NOVEL MECHANISMS OF COLLAGENASE SANTYL OINTMENT (CSO) IN WOUND MACROPHAGE POLARIZATION AND RESOLUTION OF WOUND INFLAMMATION

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Running Title: Collagenase in resolution of inflammation

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SUPPLEMENTARY FIGURES

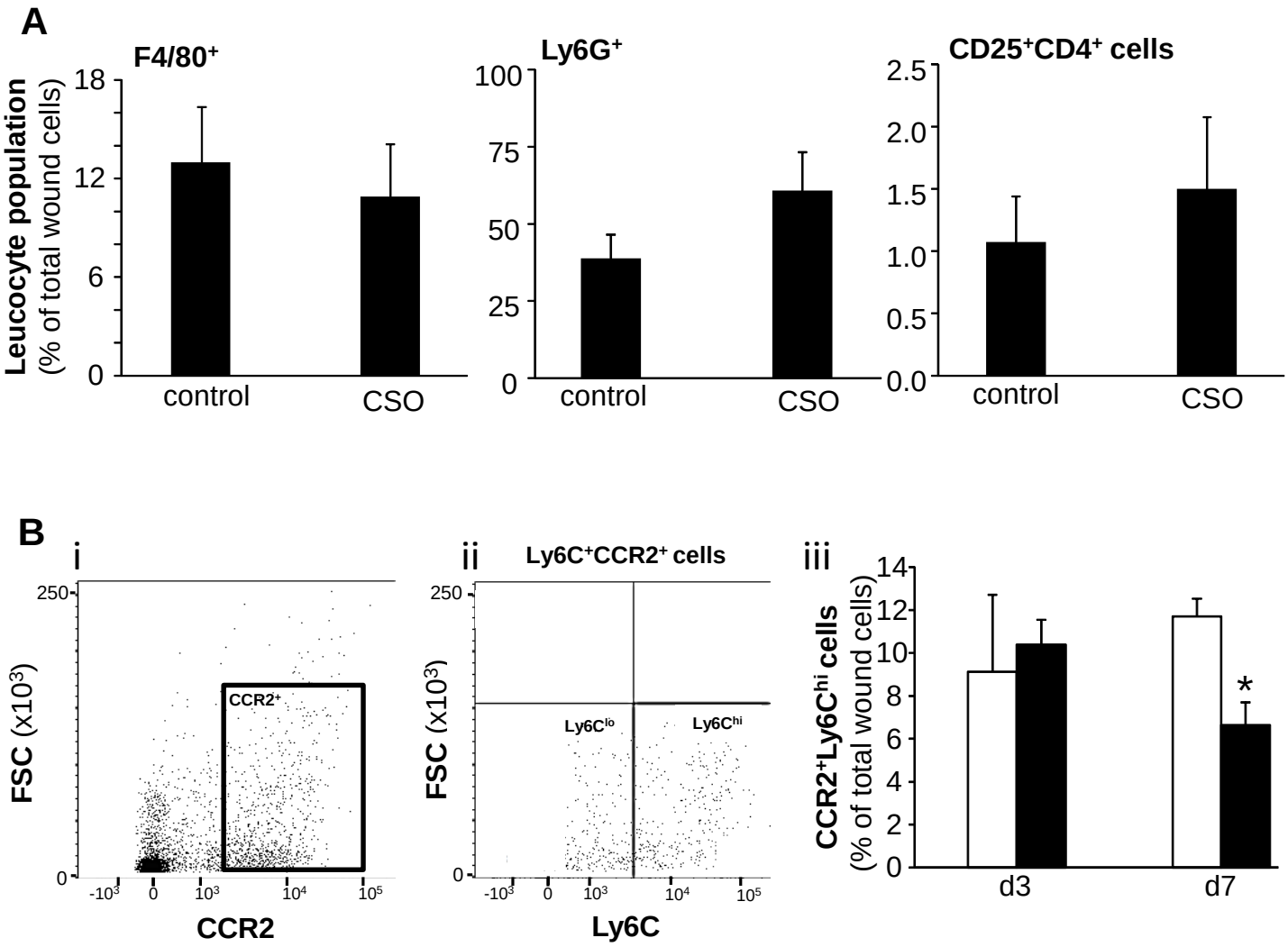

A
F4/80+
Ly6G+
CD25+CD4+ cells
