Hemolysis and Immune Activation

Karina Yazdanbakhsh, PhD
Executive Director, Research Institute
New York Blood Center
Hemolysis in Sickle Cell Disease (SCD)

- Hemolysis activates the underlying endothelium: increased expression of endothelial adhesion molecules and apoptotic markers

- Attachment of sickle RBCs and other blood components to the vessel wall; in vivo heme injection induces vascular stasis and acute chest syndrome

- Heme oxygenase 1 (HO-1) breaks down heme upregulated in SCD

- Heme scavenging/removal system (hemopexin and haptoglobin) is overwhelmed


Belcher et al.2006 J Clin Invest; 116(3):808-16
Patrolling Monocytes

- Phagocytose cellular debris derived from damaged endothelial cells
- Control endothelial damage in atherosclerosis models and clear vascular amyloid beta in Alzheimer’s disease
- SCD express high levels of HO-1 in patrolling monocytes: control T cell anti-inflammatory profile in SCD under hemolytic conditions


Hypothesis: HO-1 expressing patrolling monocytes clear heme damaged endothelial cells and sickle RBC attached to ECs in SCD, dampening inflammation

**Expanded subpopulation of circulating patrolling monocytes expressing high levels of HO-1 in SCD**

Interaction of cell free heme with endothelial cells is prerequisite for optimal induction of HO-1\textsuperscript{hi} expression in patrolling monocytes.
**HO-1\textsuperscript{hi} Patrolling Monocytes in SCD patients with Vaso-occlusive Crisis (VOC)**

- **sVCAM-1**
- **Neutrophils**
- **Total monocytes**

**HO-1\textsuperscript{hi} patrolling Mo correlate negatively with VOC in SCD**

Phagocytosed RBCs in Circulating SCD PMOs

RBC engulfed material is present in the circulating PMOs of patients with SCD which is further increased during crisis, and may lead to reduced PMo numbers.
Mechanism of Sickle RBCs Uptake by PMo

PMo uptake sickle RBC only when attached to ECs mostly through CD11a, CD18 and ICAM1; upregulate HO-1: cryoprotective; heme damaged ECs increase sickle RBC uptake by PMo
PMo Uptake Sickle RBCs In Vivo

In vivo, PMo uptake sickle RBCs, but not control RBCs in part through monocyte CD11a
PMo Uptake  EC-attached Sickle RBCs In Vivo

Blue: CD31/CD144, Red: Dil, Green: GFP

Scale = 10 µm
**In Vivo Effects of Sickle RBCs and Hemin in Nr4a1-/- mice**

Lack of patrolling monocytes drives heme-mediated endothelial activation and SCD RBC stasis

In Vivo Effects of Sickle RBCs and Hemin in Nr4a1-/- mice

GFP-Nr4a1

Patrolling monocyte protect against heme-driven endothelial activation and can inhibit hemolysis-driven SCD RBC stasis

With Patrolling monocyte

Endothelial cells

Hb

Erythrocyte

Heme-damaged Endothelial cells

ICAM-1

VCAM-1

HO-1

CD11a

HO-1

CD18

P-selectin

Patrolling monocyte

Without Patrolling monocyte

Vaso-occlusion

Classical Mo
Protection from plasma cell-free hemoglobin and heme in sickle cell disease

Victor R. Gordeuk Blood 2018;131:1503-1505
• Yunfeng Liu
• Hui Zhong
• Weili Bao
• Woelsung Yi
• Vijendra Ramlall

• Patricia Shi
• Xiuli An
• Avital Mendelson
• Francesca Vinchi

Montefiore Hospital
• Deepa Manwani
• Caterina Minniti
• Joan Uehlinger
• Ron Walsh

Children’s Hospital of Philadelphia
• David Friedman
• Stella Chou

Funding Support
NIH/NHLBI:
R01HL121415
R01HL130139
American Heart Association
**In Vivo Vascular Effects of Hemin in Nr4a1<sup>−/−</sup> Mice**

PBS or Hemin

**WT**

**Nr4a<sup>−/−</sup>**

(no patrolling monocytes)

24hr

Immunofluorescence (vascular activation: ICAM-1 expression on CD31/CD144+ endothelium)

**ICAM-1**

**HO-1**

**W T N r 4 a 1  - /-**

**PBS**

**Nra4<sup>−/−</sup>**

**+PBS**

**WT**

**+hemin**

**Nra4<sup>−/−</sup>**

**+hemin**

**New York Blood Center**
Manipulation of PMo Numbers Affects Sickle RBC Stasis In Vivo

Depletion of PMo numbers increases sickle RBC attachment to vascular endothelium and RBC stasis in SCD mice, while increasing their nos protects against tissue/organ damage

Biburger et al. Immunity. 2011;35:932