REVIEW

Cellular targets of interleukin-18 in rheumatoid arthritis

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Recent data are presented which indicate a critical role for interleukin (IL)-18 in rheumatoid arthritis (RA). The T cells and macrophages invading the synovium or in the synovial fluid are the chief cellular targets of IL-18 in RA. Neutrophils, dendritic cells and endothelial cells may also be cellular mediators of IL-18. The direct effect of IL-18 on fibroblast-like synoviocytes or chondrocytes may not be essential or important. In RA, IL-18, which is mainly produced by macrophages, activates T cells and macrophages to produce proinflammatory cytokines, chemokines, adhesion molecules and RANKL which, in turn, perpetuate chronic inflammation and induce bone and cartilage destruction.

illustrate the importance of IL-18 in the induction and perpetuation of chronic inflammation during experimental and clinical rheumatoid synovitis. IL-18 is a rational target for therapeutic regimens against rheumatoid arthritis (RA). In a phase I study, recombinant human IL-18BP was found to have a favourable safety profile and to be well tolerated in healthy volunteers and in subjects with active RA.⁹

INCREASED LEVELS OF IL-18 IN HUMANS WITH RHEUMATOID ARTHRITIS

As a proinflammatory cytokine, IL-18 has been associated with the development of several inflammatory diseases including RA.¹⁰ In 1999, Gracie *et al*¹¹ showed for the first time that IL-18 mRNA and protein were significantly more abundant in the joints of patients with RA than in those with osteoarthritis (OA). The median IL-18 concentration is comparable to the concentration of TNF α in RA synovial fluid. The results strongly suggest that mature IL-18 is present in the synovial compartment. Immunohistological analysis further showed a cytoplasmic staining pattern in RA synovial tissues, while little IL-18 protein was detected in OA synovial membranes.

Other groups also confirmed that RA synovial tissues showed increased expression of IL-18 mRNA and increased IL-18 protein synthesis compared with OA tissues.^{12–14} Moreover, western blotting revealed that RA synovial tissue expressed mature IL-18 more abundantly than OA samples.¹⁵ As in the synovial fluids, the concentration of IL-18 protein detected in the serum of patients with RA was markedly higher than that detected in the serum and synovial fluids of patients with OA.^{12–16–17} Serum and synovial fluid IL-18 levels as well as synovial tissue IL-18 expression were correlated with disease activity as measured by erythrocyte sedimentation rate, serum C-reactive protein, microscopic inflammation scores and Disease Activity Score (DAS 28) index values.^{12–14–18} After methotrexate/sulphasalazine treatment, slight decreases in synovial tissue IL-18 expression were observed which were correlated with changes in serum IL-18 and C-reactive protein.¹⁴ It was also

Interleukin-18 (IL-18), a member of the IL-1 cytokine superfamily, is an 18 kDa glycoprotein derived by enzymatic cleavage of a 23 kDa biologically inactive precursor, pro-IL-18, by caspase-1.^{1–2} Pro-IL-18 can also be intracellularly activated by caspase-1 independent mechanisms such as enzymatic cleavage by neutrophil proteinase-3.³ In addition, Fas ligand stimulated macrophages produce active IL-18 in the absence of caspase-1.⁴ IL-18 exerts its biological effects via its receptor complex. The IL-18 receptor (IL-18R) complex is a heterodimer containing an α chain responsible for extracellular binding of IL-18 and a non-binding signal transducing β chain, both members of the IL-1R family.⁵ On binding of IL-18 to IL-18R α , IL-18R β is recruited and induces signalling pathways shared with IL-1R as well as Toll-like receptors (TLRs). IL-18 signals through a pathway that involves myeloid differentiation factor 88 (MyD88), IL-1 receptor-associated kinase-4 (IRAK-4), tumour necrosis factor (TNF) receptor-associated factor 6 and NF- κ B.⁶ IL-18 also activates AP-1, MAPK and STAT3 pathways. Similar to IL-1R signalling, an accessory cell surface protein-like molecule has been identified with the ability to cooperate with IL-18R α , which is essential for IL-18 mediated signalling.⁷ IL-18 binding protein (IL-18BP) has a very high binding affinity for IL-18 but has no structural homology with the IL-18R. IL-18BP blocks binding of mature IL-18 to IL-18R.⁸ It therefore works as a soluble decoy receptor for IL-18.

