Regulators of cytokine signalling in rheumatoid arthritis

Over the last decade, dramatic improvements in the treatment of rheumatoid arthritis (RA) have resulted from increased understanding of the role played by cytokines in the rheumatoid synovium. The development of the anti-TNF-α agents infliximab [1], etanercept [2] and adalimumab [3] has been the most striking manifestation of this success, but other cytokines such as interleukin (IL)-6 and IL-1 have also been studied. It is important to recognize, however, that a sizeable proportion of patients do not respond to anti-TNF-α treatment [1–3]. For these patients, different therapeutic avenues must be explored. These may include manipulation of alternative cytokines or treatments that target cells rather than cytokines. The use of rituximab to deplete peripheral B cells is an example of the latter approach [4].

Another alternative is to look at the ways in which cytokines exert effects on their target cells and to develop agents that can modulate those intracellular signalling mechanisms. Cytokines bind to cell surface receptors, which then aggregate. The cytoplasmic domains of these receptors are constitutively associated with Janus Kinase molecules (JAKs) and receptor aggregation activates the kinase function of these JAKs. This leads to phosphorylation of a number of targets including the JAKs themselves and their associated cytokine receptors. Phosphorylated tyrosine residues on the cytokine receptors then act as docking sites for intracellular signalling molecules known as signal transducers and activators of transcription (STATs) [5, 6]. After binding to these docking sites, the STATs can themselves be phosphorylated by JAKs. The phosphorylated STATs form homodimers or heterodimers that are translocated to the nucleus and alter transcription of specific genes, which contain STAT-responsive elements in their regulatory DNA sequences. In this way, the particular pattern of phosphorylated amino acids in the cytoplasmic domain of the cytokine receptor controls which STAT molecules are involved (there are four types: JAKs 1, 2 and 3 and tyrosine kinase 2) that are activated depend both on the JAK that phosphorylates the STATs and on the receptor that has activated the JAK. The particular pattern of phosphorylated amino acids in the cytoplasmic domain of the cytokine receptor controls which STAT molecules are brought into proximity and can be phosphorylated by JAKs [5].

In 1997, Australian and Japanese groups independently discovered a new class of molecules that act as inhibitors of the JAK/STAT signalling pathway [7–9]. These molecules are termed suppressors of cytokine signalling (SOCS). There are now eight members of the SOCS family [6, 10]: SOCS 1–7 and CIS-1 (cytokine-inducible SH2-containing protein 1). The earliest to be identified, and the most intensively studied, are SOCS-1, SOCS-2, SOCS-3 and CIS-1 [6]. These molecules share a number of homologous structural domains [7, 10]. They are not expressed constitutively but are rapidly induced in response to cytokine exposure. Each SOCS then acts to inhibit the STAT signalling pathway that induced its expression [9], creating a negative feedback loop that limits the effect of cytokine signalling on the cell. Different SOCS have different methods of doing this. For example, SOCS-1 binds to JAKs and inhibits their kinase function [8], whereas CIS-1 blocks recruitment of STATs by phosphorylated cytokine receptors [10]. Furthermore, all SOCS share a consensus carboxy-terminal region called the SOCS box, which enables signalling proteins bind to SOCS to be targeted to proteasomes for degradation [10]. Transient SOCS expression can be induced by cytokines in a wide range of different tissues, and the tissues involved are different for different SOCS [7, 10, 11]. SOCS-1 is especially important in the haematopoietic system and in lymphocytes, and it is believed that expression of SOCS-1 is critical in the development of central and peripheral tolerance [6].

If SOCS regulate cytokine-induced signalling by negative feedback, does this mean that deficiency or dysfunction of SOCS proteins could be important in chronic inflammatory conditions? If SOCS are important in development of tolerance, could deficiency or dysfunction of SOCS lead to autoimmune diseases such as RA?

These questions have been considered in detailed and elegant studies in mouse models, but there are no simple answers. Most SOCS can be induced by a range of different cytokines and can inhibit the effects of those cytokines and others (reviewed in [10]). It is, therefore, difficult to predict the overall effect of a change in SOCS activity on a tissue such as rheumatoid synovium in which many different cytokines are active, sometimes in opposition to each other. However, there is some evidence from murine models that reduced SOCS activity promotes the development of arthritis. Knockout mice homozygous for deficiency of individual SOCS have been produced, but in some cases the effects are lethal at such an early stage that little can be deduced about potential development of autoimmune diseases. SOCS-1 knockout mice die by the age of 3 weeks with hepatic necrosis, macrophage infiltration of multiple organs and haemopoietic abnormalities [12]. It has been shown that these effects are dependent on hyper-responsiveness to the effects of interferon-γ (IFN-γ) [12]. Double knockout SOCS-1/− / SOCS-3/− mice do not develop this early lethal illness and it has been suggested that they do develop arthritis spontaneously [13]. When monoarthritis is induced in IFN-γ−/− mice by intra-articular injection of methylated bovine serum albumin followed by subcutaneous injection of IL-1 (the mBSA/IL-1 model), the synovitis is worse if the mice are also deficient in SOCS-1 [14]. SOCS-3−/− mice die in utero [10], but if the knockout only affects haemopoietic cells, the mice are viable. In these tissue-specific knockout mice, synovitis induced by the BSA/IL-1 method was worse when SOCS-3 was absent [15]. Mice that are homozygous for a point mutation that prevents binding of SOCS-3 to cytokine receptors (which is necessary for SOCS-3 to exert its inhibitory effects) develop a disease similar to RA [16]. There is also evidence that up-regulation of SOCS can ameliorate arthritis in mice. Administration of an adenosinexpressing SOCS-3 to the ankle joints of mice with antigen-induced or collagen-induced arthritis reduced phosphorylation of STAT-3 within the joint and led to marked reduction in synovitis [17].

