INTERLEUKIN-10 PRODUCING LYMPHOCYTES ALTER FIBROTIC RESPONSE IN INFLAMMASOME-STIMULATED DERMAL FIBROBLASTS

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Background: We recently demonstrated that Interleukin- (IL) 10-producing type 1 regulatory T cells (Tr1) decrease fibrosis in murine wounds. Tr1 are known to inhibit NLRP3 mediated production of pro-fibrotic interleukin (IL)-1beta by macrophages via IL-10 signaling. However, the capacity of Tr1 cells to modulate fibrotic phenotype driven by the NLRP3 inflammasome in dermal fibroblasts is unknown. We hypothesize that IL-10 producing regulatory T lymphocytes reverse the fibrotic phenotype of myofibroblasts which have been activated by the NLRP3 inflammasome.

Materials/Methods: Adult dermal fibroblasts (AFB) and splenocytes were harvested from C57L/B6 (WT) and 10BiT mice, respectively. Splenocytes were sorted into CD4+Foxp3+ regulatory T cells (Treg), CD4+CD44HiFoxp3- Tr1 precursors, or CD4+CD44int/loCD62L+ Tnaive using fluorescence activated cell sorting and activated (anti-CD3/28 microbeads) for 6, 9, or 12 days, with phenotype and IL-10 production evaluated by flow cytometry and ELISA. NLRP3 activation of AFB was performed using sequential administration of TGF-beta, lipopolysaccharide, and adenosine triphosphate. AFB were cultured in medium alone or with conditioned medium (CM) from 9-day-activated Tr1, Treg, or Tnaive for 24 hours, with gene expression of alpha-SMA measured by RT-qPCR. N=3 experimental replicates per time point.

Results: Flow cytometry evaluation revealed optimal polarization towards Tr1 (coexpression of CD49b and LAG3) following 9 days of activation (70.2% of live cells) vs 6 (41.9%) or 12 (41.7%) days of activation. Similarly, the proportion of cells staining positively for IL-10 was greatest at 9 days of activation (86.7% of live cells) compared to 6 (47.9%) or 12 (62.9%) days activation. CM was generated by culturing 1x10^6 lymphocytes in 3-ml of medium for 24 hours. IL-10 in CM generated by Tr1 was greater than Treg or Tnaive (1660.3 vs 154.3 vs 5.1 pg/ml). Expression of alpha-SMA was significantly increased in AFB by NLRP3 activating factors compared to untreated (1.9±0.2 vs 0.9±0.3-fold expression, $p<0.05$) (Figure 1). alpha-SMA expression was then significantly lowered by treatment with CM from Tr1 (0.6±0.03-fold expression, $p<0.01$) and Treg (0.6±0.2-fold expression, $p<0.01$).

Conclusions: IL-10 producing T lymphocytes attenuate the fibrotic phenotype of NLRP3 inflammasome-activated AFB. Developing methods to attract T cells as anti-inflammatory and anti-fibrotic mediators to the wound may result in improved patient scarring outcomes.

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