IL-18 plays an important role in host defence, particularly in the early phases. It enhances both innate and acquired immunity and also has prominently wide spectrum biological actions on non-immune systems. Excessive production of IL-18 sometimes induces pathological changes in various organs. Recent data are presented which

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Abbreviations: CIA, collagen induced arthritis; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN γ , interferon γ ; IL, interleukin; IL-18BP, IL-18 binding protein; IL-18R, IL-18 receptor; MCP-1, monocyte chemotactic protein-1; MIP-1 α , macrophage inflammatory protein-1 α ; MMP, metalloproteinase; NK, natural killer; OA, osteoarthritis; RA, rheumatoid arthritis; RANKL, receptor activator of NF κ B ligand; SCW, streptococcal cell wall; TLR, Toll-like receptor; TNF, tumour necrosis factor; VCAM-1, vascular cell adhesion molecule-1

found that serum IL-18 levels can be rapidly reduced by anti-TNF α treatment.¹⁹ These data suggest that IL-18 levels are correlated with disease activity in RA, although some authors have reported conflicting results. Bokarewa *et al*²⁰ found that the level of IL-18 was not related to markers of inflammation, although both serum and synovial fluid IL-18 levels in patients with RA with erosive joint disease were higher than in those with non-erosive RA.

Double staining indicated that cells producing IL-18 in RA synovial membranes were predominantly, but not exclusively, found within CD68+ macrophages while CD3+ lymphocytes were consistently negative.^{11–15} Notably, IL-18 protein expression in the sublining correlated with macrophage infiltration.¹³ In vitro, purified CD14+ macrophages, but not activated fibroblast cell lines, from RA synovium were able to release mature IL-18, although both cell types expressed its transcripts.¹² It was further confirmed that mRNA expression of IL-18 in fibroblast-like synoviocytes is not sufficient to indicate the production of IL-18 protein.²¹ In addition, constitutive expression of IL-18 mRNA and enhanced expression by IL-1 β were found in articular chondrocytes by our group and others using RT-PCR,^{22–23} but we failed to detect IL-18 protein in culture supernatants by ELISA either in the unstimulated condition or after stimulation with IL-1 β , TNF α or prostaglandin E₂ for 48 h.²² Taken together, these results indicate that IL-18 in RA is produced predominantly by tissue macrophages rather than by lymphocytes, fibroblasts or chondrocytes. In addition, mature IL-18 may also be released from dendritic cells (DCs)^{1,24} neutrophils²⁵ and endothelial cells.²⁶

PATHOLOGICAL ROLE OF IL-18 IN ARTHRITIS AS DETECTED IN ANIMAL MODELS

Murine collagen induced arthritis (CIA) is a useful model for RA in which the critical role of cytokines such as TNF α and IL-1 has been characterised in vivo. IL-18 also appears to play an important role in the pathophysiology of CIA, as shown by the pioneering work of Gracie *et al*.¹¹ They found that the incidence and severity of CIA in mice were exacerbated by the administration of IL-18. Shortly thereafter the central importance of IL-18 in erosive inflammatory arthritis was confirmed by the fact that CIA in IL-18^{-/-} mice was less severe than in wild-type controls.²⁷ Histologically, there was evidence of decreased joint inflammation and the destructive component of the CIA model in IL-18^{-/-} mice. Treatment with IL-18 completely reversed the disease in IL-18^{-/-} mice to that of the wild-type mice. The pro-arthritis effect of IL-18 was further supported by IL-18 gene transfer into the mouse knee joint.²⁸ Local overexpression of IL-18 resulted in pronounced joint inflammation and loss of cartilage proteoglycan. The preventive effect of systemic administration of IL-18BP or intra-articular overexpression of IL-18BP in CIA mice was demonstrated by the finding of delayed onset and reduced incidence of CIA as well as a 50% reduction in the clinical disease activity score.^{29–30} Local intra-articular IL-18BP treatment in both knees provided additional protection against the incidence and severity of CIA in distal paws.³⁰ The pro-arthritis effect of IL-18 and the protective effect of neutralising anti-IL-18 antibody were also demonstrated in CIA BB rats.³¹ Exogenous IL-18 can accelerate the clinicopathological changes in CIA models while neutralisation of endogenous IL-18 can inhibit them, which suggests that accumulation of IL-18 in the articular lesion is a cause of the pathological changes in RA rather than resulting from them.

