

## REPORT

## Role of interleukin 18 in rheumatoid arthritis

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**M**ediators produced by innate immune response cells such as macrophages can profoundly influence adaptive immunity. Recent studies have shown that interleukin (IL) 18 has an influential role in inflammatory response. Recent data are presented illustrating the importance of IL18 in the induction and perpetuation of chronic inflammation during experimental and clinical rheumatoid synovitis. These findings suggest that antagonists to IL18 may have a role in the treatment of organ specific autoimmune diseases.

There is currently considerable interest in the interactive role of innate and adaptive immunity. A large number of mediators have been implicated, particularly those derived from the innate response that can drive the adaptive immunity. In this short review, we will summarise the work carried out in our laboratory on the roles of interleukin (IL) 18 in adaptive immune response and in perpetuating chronic organ specific inflammatory reactions. We will focus primarily on rheumatoid arthritis (RA). The processes that initiate and perpetuate RA are currently unclear. Successful clinical targeting of tumour necrosis factor (TNF)  $\alpha$  therefore represents an exciting and important advance, not only in therapeutics but also in understanding the disease pathogenesis. However, non-responder or partial responder patients are not uncommon, and inflammatory disease usually flares on discontinuation of treatment.<sup>1,2</sup> This carries significant pathogenetic implications for existing disease models. Moreover, it exemplifies the clinical necessity for generation of further novel, pathogenesis led interventions. One approach to the detection of novel synovial inflammatory pathways is to establish events that regulate synovial TNF $\alpha$  production. Recently, our group has explored the expression of novel cytokine activities in RA synovial membrane that could perpetuate inflammatory synovitis, in particular through modulation of T lymphocyte function and TNF $\alpha$  expression by IL18, an innate cytokine produced principally by macrophages after activation by a range of stimuli, including infection and stress.

**IL18**

IL18 is an 18 kDa glycoprotein derived by enzymatic cleavage of a 23 kDa precursor, pro-IL18, by at least caspase 1.<sup>3</sup> Pro-IL18 expression is widespread, including monocyte/macrophages, dendritic cells, Kupffer cells, keratinocytes, articular chondrocytes, synovial fibroblasts and osteoblasts, and within adrenal cortex and pituitary gland.<sup>4</sup> IL18 mediates bioactivity through a heterodimeric receptor consisting of  $\alpha$  and  $\beta$  chains which are widely expressed on naive T lymphocyte subsets, natural killer cells, macrophages, neutrophils, and chondrocytes. IL18R $\alpha$ , characterised previously as IL1R related protein (IL1Rrp), binds IL18 at relatively low affinity (in the range of  $10^{-8}$  M). Generation of IL18R $\alpha$  deficient mice confirmed that this receptor is nevertheless essential for signalling.<sup>5</sup> IL18R $\beta$  chain, initially termed IL1 receptor accessory protein-like (AcPL), is related and similar to IL1RacP in that it does not bind ligand directly, but rather binds to the complex formed by the IL18-IL18R $\alpha$  chain<sup>6</sup> generating the likely high affinity complex. Thus far, the signal transduction pathway known for IL18R is identical with that of IL1R.

**IL18 IN RA**

We recently detected IL18 in the synovial compartment of patients with RA.<sup>7</sup> Whereas IL18 mRNA was found in synovial membranes from patients with RA or osteoarthritis, IL18 protein was reproducibly detected by histology and enzyme linked immunosorbent assay (ELISA) only in RA derived tissues. IL18 expression was localised in RA synovial membranes in cells of dendritic morphology within lymphocytic aggregates and in lining layer areas. Subsequent double staining confirmed expression in both CD68 macrophages and fibroblast-like synoviocytes. Addition of IL18 consistently induced production of TNF $\alpha$ , granulocyte/macrophage colony stimulating factor, and interferon (IFN)  $\gamma$  by RA synovial membrane or synovial fluid mononuclear cultures in vitro. IL18 induced cytokine production was significantly enhanced by coincident addition of IL12 and/or IL15, and suppressed by IL10 and transforming growth factor  $\beta$ . That IL18 was acting not only through lymphocyte activation, but also through direct effects on macrophages was confirmed using intracellular cytokine staining.<sup>7</sup> These data show that a primary function of IL18 may be direct promotion of the synthesis of TNF $\alpha$  through binding to macrophage IL18R. Importantly, dose-response studies show that only very low concentrations (down to 1 pg/ml) of IL15, IL18, or IL12 are required to induce TNF $\alpha$  production in vitro. In contrast with IL15, we found that IL18 activation of synovial T cells does not alter subsequent T cell-macrophage interactions directly. However, addition of recombinant IL18 to cytokine activated, formalin fixed T cell/monocyte cocultures synergistically up regulates TNF $\alpha$  production mediated through direct effects of IL18 on the target monocyte population (unpublished observations). Thus IL18 synergistically and potently enhances the proinflammatory potential of T cell-monocyte interactions, which are in turn induced by IL15.