Leucocyte population
(% of total wound cells)
18
12
6
0
100
75
50
25
0
2.5
2.0
1.5
1.0
0.5
0.0
control
CSO
control
CSO
control
CSO
B
i
ii
Ly6C+CCR2+ cells
iii
250
0
FSC (x10³)
CCR2+
-10³
0
10³
10⁴
10⁵
CCR2
Ly6Clo
Ly6Chi
Ly6C
CCR2+Ly6Chi cells
(% of total wound cells)
14
12
10
8
6
4
2
0
*
d3
d7

Figure S1

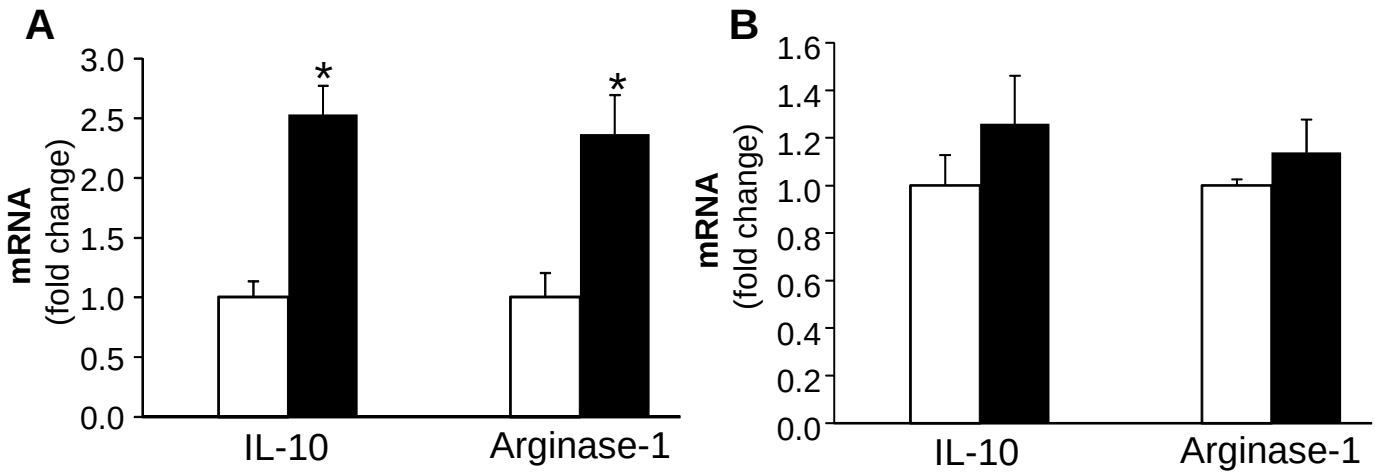
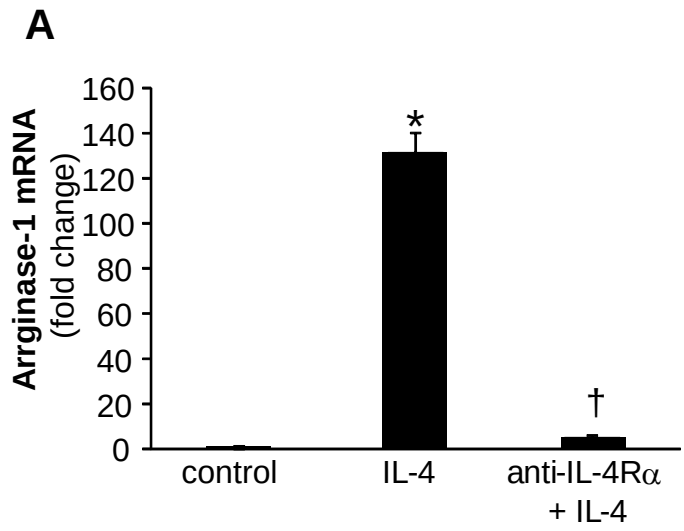