In addition to preventive effects, neutralisation of IL-18 has therapeutic effects in experimental arthritis. In mice with established CIA, the clinical severity was significantly reduced after treatment with either IL-18 neutralising antibodies or recombinant IL-18BP.³² Attenuation of the disease was associated

with reduced cartilage erosion evident on histological examination. Decreased cartilage degradation was further shown by a significant reduction in the levels of circulating cartilage oligomeric matrix protein (an indicator of cartilage turnover). Serum levels of IL-6 were significantly reduced with both neutralising strategies, which suggests that IL-18 stimulates IL-6 production in CIA. The data from animal models clearly indicate that IL-18 is of importance during developing and sustained inflammatory arthritis. However, the precise mechanism by which IL-18 mediates the pathological effect remains to be explored.

Endogenous IL-18 also plays an essential part in the development of the acute arthritis mouse model, streptococcal cell wall (SCW) induced arthritis.³³ A single intra-articular injection of SCW induces joint swelling and cartilage destruction within 2 days. Serum levels of IL-18, in addition to TNF α and IL-1 β , are increased in parallel with disease development. A neutralising anti-IL-18 antibody was injected shortly before induction of arthritis by intra-articular injection of SCW fragments into the right knee joint. Significant (>60%) suppression of joint swelling of SCW arthritis and serum TNF α and IL-1 β levels was noted after blockade of endogenous IL-18, which suggests that IL-18 plays a pivotal role in increased TNF α and IL-1 β production and joint swelling of SCW arthritis. Severe reduction of chondrocyte proteoglycan synthesis is a prominent component of SCW induced arthritis, but an almost complete reversal of depressed chondrocyte proteoglycan synthesis was observed in animals treated with anti-IL-18. These studies clearly established the pathological role of endogenous IL-18 in this model, indicating that IL-18 might be a potential target for the treatment of acute inflammatory arthritis.

SIGNIFICANCE OF PROINFLAMMATORY CYTOKINES IN MEDIATING THE ROLE OF IL-18 IN ARTHRITIS

Exogenous IL-18 exacerbates the clinicopathological scores in CIA with increased serum levels of proinflammatory cytokines including IL-6, TNF α and interferon γ (IFN γ) and anticollagen antibodies.³⁴ In CIA mice treated with IL-18BP, proliferation of collagen stimulated spleen and lymph node cells as well as the change in serum levels of IgG₁ and IgG_{2a} antibodies to collagen were decreased.²⁹ The production of IFN γ , TNF α and IL-1 β in cultured spleen cells was also reduced by in vivo treatment with IL-18BP. Cell sorting analysis showed a decrease in NK cells and an increase in CD4+ T cells in the spleen of mice treated with IL-18BP. In addition, the steady state mRNA levels of IFN γ , TNF α and IL-1 β in isolated joints were each decreased in mice treated with IL-18BP. The mechanism of IL-18BP inhibition of CIA therefore includes a reduction in cell mediated and humoral immunity to collagen as well as a decrease in the production of proinflammatory cytokines (IFN γ , TNF α , IL-1 β and IL-6) in the spleen and joints, which was further confirmed by other evidence. Production of Th1 type cytokines IFN γ and IL-2 in addition to monokines TNF α and IL-6 from splenocytes isolated from CIA BB rats was significantly enhanced by in vivo administration of IL-18 and inhibited by in vivo administration of neutralising anti-IL-18 antibody. In comparison, the Th2 type cytokine IL-10 was not affected by either IL-18 or anti-IL-18 antibody.³¹ IL-18^{-/-} CIA mice were characterised by reduced serum concentrations of TNF α and significantly reduced proliferation of cultured spleen and draining lymph node cells and production of proinflammatory cytokines (IFN γ , TNF α , IL-6 and IL-12) by spleen and lymph node cells in response to bovine type II collagen in vitro compared with wild-type mice.²⁷ These data indicate that IL-18 is a primary proinflammatory cytokine during arthritic disease that drives production of IFN γ , IL-1 β , IL-2, IL-6 and TNF α .

It was also found that IL-18 expression in active RA synovial tissue (both in the sublining and the lining) was strongly correlated with the expression of IL-1 β , TNF α and IL-32.^{13–35} IL-32 is a recently discovered cytokine that induces TNF α , IL-1 β , IL-6 and chemokines. Local overexpression of IL-18 in the mouse knee joint by gene transfer resulted in locally increased levels of IL-1 β and TNF α .²⁸ The data derived from TNF-deficient and IL-1-deficient mice show that IL-18 induced IL-1 is essential for IL-18 induced degradation of joint cartilage, and that IL-18 induced TNF is partly involved in IL-18 induced joint inflammation.²⁹ Together these results clearly show that IL-18 is required for induction of the optimal production of proinflammatory cytokines in RA, and that IL-1 β and TNF α are key mediators in IL-18 induced joint inflammation and joint destruction.

