The Role of Fibroblast-Derived CCL2 in Early Phase Wound Healing

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Introduction and Hypothesis

Introduction:

- Skin is a complex organ with multiple layers and a variety of resident cell types
- Wound healing to the skin requires robust myeloid cell recruitment and resolution of inflammation to transition into a reparative phase
- Insufficient or excessive inflammation can cause problems to the healing process
- Wound healing complications present a large global health burden.
- Fibroblasts from injured skin, 1.5 days post-wound, induce greater macrophage migration compared to those from uninjured skin, suggesting an enhanced role in directing macrophage migration to wounds in vivo.
- CCL2 is a chemokine that plays a crucial role in immune responses by attracting monocytes to inflammation or injury sites, essential for inflammation and tissue repair.
- Fibroblasts are identified as a significant source of CCL2 at wound sites, indicating their importance in the inflammatory response and tissue healing process.

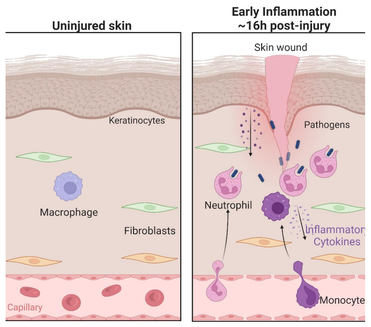

Figure 1: Cellular dynamics promoting the progression of inflammation after injury.

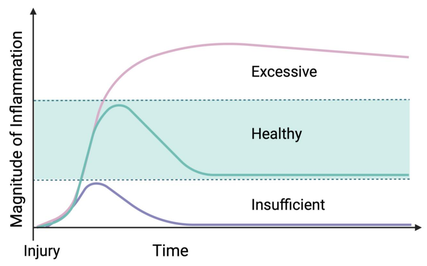

Figure 2: Healthy inflammation is necessary for optimal wound healing.

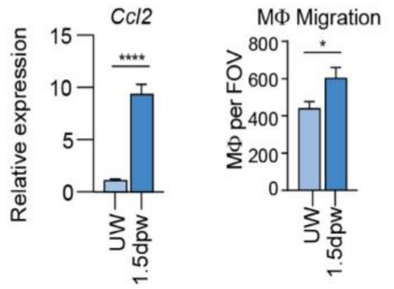

Figure 3: Ccl2 gene expression in fibroblasts from uninjured and injured skin (left) and fibroblast-induced macrophage migration (right) *p<0.05; ****,p<0.001.

Hypothesis: Fibroblast-derived CCL2 attracts myeloid cells to the site of the injury. This recruitment is essential for the subsequent stages of wound repair.

Methods

Figure 4: Timeline of tamoxifen injection into mice several days before wounding. Analysis was taken at 1.5 days and 5 days post wounding.

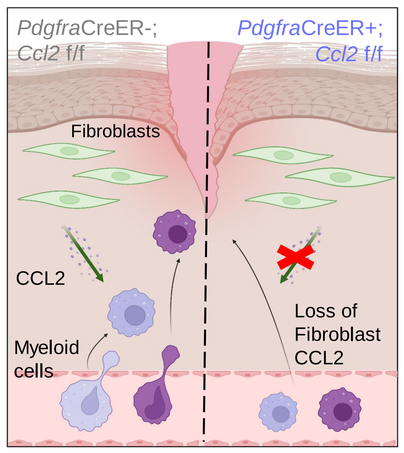

Figure 5: Model of PdgfraCre+/- determines expression of CCL2.

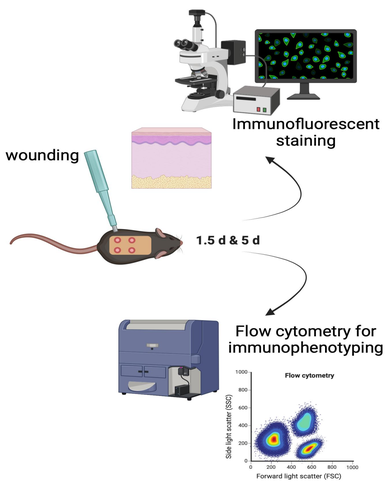

Figure 6: Mice were wounded, and skin was harvested on 1.5- and 5-days post-wounding. The skin was studied using methods of immunofluorescence and flow cytometry.

