Differential Contributions of Cell Specific STING to the T-cell Immune Response in Doxorubicin Cardiotoxicity

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Introduction: Doxorubicin (DR), the most commonly used chemotherapy, results in dose-dependent cardiotoxicity, however current therapies are not widely effective. DR causes oxidative stress, cardiomyocyte death, and DNA damage which activates the stimulator of interferon genes (STING) across broad cell types. STING activation induces type I interferon (IFN-I) secretion and downstream IFN-I stimulating genes (ISGs) including the chemokines CXCL9/10. We found that DR induces cardiac and systemic CXCL9/10 and a cytotoxic $CD8^+$ T-cell response, but whether this response is dependent on cell specific activation of STING by DR remains unknown. We **hypothesized** that DR activation of vascular, stromal and immune cell STING differentially mediates adverse cardiac remodeling and $CD8^+$ T-cell cardiotropism in DR cardiomyopathy. **Results:** We treated WT and $STING^{-/-}$ mice with 5.0 mg/kg DR for 4 weeks and found that $STING^{-/-}$ mice were protected from systolic dysfunction and cardiac fibrosis. Further, DR increased total cardiac $CD8^+$ T-cell numbers, $CXCR3^+CD8^+$ T-cells, and cardiac *Cxcl9/10* in WT but not $STING^{-/-}$ mice. *In vivo* using flow cytometry, DR enhanced IRF3 phosphorylation, immediately downstream of STING, in endothelial cells (ECs), fibroblasts (CFB), and macrophages, in WT but not $STING^{-/-}$ mice. *In vitro,* treatment of all cell types with 1.0 µg/mL DR increased IFN-I transcription, corroborating STING activation. Cell-specific ablation of STING in ECs ($STING^{fl/fl}Vecad^{Cre+}$), CFBs ($STING^{fl/fl}Tcf21^{Cre+}$), or myeloid cells ($STING^{fl/fl}LysM^{Cre+}$) resulted in improved systolic dysfunction and cardiac fibrosis after DR compared to $STING^{fl/fl}Cre^-$ controls. However, we observed distinct mechanisms of protection, as only EC-STING and Myeloid-STING contributed to DR-induced $CD8^+$ T-cell cardiotropism. Mechanistically, we found that direct treatment of primary ECs and macrophages *in vitro* increased *Cxcl9/10* expression and induced subsequent $CD8^+$ chemotaxis and transendothelial migration across primary cardiac ECs in a STING dependent manner. Our data outline a significant role for endothelial and macrophage STING in mediating $CD8^+$ cardiotropism in response to DR.