Macrophages and Wound Healing and Skin Regeneration

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Disclosure

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Macrophages and Wound Healing and Skin Regeneration

Participants will be able to:

• Understand the role of macrophages in the wound healing process
• Understand how macrophage dysfunction contributes to delayed wound repair
Physiology of Wound Healing

- Blood clotting
- Exudation/inflammatory phase
- Proliferation and granulation phase
- Migration/re-epithelialization
- Maturation
Inflammatory Cells in Wound Healing

- Platelets
- Neutrophils
- Mast Cells
- Macrophages

Cells are chemoattracted to sites of injury by release of small molecules from damaged cells and degranulating platelets.
Macrophage function

Phagocytes

– Clearing of matrix and cell debris

Macrophages are potent cellular sources of cytokines, growth, and angiogenic factors.
Why focus on macrophages?

Macrophages are elevated
– in human chronic leg ulcers
– in wounds in genetically diabetic (db/db) and obese (ob/ob) mouse models

Depletion of wound macrophages dampens excessive inflammation and restores tissue regeneration in ob/ob mice.
Macrophages - Classic studies

- Anti-sera and hydrocortisone induced monocytopenia resulted in –
  - Poor clearance of dead and damaged cells, fibrin, and tissue debris
  - Delayed healing

*under sterile conditions

**guinea pig model

Wound Healing in the PU.1 Null Mouse—Tissue Repair Is Not Dependent on Inflammatory Cells

Paul Martin,¹ ¹ Deana D’Souza,¹ ¹ Julie Martin,¹
Richard Grose,² Lisa Cooper,¹ Rich Maki,³
and Scott R. McKercher³
PU.1 knockout mice

- PU.1 knockout mice lack neutrophils, macrophages, and B cells
  - No delay in wound healing
  - Slight enhanced rates of re-epithelialization

Caveat: Experiments are in embryo or neonatal pups (day 1-4) under sterile conditions
Chemokines and Macrophages

- Macrophage-inflammatory protein 1 (MIP-1$\alpha$) knockout have normal wound repair

- Monocyte chemotactic protein 1 (MCP-1) knockout mice have
  - delayed re-epithelialization
  - delayed angiogenesis
  - reduced collagen synthesis

Low et al (Dipietro L). Am j Pathol 2001
A Transgenic Mouse Model of Inducible Macrophage Depletion

Effects of Diphtheria Toxin-Driven Lysozyme M-Specific Cell Lineage Ablation on Wound Inflammatory, Angiogenic, and Contractive Processes

Goren et al. Am J Pathol 175(1) 2009
Wound closure in DTox-treated lysM-Cre/DTR mice
Summary of findings

- lysM-Cre/DTR mice with reduced wound tissue macrophages display
  - Exacerbated inflammatory response
    - Increased MIP-2, MCP-1, Cox-2, and IL-1β
    - Reduced TGF-β
  - Dysregulated VEGF
  - Loss of wound contraction
  - Absent myofibroblast differentiation

*Caveat: lysM is shared by both macrophages and neutrophils*
Physiology of Wound Healing

- Blood clotting
- **Exudation/inflammatory phase**
- Proliferation and granulation phase
- Migration/re-epithelialization
- Maturation

Macrophages persist in all the phase of repair
Differential Roles of Macrophages in Diverse Phases of Skin Repair


Tina Lucas,* Ari Waisman,† Rajeev Ranjan,* Jürgen Roes,‡ Thomas Krieg,*§ Werner Müller,¶ Axel Roers,‖ and Sabine A. Eming*
A: DT injection regime A

B: DT injection regime B

C: DT injection regime C
Early depletion of macrophages

- attenuates epithelialization and granulation tissue formation, wound contraction, angiogenesis, and myofibroblast differentiation
Mid-stage depletion of macrophages

• abrogates transition into maturation stage
Late stage depletion of macrophages

• does not affect wound healing
Macrophage Dysfunction Impairs Resolution of Inflammation in the Wounds of Diabetic Mice

Savita Khanna, Sabyasachi Biswas, Yingli Shang, Eric Collard, Ali Azad, Courtney Kauh, Vineet Bhasker, Gayle M. Gordillo, Chandan K. Sen, Sashwati Roy*

Background
• Diabetes impairs phagocytic function of macrophages
• Diabetic wounds have elevated apoptotic cell count
Efferocytosis at the wound site is a pre-requisite for resolution of inflammation and wound healing.
Key findings

• Diabetic wounds have increased load of apoptotic cells
  – Impaired clearance activity of macrophages at wound site
  – Increase apoptotic cell count of PMNs
Key findings

• Increase apoptotic cell burden in diabetic wounds augment inflammatory response

• Impaired dead cell clearance activity in diabetic wound macrophages compromises resolution of inflammation

*benefits of debridement
**environmental stimuli provide cues for macrophage activation
skin injury and chronic ulcers

- Inflammatory and cytotoxic
  - Good infection control
  - Tissue destruction
  - TNF-α
  - Nitric oxide

- Anti-inflammatory and tissue repair
  - Good wound healing
  - IL-4, IL-13, or glucocorticoid, immune complex
  - Pro-angiogenic factors
  - IL-10
  - Collagen precursors

Classically activated Mφ

Precursor monocyte

Alternatively activated Mφ
Metabolism of arginine by macrophage subsets

Classically activated Mφ

L-Arginine → L-OH Arginine

NOS2

L-ornithine → polyamines

Arginase-1

Nitric oxide

Citrulline

Cell proliferation

Collagen production

Alternatively activated Mφ

IL-4/IL-13
IL-10
GM-CSF

Ornithine aminotransferase

proline
Delayed wound healing in KLF-4 deficient mice

Average Percent of Initial Wound (mm²)

Days

KLF4-/-

WT

n=9
TNF-α and iNOS are elevated in the skin of KLF-4 deficient mice 48 hours post wounding.
No accelerated healing in wildtype animals with combined treatment (UVB + PPAR γ). Tissue destruction and severe delayed wound healing in combined treatment of IL-6−/− mice.
IL-6-dependent PPAR-γ activation results in tissue destruction and delayed wound repair

(*1 p<0.01, *2 p<0.005, *3 p<.001, *4 p<.01, *5 p<.05)
Co-localization of iNOS with inflammatory monocytes and macrophages in wounded skin

Red = CD11b
Monocyte

Green = F4/80
Macrophage

Blue = iNOS

C57BL/6  IL6-/-
SUMMARY

• Macrophages play important roles in the early and mid phases of wound healing
• Effective phagocytosis of apoptotic cells by macrophages are important for resolution of inflammation
• Macrophage function is altered in diabetes and diabetic wounds
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