Evaluation of Anti-Inflammatory Signaling of Resolvin D2 on Murine Thoracic Aortic Fibroblasts

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Introduction: Inflammatory signaling, fibroblast phenotypic changes, and extracellular matrix remodeling play an important role in thoracic aortic aneurysm (TAA) formation. D-series Resolvins, including Resolvin D2 (RvD2), have effectively decreased the progression of abdominal aortic aneurysms (AAA) by promoting fibroblast anti-inflammatory signaling. However, no studies have investigated whether this holds for TAA.

Methods: Thoracic aortic fibroblasts were extracted from 12-to-14-week-old C57/B6 male wild-type mice and harvested for cell culture. Cells were grown in fibroblast growth factor media until they reached optimal confluency and then were transferred to a 6-well plate. Groups of fibroblasts were treated with vehicle, 5 ng/mL TGF-β1, 30 ng/mL RvD2, and both TGF-β1 and RvD2 for 24 hours. Cell lysate samples were loaded onto a Western blot and immunoblotted for endogenous TGF-β1 and β-actin (control). The supernatant was loaded onto a 40-plex murine inflammatory cytokine assay (Abcam). All samples were assessed for staining signal intensity post-hoc.

Results: Fibroblast treatment with RvD2 attenuates the production of TIMP-1 and TIMP-2, especially in the presence of exogenous TGF- β. It decreases pro-inflammatory GM-CSF, but not other macrophage attractants such as lymphotactin and CCL2. Treatment decreases the production of IL-12p70 but does not decrease the extracellular expression of other pro-inflammatory interleukins.

Conclusions: Preliminary results suggest that RvD2 treatment attenuates the endogenous expression of TIMPs to counteract the breakdown of the ECM, especially in the presence of exogenous TGF- β. RvD2 treatment decreases macrophage proliferation and maturation through GM-CSF but does not decrease the extracellular expression of other macrophage attractants or pro-inflammatory interleukins.

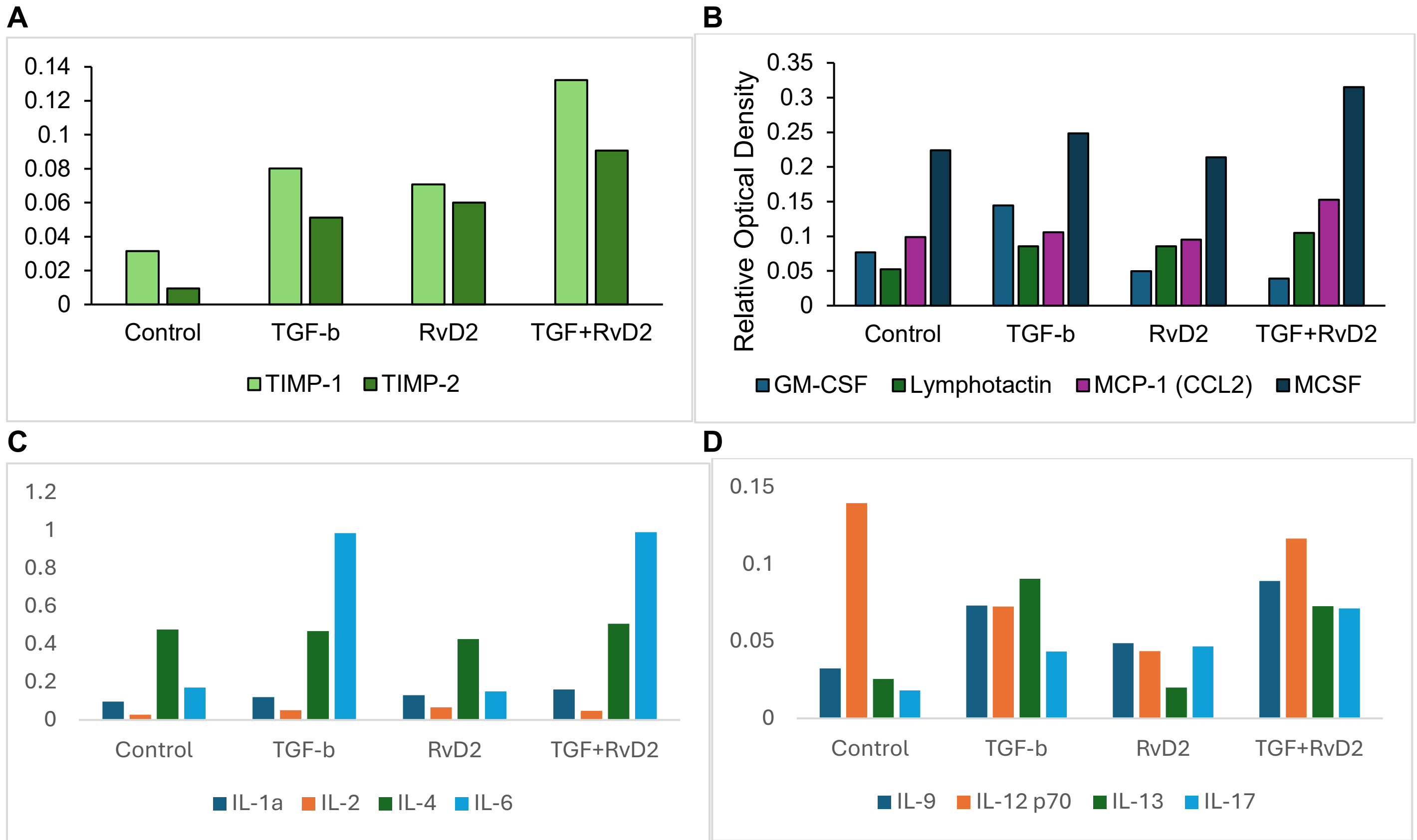

Figure 1. Cytokine array staining density analysis for (A) TIMPs, (B) macrophage proliferation and maturation, and (C and D) pro-inflammatory interleukins. Staining intensity was measured by relative optical density normalized to the control. As shown in A and B, fibroblast treatment with RvD2 at this concentration attenuates the production of TIMPs and certain pro-inflammatory cytokines like GM-CSF. However, it had varying changes in the expression of pro-inflammatory interleukins (C, D) and other macrophage attractants such as lymphotactin, CCL2, and MCSF (B).