Role of pro-inflammatory cytokines in rheumatoid arthritis

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Abstract. Rheumatoid arthritis (RA) is well known to be a chronic autoimmune/inflammatory disease which leads to progressive joint damage and destruction. Less well known is the fact that in severe cases of RA, with extra-articular manifestations and multiple joint involvement, there is also a significant reduction in life expectancy [28]. Hence the need for new therapeutic agents. With the cloning of cDNAs encoding cytokines in the early to mid 1980s, it became possible to use new assays to evaluate cytokine expression in the local site of autoimmunity, the rheumatoid synovium. There were two goals. First would understanding cytokine expression help us understand the pathogenesis of RA? Secondly, would it be possible to learn enough about the cytokine network to establish possible therapeutic targets? While a complete understanding of either of these questions remains elusive, here we review the state of knowledge in early 1998, which shows that much progress has been made and that these goals have been partly reached. The clinical benefits of this knowledge are documented elsewhere in this compilation, as is the role of chemokines, anti-inflammatory cytokines and the cytokines involved in neovascularisation.

Background

In the 1970s the biochemical purification of cytokines began in earnest. This was not an easy task, as the active concentrations of most cytokines are low, in the ng/ml to pg/ml range and hence there is little protein to purify in biological fluids. With the advent of molecular cloning, this task became easier, and the interferons were cloned first [44, 45, 83], followed by IL-2, TNF-α and its related protein lymphotoxin (LT), also known as TNF-β [47, 100, 106]. At the time we began defining cytokine expression and regulation in rheumatoid joints in 1985, there were less than a dozen cloned molecules to be tested, but obviously many more in the pipeline. In those days, prior to the polymerase chain reaction (PCR) and its variants, the key question was whether the available assays for mRNA (Northern and slot blots) would be sensitive enough to detect the low abundance of cytokine mRNA expression in rheumatoid tissue. These
fears were groundless, and it was possible to detect mRNA for IFN-γ, IL-1, TNF-α, LT, IL-2, etc., using arthroscopic biopsy and operative samples of rheumatoid synovium, using either Northern or slot blot analysis [12, 13].

The next problem was how to interpret the data. While mRNA is part of the biochemical pathway from DNA gene expression to the generation of protein, it was not clear if these processes were always directly linked. In other words, does the amount of mRNA for a cytokine accurately predict its protein expression? Our early studies involved extraction of fresh tissues with guanadinium isothiocyanate, and hence protein expression could not be simultaneously assayed. Two procedures were developed to address this question. First, we set up in vitro cultures of total dissociated synovial tissue cells, in the absence of extrinsic stimulation. These cultures contained ~30% T cells, 30% monocytes/macrophages as the major cell types, with smaller numbers of B cells, plasma cells, dendritic cells, fibroblasts and endothelial cells (not in that order). These cells rapidly formed the clumps which are an indication of active immune responses, for example a response of blood mononuclear cells to phytohaemagglutinin (PHA) and probably reflect the cell interactions which occurred in vivo. Supernatants of these cultures were assayed for the production of cytokines, and it was found that 24-h supernatants contained most of the cytokines whose mRNA had been detected. These included TNF-α, IL-1α and β, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [6, 9, 13, 16]. However, some were not detectable in the amounts ‘predicted’ from the mRNA levels detected. These included IFN-γ, IL-2 and LT [7, 12]. Firestein and colleagues [34, 35] have made much of this observation and elaborated on the possible implications, particularly favouring the possibility that T cells may not be important in the late stages of rheumatoid arthritis (RA) from which these samples are obtained [34]. We believe that this discrepancy may reflect immunoregulatory and possibly also therapeutic effects.

The cataloguing of cytokine expression in rheumatoid synovium, by ourselves and other groups [3, 4, 24, 25, 29, 30, 35–37, 65–69, 116] using a variety of complementary techniques revealed that most (if not all) cytokines sought were detectable in all biopsy or operative samples of synovium, although at variable levels (Table 1) and (reviewed in [31]). We noted that there was no obvious correlation of the degree of cytokine expression with concomitant drug therapy or disease duration. In experimental systems in vitro or in vivo, cytokine expression is typically transient, for example IL-1α production lasting 24–48 h after mitogen stimulation. If cytokine production in RA joints was similarly transient, then there should have been many samples in which any given cytokine was not expressed, and hence considerable variation in cytokine expression profiles. The reproducibility of cytokine expression suggested that in RA joints cytokine expression may be prolonged or continuous. Testing that hypothesis led to our current concepts of the cytokine network in rheumatoid joints.

The cytokine network in rheumatoid joints

Unstimulated total rheumatoid synovial joint cells (a very heterogeneous collection of cells) spontaneously form aggregates and produce cytokines without the requirement for extrinsic stimulus in vitro (reviewed in [31]). This provided a system in which our hypothesis that there was prolonged expression of cytokines in the diseased tissue could be tested. Prolonged cytokine expression was detected, at both mRNA and protein level, mimicking what was presumed to occur in vivo. Most importantly this in