A
mRNA
(fold change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
IL-10
Arginase-1
B
mRNA
(fold change)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
IL-10
Arginase-1

Figure S2


A
Arrginase-1 mRNA
(fold change)
160
140
120
100
80
60
40
20
0
*
†
control
IL-4
anti-IL-4Rα
+ IL-4

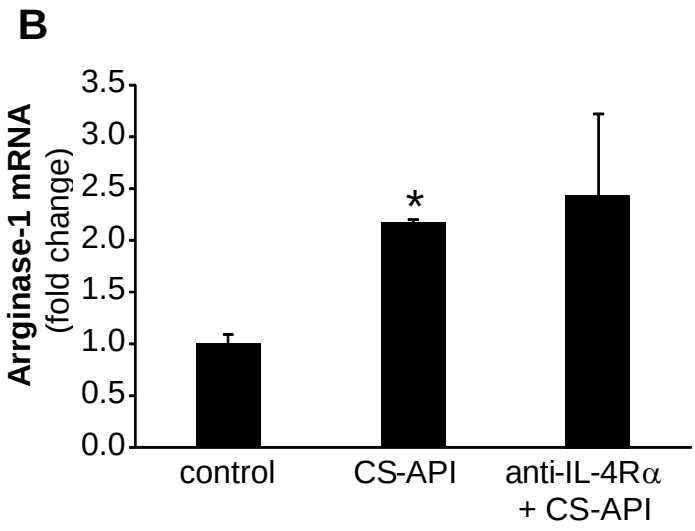

B
Arrginase-1 mRNA
(fold change)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
control
CS-API
anti-IL-4Rα
+ CS-API

Figure S6

Supplementary Figures.

Figure S1. Flow cytometry analysis of wound cell populations following CSO treatment. Wound cells were harvested from PVA sponges coated with CSO (300 mg), implanted subcutaneously in C57bl/6 mice and were immunostained with leukocyte markers and subjected to flow cytometry analysis. (**A**) d7 leukocyte populations $F4/80^{+}$, $Ly6G^{+}$ and $CD25^{+}CD4^{+}$ were quantified from the gated cell populations. (**B**) d3 and d7 populations of $CCR2^{+}Ly6C^{hi}$ cells. **i**) $CCR2^{+}$ (PE positive) cells were gated against relevant IgG control; **ii**) scatter blot analysis of $Ly6C^{+}/CCR2^{+}$ gated cells to determine the expression $Ly6C^{hi}$ or $Ly6C^{lo}$ cells. iii) quantification of cells shown in ii. CSO, solid bars; control, blank bars. Data are mean ± SEM (n = 5-8); *p<0.05 compared to d7 wound mϕ treated with equal amounts of white petrolatum (control).

Figure S2. CSO induces macrophage polarization to a pro-resolving phenotype in human blood monocyte derived macrophages (hMDM) but not in pro-resolving (M IL-4) polarized hMDM. A. hMDM were treated with CS-API (250ng/ml) for 24h. Total RNA was isolated and IL-10 and Arginase-1 mRNA expression was measured using RTPCR. Data are expressed as mean ± SEM (n=4). *p<0.05 compared to control. **B.** hMDM were polarized to pro-resolving {M(IL-4)} macrophages using IL-4 (20ng/ml) and IL-13 (20ng/ml) for 48h and then treated with CS-API (250ng/ml) for 24h. Total RNA was isolated and mRNA expression of IL-10 and Arginase-1 was measured using RTPCR. CSO, solid bars; control, blank bars. Data are expressed as mean ± SEM (n=4). *p<0.05 compared to control.

Figure S6. CS-API mediated macrophage polarization is independent of classical IL4- IL4R activation pathway. Cultured mouse mϕ were treated with IL-4R-α neutralizing antibody (10µg/ml) for 1 hour followed by **(A)** IL-4 (20ng/ml) or **(B)** CS-API (250 ng/mL) treatment for 24 hours. Arginase-1 mRNA expression was measured using RTPCR. Data are expressed as mean ± SEM (n=3); *p<0.05 compared to control, $^{\dagger}p$<0.05, compared to IL-4 treated group.