Defining the Role of CD169/Siglec-1 in Macrophages During Cardiac Wound Healing

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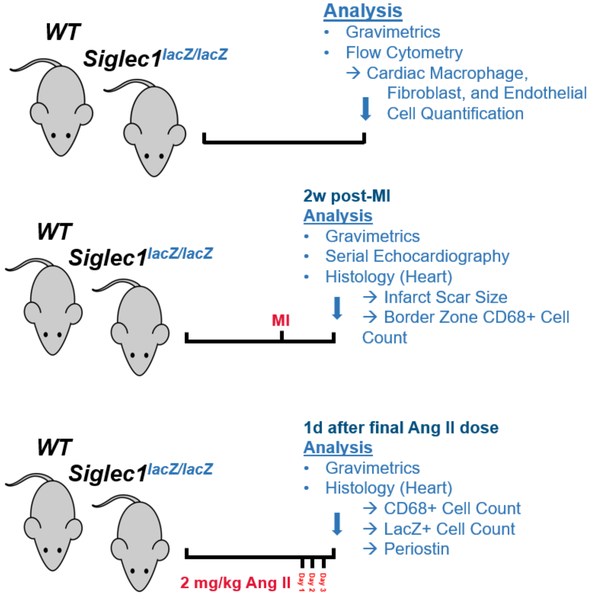
Background

- Macrophages modulate wound healing, fibrosis, and tissue remodeling after injuries such as myocardial infarctions (MI).
- Embryonic tissue-resident macrophages are critical for optimal tissue healing.
- CD169/Siglec1 has been identified as a useful marker for identifying this population.
- However, the functional role of Siglec1 in cardiac macrophages has not been characterized.

Methods

- We generated global Siglec1 knockout (KO) mice and assessed these animals for baseline heart function, cellular content, recovery after surgically-induced MI, and response to hypertensive stress induced by angiotensin II (Ang II).



Results

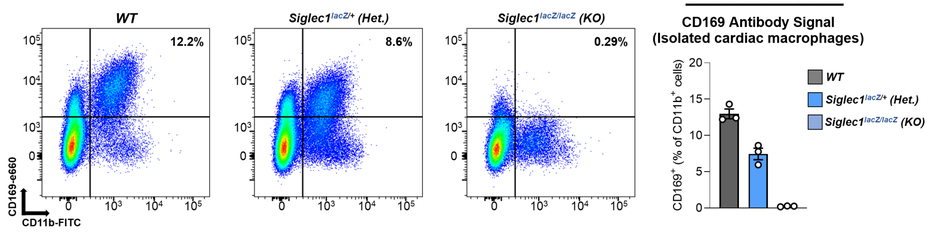


Figure 1: Cardiac macrophages from 9-week old C57/BL6 (wild-type; WT), Siglec1$^{lacZ/+}$ (Het), and Siglec1$^{lacZ/lacZ}$ (KO) mice were assessed via flow cytometry for their expression of CD169/Siglec1. Cells from dissociated hearts were stained for CD11b to mark cardiac macrophages, as well as CD169. Representative flow cytometry plots and their quantitation are shown; *n*=3 per group.

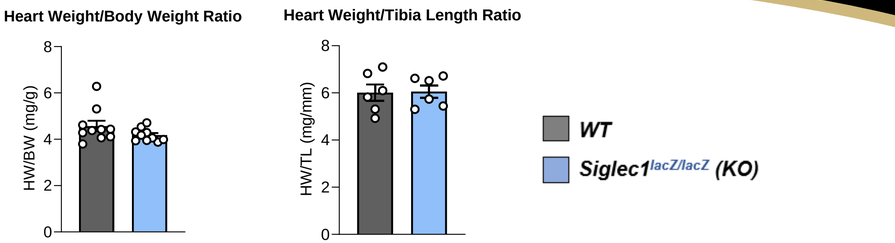


Figure 2: Gravimetric data obtained from 15- to 18-week old WT and Siglec1 KO mice at baseline; *n*=10 per group for heart weight to body weight ratios (HW/BW), and *n*=6 per group for heart weight to tibia length ratios (HW/TL).

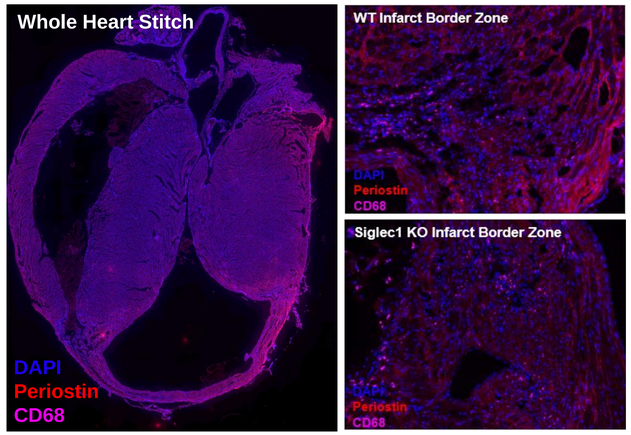


Figure 4: Representative immunofluorescent (IF) images of WT and Siglec1 KO hearts, harvested two weeks after surgically-induced MI. Cardiac tissue sections were stained with DAPI (blue) and for CD68$^+$ macrophages (pink). Whole heart images (left) were obtained to analyze infarct size; 20x images of infarct border zones (right) were obtained to analyze macrophage content.

