

20 **THE RHEUMATOID ARTHRITIS SYNOVIAL MICROENVIRONMENT PROMOTES DIFFERENTIATION OF MONOCYTES INTO PRO-ANGIOGENIC MACROPHAGES RESPONSIVE TO ANGIOPOIETIN SIGNALING**

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Background and objectives Vascular remodeling promotes immune cell infiltration and provides nutrients to hyperplastic tissue in the synovial tissue of patients with rheumatoid arthritis (RA). In solid cancers, angiopoietin (Ang-1 and Ang-2) signaling to Tie2-expressing immunosuppressive monocytes (TEMs), is critical in allowing tumor establishment. A similar role for TEMs in chronic inflammatory disease has not been assessed, but the authors have recently found that macrophages are the primary cell types in which Tie2 is activated in RA synovial tissue. Here, the authors examined how the differentiation stimuli and the RA synovial microenvironment might regulate expression of Tie2 on macrophages, as well as their effects on expression of macrophage angiogenic factors and gene expression responses to Ang stimulation.

Material and methods Human healthy donor peripheral blood monocytes were differentiated in the presence of pro-inflammatory/classically activating (GM-CSF, IFN- γ), anti-inflammatory/alternatively activating (M-CSF, IL-10) cytokines

and RA synovial fluid (SF). Tie-2 expression was analysed by flow cytometry and quantitative PCR. Macrophage mRNA expression of 84 angiogenic factors in the absence or presence of TNF stimulation, alone or in combination with Ang-1 was analysed using low density quantitative PCR Array.

Results Tie2 protein and mRNA expression was observed under all conditions, but expression levels failed to correspond to pro- or anti-inflammatory phenotypes – expression levels were equivalent and significantly highest in macrophages differentiated in IFN- γ and IL-10. Tie2 expression was also maintained in macrophages differentiated in the presence of RA SF. Gene expression analysis of angiogenic factors demonstrated distinct expression profiles under each polarisation condition, although macrophages differentiated in RA SF (RASf macrophages) showed significantly enhanced expression of pro-angiogenic chemokines (CXCL3, 5 and 6, IL-8 and CCL2) and decreased expression of antiangiogenic chemokines (CXCL2, 9, 10 and 11) (p values ranging from < 0.05 to 0.0005 for each chemokine). TNF-treatment of RASf macrophages enhanced chemokine expression, and synergistic effects of TNF and Ang-1 were observed in promoting CXCL3, 6, and IL-8 expression.

Conclusion Our results suggest that the RA synovial environment promotes pro-angiogenic macrophage differentiation, and potentiates macrophage production of chemokines which perpetuate synovial monocyte influx in response to TNF and Ang-1.