Interleukin-20 is associated with delayed healing in diabetic wounds

Phillip Finley, PhD
Integrated and Applied Sciences Program
Biology and Statistics/Research Methodology
Normal Healing

- Body’s natural process of regenerating skin – Complex
  - Inflammatory response
  - Formation of granulation tissue
  - Angiogenesis
  - Collagen deposition
  - Epithelialization
  - Remodeling

- All of these events are impaired during healing in diabetic wounds.
Background

- Impaired wound healing in diabetic patients = significant health problem
- 80,000 amputations per year at $45,000 per amputation.
- Approximately 20.8 million people with diabetes
- 54 million Americans have pre-diabetes.
- ↑ in obesity = ↑ diabetes
Background

- Neuropathy, prevents diabetic patients from feeling discomfort in their lower extremities.
- Thus, a minor abrasion can go unnoticed leading to a very serious chronic wound
Outline

Diabetic wounds
  Delayed inflammatory cell influx
  Increased expression of Pf4 and IL-20

Silver dressings improve diabetic wound healing
  Do not affect bacterial burden
  Decrease both Pf4 and IL-20 expression
Neutrophils

- First inflammatory cells to respond to the site of injury within hours
- Remain the predominant cell in the wound bed for the first 2 days
- Main role to clean wound bed:
  1. Phagocytose debris and bacteria
  2. Release microbicidal compounds to kill bacteria
  3. Secrete proteases that break down damaged tissue
  4. Produce cytokines to promote inflammatory response
- Depleted in the wound after 2-3 days.
Macrophages

- Replace Neutrophils as the predominant cell in the wound around day 2 or 3.
- The macrophage (a type of white blood cell) derived from peripheral blood monocytes
- Monocytes migrate from bloodstream and differentiate upon entering the tissue
- Role – Similar to Neutrophils
  1. Release microbicidal compounds to kill bacteria
  2. Phagocytose debris and bacteria including dead neutrophils
  3. Produce cytokines to promote inflammatory response
# Macrophage and Healing

<table>
<thead>
<tr>
<th>Normal Wound Healing</th>
<th>Diabetic Wound Healing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depletion of macrophages resulted in defective healing</td>
<td>Depletion of macrophages significantly improved healing</td>
</tr>
</tbody>
</table>

Russell Ross – University of Washington

Stefan frank – 2007
Macrophage Activation

**Classically Activated Macs**
- Provide antimicrobial protection by eliminating various pathogens
- Clean the wound by removing dead cells and debris.
- Produce pro-inflammatory cytokines to sustain response
- Helps maintain inflammation

**Alternatively Activated Macs**
- Associated with the resolution of inflammation
- Promotes tissue remodeling and angiogenesis
- Produce anti-inflammatory cytokines

Differentiation depends on proteins in the microenvironment
Hypothesis

- Macrophage activation will be different between normal and diabetic wounds
Mice Homozygous for diabetes spontaneous mutation were used as diabetic mice. C57BLCK6 db/db

• Depletion of beta-cells
• Increased blood glucose at 4 weeks

WT mice were C57BLCK6 with the genetic background as diabetic mice
Mouse skin
H&E Staining

Day 2

Day 5

Day 7

WT

Diabetic
# Neutrophil Staining

<table>
<thead>
<tr>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
YM1 Staining

Day 2  Day 5  Day 7

WT

Diabetic
Summary

• Influx of inflammation cells is delayed in diabetic wounds
• Neutrophil Influx is delayed in diabetic wounds
• Influx of alternatively activated macrophages was delayed in diabetic wounds
• Need to do IHC for classic Macs
Results of PCR array – Day 5

Based on histology findings examined 4 DB and 4 WT samples at day 5 of healing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change (diabetic/control)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-20</td>
<td>9.75</td>
<td>0.0348</td>
</tr>
<tr>
<td>Pf4</td>
<td>2.17</td>
<td>0.0323</td>
</tr>
<tr>
<td>Scye1</td>
<td>-1.52</td>
<td>0.0414</td>
</tr>
<tr>
<td>CXCL9</td>
<td>-2.95</td>
<td>0.0195</td>
</tr>
<tr>
<td>IL-1f8</td>
<td>-2.99</td>
<td>0.0035</td>
</tr>
<tr>
<td>CCL24</td>
<td>-3.35</td>
<td>0.0231</td>
</tr>
<tr>
<td>IL-18</td>
<td>-3.67</td>
<td>0.0241</td>
</tr>
<tr>
<td>IL-13</td>
<td>-3.8</td>
<td>0.0231</td>
</tr>
<tr>
<td>CCL19</td>
<td>-4.09</td>
<td>0.0046</td>
</tr>
<tr>
<td>XCR1</td>
<td>-6.3</td>
<td>0.0308</td>
</tr>
</tbody>
</table>
IL-20 is associated with inflammation in the skin

- Pro-inflammatory protein
- Associated with many chronic inflammatory diseases including psoriasis
- Over expressed in psoriatic lesions
- IL-20 expression decreases as a function of psoriatic treatment
- Transgenic mice overexpression IL-20 in the skin develop skin inflammation
Conclusion Part I

- Inflammatory cell influx is delayed in diabetic wounds
- Diabetic wounds express higher levels of Pf4 and IL-20
- Interleukin-20 may be a useful therapeutic target to improve wound healing in diabetic patients
Silver dressings are a popular wound treatment
- Effective antimicrobial agent
- Silver may improve wound healing by reducing inflammation
- Thicker granulation beds, and limited inflammation.
- Lower wound contracture
- Faster healing
There is insufficient evidence to establish whether silver dressings promote wound healing or prevent wound infection in normal or diabetic wounds.

These studies did not distinguish between anti-inflammatory activity above its antimicrobial activity.
The diabetic mice (mean = 425.52mg/dl, SD = 109.81) had a statistically significant higher blood glucose level than WT mice (mean = 108.35mg/dl, SD = 22.43) (t = 16.63, p < .001)

The diabetic mice (mean = 46.63g, SD = 1.98) had a statistically significant higher body weight compared to WT mice (mean = 21.65, SD = 4.48) (t = 26.39, p < .001)
Zone of Inhibition testing with *Serratia marcescens* ATCC 60 (starting concentration: $1.00 \times 10^6$ cfu/mL) and *Staphylococcus aureus* ATCC 6538 (starting concentration: $2.00 \times 10^8$ cfu/mL) across silver nylon and gauze dressings.
Delayed wound healing in wounds treated with a gauze dressing compared to wounds treated with a silver nylon dressing across Diabetic and wild type mice.
Corrected zone of inhibition testing with *S. xylosus* across silver and gauze dressings. Silver dressing had an average CZOI of 5mm and the gauze dressing had a zero CZOI. Initial bacterial concentration of *S. xylosus* was $2.0 \times 10^8$ (cfu/ml).
PCR Data
Diabetic wounds
Day 2
PCR Data
Diabetic Wounds
Day 5
Conclusions

- Faster healing in the silver treated wounds of both diabetic and control mice.
- Reduction of bioburden in silver treated wounds in wild type mice but not in diabetic wounds.
- *In vitro* *S. xylosus* produced biofilms faster in higher glucose environments, which may explain the increased bioburden in the diabetic wounds compared to wild type.
- IL-20 and Pf4 mRNA expression was decreased in silver treated diabetic wounds.