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Background Acute serum amyloid A (A-SAA) is strongly expressed in rheumatoid arthritis (RA) synovial tissue (ST) and is critically involved in regulating cell migration and angiogenesis. Cell migration and endothelial cell (EC) morphology is dependent on complex signalling interactions which link cytoskeletal rearrangement to extracellular matrix. These processes are also dependent on NOTCH signalling pathways which regulate cell survival and cell shape.

Methods NOTCH1-IC and downstream signaling components HRT1, HRT2 were quantified in synovial tissue/cells by immunohistology and realtime PCR. RAST explants and human microvascular endothelial cells (HDEC) were stimulated with A-SAA (10 and 50 μg/ml) or TNFα (10 ng/ml) for (3–24 h), NOTCH-1C, its ligand DLL-4 and downstream signaling components HRT1, HRT2 were quantified by real-time PCR. NOTCH-1IC, and growth factors (*vascular endothelial growth factor (*VEGF), angiopoietin-2) were assessed by western blot and ELISA. A-SAA modulation of filamentous actin (F-actin) and focal adhesions (vinculin) was examined by dual immunofluorescence. A-SAA induced angiogenesis and migration were assessed by Matrigel tube formation assays and scratch assays. To examine if A-SAA induced angiogenesis, altered cell shape and migration were mediated by NOTCH signaling, functional assays were performed with A-SAA in the presence of siRNA against NOTCH-1IC.

Results NOTCH 1IC, HRT1, HRT2 were expressed in the lining layer and perivascular regions of RA synovial tissue. A-SAA (10 μg/ml) increased NOTCH1IC and VEGF mRNA and protein expression. A-SAA induced HRT-1 mRNA with no effect on HRT2 mRNA. A-SAA inhibited DLL-4 mRNA, which is consistent with a negative feedback loop that controls the interactions between NOTCH-1IC and DLL-4 in the regulation of EC tip versus stalk cells. No effect was observed for angiopoietin 2. A-SAA induced disassembly of EC F-actin cytoskeleton and loss of focal adhesions as demonstrated by a reduction in vinculin staining. Alterations in cytoskeletal dynamics in response to A-SAA was observed as early as 15 min with maximal effect at 24 h, where dramatic formation of filopodia protrusions were demonstrated. Finally, A-SAA induced angiogenesis and cell migration were inhibited when cells were transfected with NOTCH1IC siRNA.

Conclusion A-SAA induces the NOTCH signalling pathway, VEGF and cytoskeletal rearrangement which allows temporal and spatial reorganisation of cells during cell migratory events and EC morphology. Together these results suggest a critical role for A-SAA in driving cell shape, migration and invasion in the inflamed joint.

A28 ACUTE SERUM AMYLOID A THE NOTCH SIGNALING PATHWAY AND CYTOSKELETAL DYNAMICS IN RHEUMATOID ARTHRITIS

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