

# MACROPHAGES ARE CRITICAL DETERMINANTS OF VASCULAR PROGENITOR CELL LINEAGE DIFFERENTIATION IN ARTERIOSCLEROSIS THROUGH TNF- $\alpha$ MEDIATED CANONICAL NF- $\kappa$ B ACTIVATION

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doi:10.1136/heartjnl-2013-304019.164

Macrophages are critical determinants of vascular progenitor cell lineage differentiation in arteriosclerosis through TNF- $\alpha$  mediated canonical NF- $\kappa$ B activation Mei Mei Wong, Andriana Margariti, Yanhua Hu, Qingbo Xu. Cardiovascular Division, King's College London BHF Centre, London U.K.

**Background** Recent data indicates the existence of a population of tissue-resident progenitors that can give rise to cells of both the endothelial and smooth muscle lineages. Macrophages have been established as key inflammatory cells that can promote atherosclerotic plaque progression. The role of macrophages in controlling lineage differentiation of vascular progenitors is unknown and may play an important role in arteriogenesis.

**Methods and Results** Here we examined the ability of macrophages to directly regulate progenitor differentiation in vitro and in vivo. Either murine adult vascular progenitor cells derived from vessel wall or embryonic stem cells were cultured with the peritoneal macrophages or cell line J774A. Analysis of gene and protein expression with endothelial-specific markers (CD31, CD144, eNOS and Flk-1) indicated the profound capacity of the macrophages to induce endothelial differentiation from the stem/progenitor cells. In vivo Matrigel plug assay indicated the endothelial cell-like phenotype derived from stem cells in the presence of macrophages. Interestingly, macrophages could promote the simultaneous suppression of stem/progenitor cell differentiation towards the smooth muscle lineage. Further analysis revealed that both the induction of endothelial cell and inhibition of smooth muscle differentiation were mediated by macrophage-derived TNF- $\alpha$  via TNF- $\alpha$  receptor 1 and specific activation of the canonical NF- $\kappa$ B signalling pathways. The over-expression of NF- $\kappa$ B (p65) similarly enhanced endothelial differentiation whereas NF- $\kappa$ B (p65) or TNF- $\alpha$  receptor 1 knockdown led to a significant decrease in TNF- $\alpha$  mediated-endothelial differentiation. Consistently, ex vivo experiments using a decellularised vessel system indicated both an increase in the number of endothelial cells as well as a reduction in smooth muscle marker expression in the presence of TNF- $\alpha$ . Functionally, studies performed on a knockout mouse model of vein graft showed that the lack of TNF- $\alpha$  resulted in significant reduction of endothelial repair that led to thrombus formation.

**Conclusion** These data provide the first evidence that macrophages are key players in controlling the lineage commitment of vascular stem cells in vitro and in vivo, in which TNF- $\alpha$ /TNFR1/NF $\kappa$ B signal pathways are crucial. Our study highlights the role of macrophages in directing vascular stem cells toward endothelial cells that is required for vascular repair in vein grafts.