Macrophages, Growth Factors & Cytokines

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Disclosure

- No commercial interest or bias
- No conflict of interest
- No relevant financial relationships
Participants will be able to:

• Understand the role of macrophages in the wound healing process

• Recognize key growth factors and cytokines involved in wound healing
Physiology of Wound Healing

- Blood clotting
- Exudation/inflammatory phase
  - Macrophages replace PMNs at 48 hours as the principle inflammatory cell
- Proliferation and granulation phase
- Migration/re-epithelialization
- Maturation
Physiology of Wound Healing

- Blood clotting
- Exudation/inflammatory phase

increased vascular permeability

infiltrate of PMNs, platelets, plasma proteins
Recruitment of Inflammatory cells

• Small molecules released by damaged cells
  – ATP, adenosine, uric acid, arachidonic acid derived products, bioactive lipids

• Growth factors released by degranulating platelets
  – EGF, FGF-2, TGF-β, PDGF, VEGF
Recruitment of Inflammatory cells

- Histamine release by neighboring mast cells
- Chemokine release
- Cytokine release
Growth factors and cytokines in wound healing

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Epidermal Growth Factor
Fibroblast Growth Factor-2
Transforming Growth Factor
Platelet Derived Growth Factor
Vascular Endothelial Growth Factor
Connective Tissue Growth Factor
Pro-Inflammatory Cytokines
Chemokines
TGF-β

- Produced by macrophages, fibroblasts, keratinocytes, and platelets
- Increased in acute wounds and decreased in chronic wounds
- Chemoattractant for monocytes
- Topical TGF-β applied to wounds result in
  - Increased inflammation
  - Angiogenesis and fibrosis
  - Increased matrix deposition

Roberts A.B. et al (1986) PNAS
TGF-β1

- Studies show inhibition of keratinocyte proliferation (during reepithelialization)*
- Other studies show promotion of a migratory keratinocyte phenotype**
- Overexpression of TGF-β1 increases proliferative phenotype of keratinocytes in late stages of wound healing

*Sellheyer K et al (1993) PNAS, Roop Group
**Li Y et al (2006) JID, Woodley Group
More on TGF-β

- Mice with knockout of downstream mediator of TGF-β (Smad-3) have blunted response to TGF-β

More on TGF-β

- Smad-3 knockout mice
  - Faster re-epithelialization of incisional wounds
  - Decreased monocyte infiltration in wounds
  - Increased keratinocyte proliferation
  - Reduced scarring

Excisional wound healing in Smad3 mice

Wound healing depends on underlying tissue support

Arany P R et al. PNAS 2006;103:9250-9255
Inflammatory Cells in Wound Healing

- Platelets
- Neutrophils
- Mast Cells
- Macrophages
Platelets

- Anti-sera to platelets to induce thrombocytopenia results in increased macrophages and T cell
  - No changes
    - In proliferative phase of repair
    - Wound closure
    - Angiogenesis or collagen synthesis

Neutrophils

- Anti-sera studies in the 1970s* and recent neutrophil depletion** show that chemokines released are not essential

- In fact, neutrophils can release factors that impair the healing process

Mast Cells

- Mice deficient of mast cells (WBB6F1/J-kit$^w$/Kit$^{w-}$) show
  - Reduced neutrophils at wound site
  - Normal repair

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

Macrophage function in wounds

• Phagocytes
  – Clearing of matrix and cell debris

• Cellular source of numerous factors
  – Cytokines, growth and angiogenic factors
Macrophages function as phagocytes

Macrophages function as phagocytes

Monocytes are activated to become macrophages

quiescent

activated
Macrophages – New Studies

• PU.1 knockout mice lack neutrophils, macrophages, and B cells
  – No delay in wound healing
  – Slight enhanced rates of re-epithelialization
  – Heal without fibrosis
Wound Healing in the PU.1 Null Mouse—Tissue Repair Is Not Dependent on Inflammatory Cells

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and Scott R. McKercher³
PU.1 knockout mice

- **decreased** clearance of cell and matrix debris
- do not have neutrophils which themselves comprise a bulk of the debris
- decreased growth factors

- **Caveat**
  - Experiments are in embryo or neonatal pups (day 1-4)
Other considerations

- Wounds under germ-free conditions are heal slower than under normal conditions
  - Commensal bacteria may enhance innate immune processes and wound repair
  - Synergy between TLR and adenosine A2a receptors switches macrophages from pro-inflammatory to an angiogenic phenotype

Tipton J.B. et al (1966)  
Chemokines and Macrophages

- Macrophage-inflammatory protein 1 (MIP-1α) knockout have normal wound repair

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

skin injury and chronic ulcers

Classically activated Mφ
- Good infection control
- Tissue destruction
- TNF-α
- Nitric oxide

Alternatively activated Mφ
- Pro-angiogenic factors
- IL-10
- Collagen precursors

Precursor monocyte
- IL-4, IL-13, or glucocorticoid, immune complex

IFN-γ
- Anti-inflammatory and tissue repair
- Good wound healing

disrupted barrier
Monocyte precursor

Classically activated Macrophages
Pro-inflammatory and cytotoxic

Monocyte precursor

Nitric oxide and citrulline

IL-4 or IL-13 or glucocorticoids

IL-4 or IL-13 or glucocorticoids

IL-10 and IL-1R antagonist

IL-10

Type II-activated Macrophages
Anti-inflammatory and tissue repair

Alternatively activated Macrophages
Anti-inflammatory and tissue repair

Mannose receptor, CD23, CD14, scavenger receptor

IL-10

Mannose receptor, CD86

MHC class II, CD86

Pro-inflammatory cytokines TNF-α, IL-12, IL-1, IL-6

MHC class II, CD86

TNF-α, IL-1, IL-6

IL-10

1st signal - Fc-γR ligation
diverse 2nd signal (TLRs, CD40, CD44)

1st signal - Fc-γR ligation
diverse 2nd signal (TLRs, CD40, CD44)

iNOS2

L-arginine

L-arginine

Nitric oxide and citrulline

Mannose receptor

2nd signal TNF-α

1st signal IFN-γ, PAMPs

Murine macrophage subpopulations
Metabolism of arginine by macrophage subsets

**Classically activated Mφ**

- IFN-γ
- TNF-α
- IL-1

L-Arginine → L-OH Arginine → NOS2 → Citrulline → Nitric oxide

**Alternatively activated Mφ**

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

L-Arginine → Arginase-1 → L-ornithine → Ornithine aminotransferase

- Polyamines
- Cell proliferation
- Collagen production
- Proline
Thank you