Factors regulating IL18 in synovial membrane are as yet unclear. IL18 expression in monocytes is complicated by constitutive expression of mRNA. IL18 mRNA and protein expression are, however, up regulated in vitro in fibroblast-like synoviocytes by IL1 $\beta$  and TNF $\alpha$ , suggesting the existence of positive feedback loops linking the well recognised monokine predominance with cytokine production and Th/c 1 cell activation in synovial immune responses. Finally, IL18 also induced nitric oxide release by RA synovial membranes in vitro. As nitric oxide inhibits caspase 1 activity, this provides a potential feedback loop, whereby IL18 may regulate its own cleavage.<sup>4,8</sup>

**IL18 IN EXPERIMENTAL ARTHRITIS**

We then investigated the mechanism of IL18 induced arthritis in vivo using a murine model of collagen induced arthritis (CIA) in DBA/1 mice.<sup>9</sup> Mice primed intradermally with type II

**Abbreviations:** CIA, collagen induced arthritis; IFN, interferon; IL, interleukin; RA, rheumatoid arthritis; RT-PCR, reverse transcriptase-polymerase chain reaction; TNF, tumour necrosis factor

bovine collagen and then boosted intraperitoneally 21 days later with this type of collagen in saline developed a mild form of CIA. The incidence and severity of the disease was considerably increased when the mice were treated intraperitoneally with 100 ng IL18/mouse/day from day -1 to 4 and then again on days 20-24. The cytokine treatment led to significantly enhanced synovial hyperplasia, cellular infiltration, and cartilage erosion compared with controls treated with phosphate buffered saline. IL18 treated mice produced significantly more IFN $\beta$ , TNF $\alpha$ , and IL6 than the controls. Furthermore, splenic macrophages from DBA/1 mice cultured in vitro with IL18, but not IL12, produced substantial amounts of TNF $\alpha$ . Together, these results show that IL18 can promote CIA through mechanisms that may be distinct from those induced by IL12. To investigate directly the effect of endogenous IL18 expression, we generated IL18 deficient mice on a DBA/1 background. These mice developed appreciably delayed onset and milder severity of CIA than wild type littermates. The reduced disease is characterised by decreased TNF $\alpha$  concentrations in serum and spleen cultures in vitro and by suppressed type II collagen specific Th1 responses in vitro.<sup>10</sup> Importantly, the reduced CIA in the IL18 knockout mice can be reversed by the administration of recombinant murine IL18. Compatible with this, antibody mediated IL18 neutralisation suppressed streptococcal cell wall induced arthritis through an IFN $\gamma$  independent mechanism,<sup>11</sup> and IL18BP-Fc fusion retarded established CIA to an extent comparable to etanercept.<sup>12</sup> Finally, we have shown that antibody to IL18 suppressed development of carrageenin induced paw inflammation by directly suppressing TNF $\alpha$  expression,<sup>13</sup> suggesting that IL18 can operate upstream of TNF $\alpha$  production in vivo. Together, these data strongly suggest that the net effect of IL18 expression is proinflammatory, at least in antigen driven articular inflammation.

