression in “no flow ischemia” model (ex vivo)

Sham or MI

Perfusion for 15 min
No flow ischemia for 1 Hr.

RT-PCR

mRNA expression in the heart
(Fold change compared to control)

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<th>CON</th>
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<tbody>
<tr>
<td>S100a8</td>
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* indicates statistical significance
Model of Nlrp3 inflammasome induction and activation by DAMPs

DAMPs