Acknowledgements

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Results

Fig. 7: Fibroblast-derived CCL2 supports macrophage numbers during the inflammation phase of wound healing.

(A) Gating strategy for immune cell populations in wound beds. First, on only live cells. CD45 and CD11b to identify activated immune cells (first plot). Differentiated cells into neutrophils (LY6G+) , Macrophages (F4/80+) , and monocytes (F4/80-). CD45+, CD11b+ cells were identified as neutrophils (red gate), monocytes (purple gate), or macrophages (yellow gated) based on F4/80 and LY6G expression (second plot).
(B) Using immunofluorescence comparison of total numbers of immune cells (CD45+), macrophages, monocytes, and neutrophils in wounds 1.5dpw between *Pdgfra*CreER+; and *Pdgfra*CreER-; *Ccl2*f/f mice . *n* = 7-10 animals per group. (*$p < 0.05$; **,$p < 0.01$)

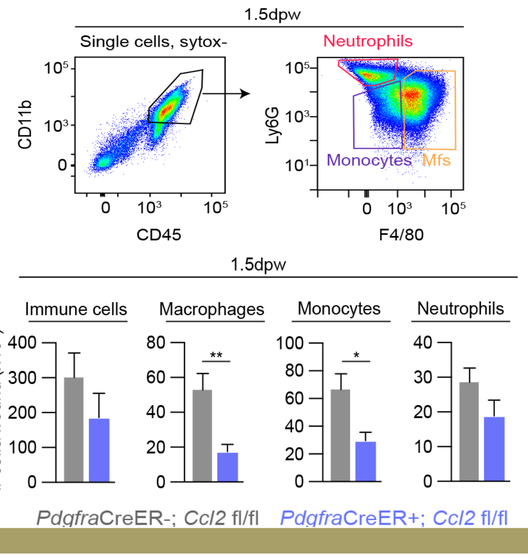
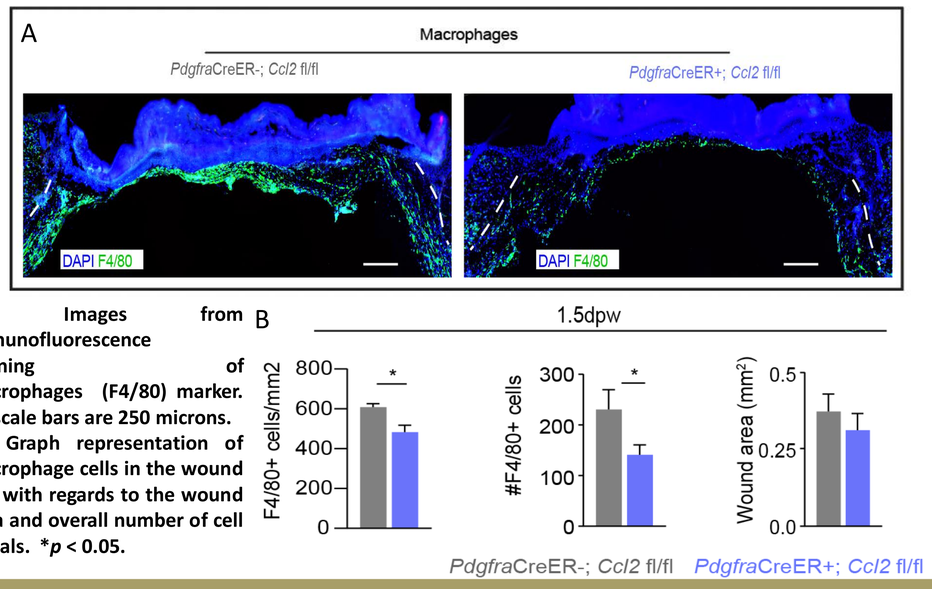
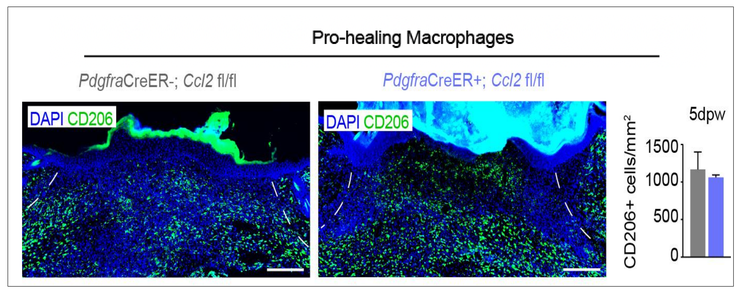