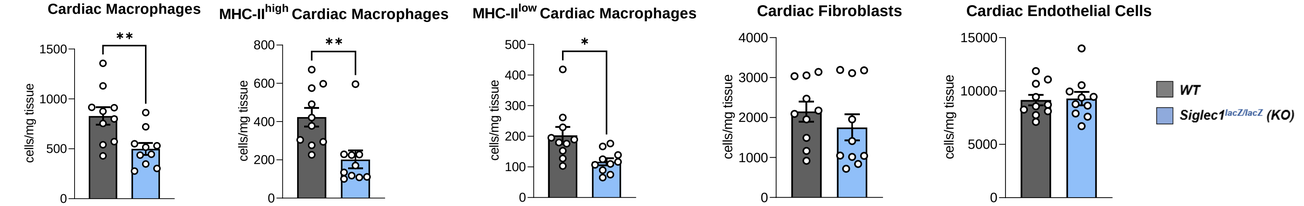


Figure 3: Quantitation of flow cytometry plots analyzing the cellular content of dissociated hearts from 15- to 18- week old WT and Siglec1 KO mice, with *n*=10 per group. CD11b, F4/80, and MHC-II were used to mark cardiac macrophages, MEFSK4 was used to mark cardiac fibroblasts, and CD31 was used to mark cardiac endothelial cells. *p<0.05 by unpaired two-tailed t-test; **p<0.01 by unpaired two-tailed t-test.

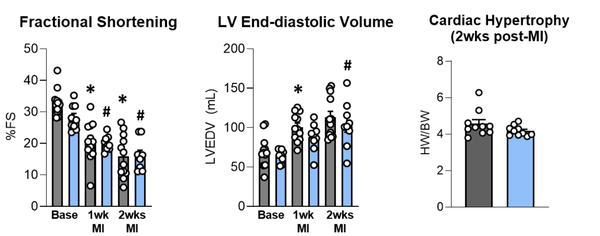


Figure 6: Fractional shortening, LV end-diastolic volume, and HW/BW of WT and Siglec1 KO mice after surgically-induced MI. Serial echocardiography was performed one and two weeks post-MI to obtain functional data. *p<0.05 vs baseline WT; #p<0.05 vs. baseline KO.

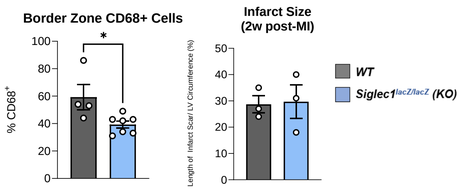


Figure 5: Quantification of infarct scar size (% of left ventricular [LV] circumference) and border zone CD68$^+$ cell count (% of DAPI$^+$ cells) in immunofluorescent images of WT and Siglec1 KO hearts 2 weeks post-MI. *p<0.05 by unpaired two-tailed t-test. Image analysis performed in ImageJ.

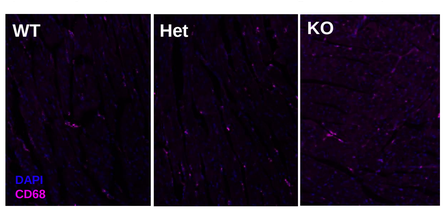
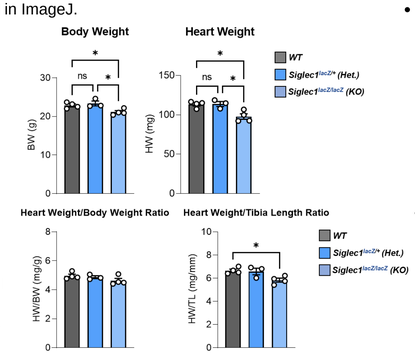


Figure 7: Gravimetric data (right) obtained from WT, Siglec1 Het, and Siglec1 KO mice after 3 doses of Ang II. Representative IF images of cardiac tissue obtained after Ang II treatment shown (above); CD68 (pink) was used to mark cardiac macrophages. *n*=4 for WT and KO groups; *n*=3 for Het group. *p<0.05 by unpaired two-tailed t-test.



Conclusions

- Siglec1 KO mice had reduced CD11b+F4/80+ cardiac macrophage counts at steady-state.
- After MI injury, cardiac dysfunction and pathological remodeling were similar between Siglec1 KO mice and controls.
- Siglec1 KO did not affect infarct scar size as measured by histology; however, the infarct border zone of CD169 KO mice contained fewer CD68+ macrophages, which may impact the long-term outcome of the cardiac wound healing response.
- Further studies are required to determine the effect of Siglec1 knockout on myocardial fibrosis post-MI and Ang II, as well as on T-cell populations in the context of cardiac development and wound healing.

Implications

- A better understanding of CD169/Siglec1 functionality in cardiac macrophages will elucidate how this tissue-resident macrophage population might be modulated to improve the cardiac wound healing response.