These murine studies might lead us to predict that RA would be characterized by reduced levels of SOCS-1 and/or SOCS-3 in lymphocytes and synovial cells. In the relatively few human studies available, however, the reverse is generally true. Shouda et al. [17] found increased expression of SOCS-3 mRNA in synovium from patients with RA compared with patients with osteoarthritis. Yamana et al. [18] found that CD4-positive T lymphocytes from peripheral blood of patients with RA had increased expression of SOCS-1 but decreased expression of SOCS-3 at the mRNA level compared with CD4-positive cells from healthy controls.

Why should raised levels of inhibitory SOCS molecules be associated with RA in humans? SOCS can inhibit signalling by anti-inflammatory cytokines and if this were the dominant effect, raised SOCS activity might indeed promote inflammatory disease. The T lymphocytes from patients with RA studied by Yamana et al. [18] were resistant to the immunosuppressive effects
of IL-10. It was postulated that this might be due to the imbalance between levels of SOCS-1 (which inhibits the activation of STAT-3 caused by IL-10) and SOCS-3 (which does not inhibit this activation) [18]. An alternative explanation is that even though SOCS expression is raised in RA, it is not raised sufficiently to counteract the pro-inflammatory effects of the activated JAK/STAT pathways in those cells. A third possibility is that much of the pro-inflammatory signalling in RA might occur via non-JAK/STAT pathways, which are less sensitive to regulation by SOCS. To distinguish these possibilities, there is a clear need for further studies of SOCS expression in patients with RA.

In the October 2007 issue of *Rheumatology*, Isomaki and colleagues [19] reported on levels of expression of mRNA of SOCS-1, SOCS-2, SOCS-3 and CIS-1 in cells from patients with active RA. In an important advance, they studied levels in T lymphocytes and monocytes separately and compared peripheral blood cells with synovial fluid cells derived from the same patients. They found that SOCS expression varies with both cell type and origin of the cells. In the peripheral blood, SOCS-1 and SOCS-2 expression were mainly raised in T cells and SOCS-3 in monocytes but CIS-1 expression was not elevated in either cell type. Expressions of SOCS-1, -2 and -3 were higher in synovial fluid macrophages than in peripheral blood monocytes. Conversely, RA synovial fluid T cells expressed less SOCS-1, -2 and -3 mRNA than peripheral blood T cells from the same patients, but more than peripheral blood T cells from healthy controls. This difference between cells from blood and synovial fluid seems likely to be due to stimulatory cytokines being present in the latter. Exposure of peripheral blood mononuclear cells (PBMCs) from healthy controls to synovial fluid from patients with RA for 1 h led to increased expression of all SOCS. This effect was reduced after longer term exposure, as one would expect if a negative feedback loop was operating. Similar effects on SOCS expression were also seen after exposure of PBMCs to the individual cytokines IL-6, IL-10 and IFN-γ, but not TNF-α. Finally, the authors studied synovial tissue obtained from patients with RA or OA at the time of orthopaedic surgery. These RA patients were rather older than most patients with RA (median age 66 yrs) and did not have highly active disease. Only SOCS-1 mRNA expression was significantly higher in RA synovium than OA synovium. At the protein level, immunohistochemistry showed expression of SOCS-1 and SOCS-3 in macrophages and endothelial cells, but most T cells were SOCS negative.

Overall, these results show clearly that SOCS-1 and SOCS-3 tend to be elevated in all cell types in RA. This is in contrast to the finding of Yamana et al. [18] that SOCS-1 was raised but SOCS-3 was reduced in T cells from patients with RA. However, those authors studied only peripheral blood cells, whereas the paper by Isomaki and colleagues [19] demonstrates that the properties of cells in the joint itself may be different due to effects of intraarticular cytokines. Both the mRNA and protein studies seem to suggest that SOCS are more important in monocytes than in T cells within the joints, but there was wide variation between patients so further studies would be needed to confirm this. This article does not investigate the downstream effects of the raised SOCS levels that were demonstrated. Are the cells responding to the SOCS levels appropriate, and what is the balance between effects of these SOCS on signalling by pro-inflammatory and anti-inflammatory cytokines? It would be interesting to see data on phosphorylation of STATs in the synovial T lymphocytes and macrophages of these patients and the responsiveness of these cells to cytokines. The authors found no relationship between SOCS expression and parameters of disease activity, and treatment with anti-TNF-α agents also had no discernible effect on SOCS expression. Again, studies in larger numbers of patients would be necessary to confirm these findings.

In conclusion, this study has extended our knowledge of expression of SOCS in RA, but there remains much to be done to elucidate whether they play a role in disease pathogenesis. Since SOCS levels already seem to be high in RA, it is not yet clear whether further modulation of these levels will be a fruitful therapeutic avenue.

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