Fig. 8: Absence of CCL2 from fibroblasts recruit less macrophages to the wound site



(A) Images from Immunofluorescence staining of macrophages (F4/80) marker. All scale bars are 250 microns.
(B) Graph representation of macrophage cells in the wound site with regards to the wound area and overall number of cell signals. *$p < 0.05$.

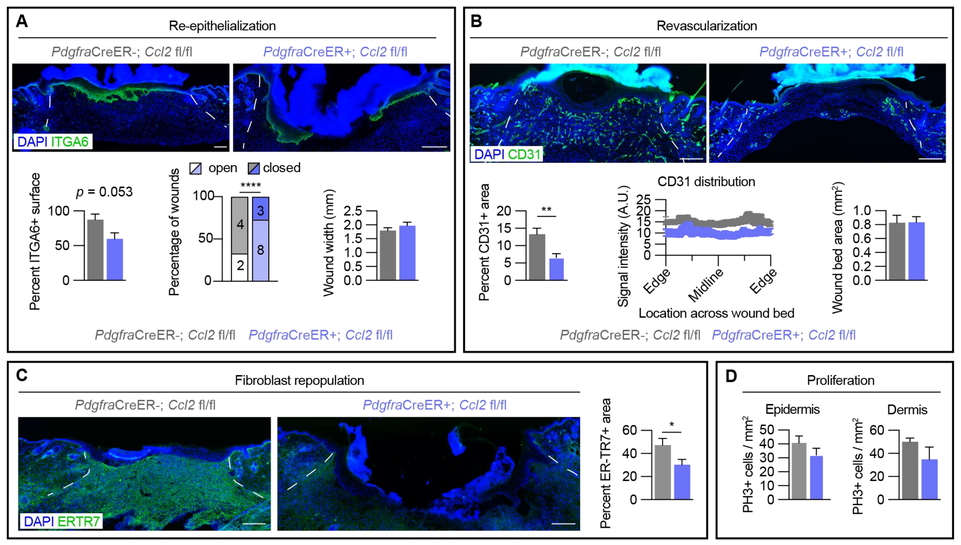
Fig. 9: At 5 days post-wounding, the number of macrophages stabilized.



Quantification of CD206+ cells (anti-inflammatory, pro-healing macrophages) in the wounds at 5-days post wounding. 3-4 mice per group. All scale bars in these images are 250 microns

Figure 10: Loss of Fibroblasts CCL2 delays the healing process

(A) Re-epithelialization analysis in the wound beds of PdgfraCreER-; and PdgfraCreER+; Ccl2f/f mice 5dpw. White lines delineate wound edges. Scale bars 250µm.
(B) Revascularization analysis in the wound beds 5dpw. Images from immunostained tissue sections and quantification of CD31+ area, distribution of CD31 signal intensity from the wound edge to center, and wound area. A.U. Arbitrary fluorescence units.
(C) Images of wound beds of PdgfraCreER-; and PdgfraCreER+; Ccl2f/f mice 5dpw immunostained for ER-TR7 (green) and DAPI. The percentage of ER-TR7+ area was compared between groups (right).
(D) Analysis of PH3+ cell proliferation in the epidermis and dermis of wound beds 5dpw.
Error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, ****p < 0.0001.



Major Findings

- **Blockage of CCL2 lowers the recruitment of macrophages and myeloid cells in the wound bed 1.5 days PW**
- **Immune cell numbers begin to stabilize at 5 days PW**
- **At 5 days PW, there is decreased revascularization and contraction of the wound indicating that there is delayed healing in the PdgfraCre+ mice**

FUTURE DIRECTIONS:

- **Increase n value by using female mice**
- **Verify if there are significant variations in the healing process and the 7-day timepoint**
- **Test other chemokines produced by fibroblasts that affect macrophage or neutrophil recruitment**