CYTOKINE MRNA PROFILING IDENTIFIES B CELLS AS A MAJOR SOURCE OF RANKL IN RHEUMATOID ARTHRITIS

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Background and objectives Cytokines are important mediators involved in chronic inflammation and tissue destruction in rheumatoid arthritis (RA). Animal models of arthritis, in vitro studies, and observations made in patients treated with anticytokine antibodies indicate that cytokines act as part of a complex regulatory network. Multiple cell types present in the synovium contribute to the cytokine production that is responsible for maintaining the inflammatory environment in the joints. Elucidating the cytokine network in RA therefore presents a considerable challenge; arguably the first step towards furthering our understanding is to determine the cellular sources of the cytokines found in the RA synovium.

We have developed a method to simultaneously detect cytokine mRNA expression by the five largest cell populations in synovial fluid (SF) and peripheral blood (PB) of RA patients. Cells are neither cultured nor stimulated ex vivo to reflect their in vivo cytokine profile as closely as possible.

Materials and methods SF and PB were obtained from 14 patients with RA. CD3/CD4/CD45RO+ T cells, CD3/CD8/CD45RO+ T cells, CD19+ B cells and CD14+ macrophages were sorted with a cell sorter, and neutrophils were isolated using magnetic anti-CD15 beads, to at least 95% purity. mRNA expression of 40 cytokines was quantified using real-time PCR microfluidic cards. Bias due to sample preparation, cell number and staining techniques were carefully excluded in a series of validation experiments.

Results We have characterised patterns of cytokine mRNA expression by the five largest populations present in RA SF and PB. Detection of cytokines characteristic for T cells and for myeloid cells in the expected populations indicates that we are able to separate cytokine profiles of the investigated populations, thus validating our methodology. Beyond the expected cytokine patterns, we made several novel observations. One intriguing observation was that mRNA expression of RANKL was significantly higher in B cells compared to the other SF populations. This observation was confirmed at the protein level by flow cytometry and in RA synovial tissue by immunofluorescence.

Conclusion Patterns of cytokine production have been identified and are now allowing us to map which cytokines are expressed in individual cell populations in RA. We have identified synovial B cells as producers of RANKL, a key cytokine driving joint destruction by osteoclast activation. This observation is of importance for our understanding of the role of B cells in RA, and may help to explain the success of B cell therapeutic targeting in limiting bone erosion.