### SOLUBLE IL18R $\alpha$ (sIL18R $\alpha$ )

We have previously shown that soluble IL15R $\alpha$  is a potent antagonist of IL15 in vitro and significantly attenuated CIA in DBA/1 mice.<sup>14</sup> Building on this concept, we cloned a truncated version of the extracellular domain of human IL18R $\alpha$  using reverse transcriptase-polymerase chain reaction (RT-PCR), based on a published sequence of IL18R $\alpha$ .<sup>15</sup> mRNA from human peripheral blood mononuclear cells stimulated with *Staphylococcus* enterotoxin B was analysed with primers that amplify the extracellular domain without the leading sequence. Unexpectedly, two PCR products were obtained. The smaller fragment was the expected size for IL18R $\alpha$ . The larger fragment contained an extra 57 bp, inserted from bp 492 of the human IL18R $\alpha$  cDNA, suggesting that a novel exon may have contributed to this larger IL18R $\alpha$  mRNA. Using a Blast-2 sequence search, this novel exon was matched 100% with a 57 bp sequence located in the human IL18R $\alpha$  intron between exon 3 and 4. We called this new exon, exon 3'. These results also indicate that the novel human IL18R $\alpha$  mRNA and previously reported membrane bound IL18R $\alpha$  are transcribed by differential splicing. Further analysis found a stop codon (TGA) in the inserted coding region of the novel IL18R $\alpha$ . As this TGA would make a translational stop before the transmembrane domain of IL18R $\alpha$ , it is likely that this novel fragment is a naturally produced soluble IL18R $\alpha$ . Another primer was then used to span the inserted region to clone out this novel sIL18R $\alpha$ . RT-PCR with primers WR1 and WR4 produced only a single band at the expected 400 bp. The novel sIL18R $\alpha$  was then expressed by transfecting COS-7 cells with sIL18R $\alpha$  fused with green fluorescent protein (GFP) and tagged with myc/6xhis under tetracycline induction. Transfectants produced sIL18R $\alpha$ -GFP detectable with anti-Myc as well as anti-IL18R IgG by Western blot. Moreover, the soluble product strongly suppressed IL18 induced IFN $\gamma$  synthesis by KG1 cells.

We then investigated whether sIL18R $\alpha$  is secreted by physiologically relevant cells. The human monocytic cell line, KG1, was cultured in vitro with graded concentrations of TNF $\alpha$  and mRNA, and culture supernatants were collected for analysis. sIL18R $\alpha$  was detected intracellularly and as secreted protein in the culture supernatant in a time and dose dependent manner. Further experiments show that, under different activating conditions, human mononuclear cells can produce a number of spliced variants of sIL18R $\alpha$ . For example, when peripheral blood mononuclear cells were activated with *Staphylococcus* enterotoxin B and IFN $\gamma$ , six species of IL18R $\alpha$  with size ranging from 250 to 900 bp were detected by RT-PCR Southern blot. The existence of spliced variants of cytokine receptors has been observed previously. For example, three isoforms of IL15R $\alpha$  mRNA have been described that result from alternative splicing of exon 3 and/or alternative usage of exon 7 or 7'.<sup>16</sup> More recently, eight further variants of IL15R $\alpha$  resulting from splicing of exon 2 have been identified.<sup>17</sup> Natural spliced variants of cytokines have been studied extensively, and some are found to have considerable inhibitory effects on clinical inflammation.<sup>18</sup> Biochemical studies have shown that IL18R $\alpha$  protein exists as heterogeneous molecules ranging from 60 to 110 kDa, which could not be explained by deglycosylation alone.<sup>19</sup> Thus, there may be a family of spliced variants of naturally produced sIL18R $\alpha$ , possibly with distinct functions in the regulation of IL18 activity for the maintenance of immunological homeostasis. Such molecules could be exploited for treatment of inflammatory diseases associated with the overexpression of IL18.

### CONCLUSION

Our studies show the potential for striking cytokine synergy in promoting synovial inflammation. Our choice of IL18, an IL1-like cytokine (NF- $\kappa$ B dependent), and IL15, an IL2-like cytokine (STAT3/STAT5 dependent), may partially explain this synergism, although this remains to be explored. Enhanced expression of IL15 and IL18 extends beyond RA to include several human inflammatory diseases. Thus, the biological activities elucidated here for IL18 will probably be of general importance. The ultimate significance of IL18 expression in autoimmune disease in vivo, however, requires confirmation in humans before therapeutic intervention can be performed.

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