BIOLOGICAL FUNCTION OF IL-18 ON TARGET CELLS IN SYNOVITIS

T lymphocytes

IL-18 was initially characterised as IFN γ inducing factor for its ability to augment IFN γ production by activated T cells.¹ Shortly after it was found that IL-18 can induce Th2 responses (ie, IgE, IL-4, IL-13). At early stages of T cell differentiation, IL-18 can promote either Th1 or Th2 responses, dependent on IL-12 or IL-4.³⁶ However, data primarily derived from knock-out experiments indicate that IL-18 might indeed favour a Th1 response *in vivo*.³⁷ IL-18 alone fails to induce IFN γ production from naive T cells, whereas IL-18 in combination with IL-12 induces massive production of IFN γ from T cells as a result of upregulation of IL-18R expression on naive T cells, Th1 cells and B cells by IL-12. This synergism is important to the production of IFN γ in RA synovial T cells and natural killer (NK) cells.^{10–11–15} Although IL-18 alone is not sufficient to drive NK cell proliferation, it is able to act synergistically with IL-15 in stimulating NK cell proliferation *in vitro*.³⁸ It also helps activate NK cells to express Fas ligand and consequently enhances NK cell cytotoxicity.³⁷ IL-18 enhances the proliferation of anti-CD3 monoclonal antibody-stimulated human T cells and may also induce IL-17 and TNF α production by T cells.^{13–39} Our data show that IL-18 upregulates membrane bound receptor activator of NF κ B ligand (RANKL) expression and soluble RANKL production by RA synovial T cells.⁴⁰ Compared with healthy controls, peripheral blood mononuclear cells from patients with RA have decreased IFN γ production in response to IL-12 and IL-18,⁴¹ which suggests that RA disease combines a polarised immune response with an active Th1 in inflamed joints and a reduced Th1 pattern in the peripheral circulation.

Chemoattraction of T cells by IL-18 was demonstrated both *in vitro* and *in vivo*. It was found that IL-18 induced chemokines IL-8, macrophage inflammatory protein (MIP)-1 α and monocyte chemoattractant protein (MCP)-1 in human peripheral blood mononuclear cells in the absence of any co-stimuli.³⁹ IL-18 alone or in combination with IL-12 induces T cell adhesion to inflamed sites to regulate early inflammatory events which were mediated by specific adhesion molecules (β_1 integrins and CD44) expressed on the T cell surface.⁴² IL-18 increased the proportion of T cells in polarised morphology *in vitro* and stimulated their subsequent invasion into collagen gels in an IL-18 concentration gradient-dependent manner. Furthermore, RA synovial CD4+ but not CD8+ T cells also migrate to IL-18. Injection of IL-18 into the footpad of DBA/1 mice led to local accumulation of inflammatory cells.⁴³ These data suggest that IL-18 can contribute to the development of an acquired immune response and the maintenance of chronic immune stimulation in diseases such as RA by promoting chemotaxis of activated T cells.

Macrophages/monocytes

Macrophages derived from RA synovial membrane but not peripheral blood monocytes respond directly to IL-18 with TNF α production.⁴⁴ IL-18 upregulated intercellular adhesion molecule-1 (ICAM-1) expression on monocytes in human peripheral blood mononuclear cells. Antibodies to ICAM-1 and its ligand LFA-1 not only prevented IL-18 induced aggregation of peripheral blood mononuclear cells, but also exhibited a significant inhibitory effect on enhanced production of IFN γ , IL-12 and TNF α in peripheral blood mononuclear cells by IL-18.⁴⁵ These results as a whole indicated that the production of IFN γ —as well as IL-12 and TNF α induced by IL-18—was dependent on the cell-cell interaction through ICAM-1 on monocytes and LFA-1 on T or NK cells. The cell-cell interaction was further highlighted by our studies.⁴⁶ IL-18 enhances IL-1 β and TNF α (but not IL-10) production by monocytes in a dose dependent manner following direct contact with paraformaldehyde fixed RA synovial T cells. Neutralising antibodies to IL-18 inhibited monocyte production of IL-1 β and TNF α induced by direct contact with activated T cells. As a result of cell-cell contact, activated T cells increased autocrine production of IL-18 by monocytes and upregulated IL-18R expression on monocytes.⁴⁶ These data also imply that IL-18 can act in an autocrine fashion on the monocyte population. IL-18 upregulated the expression of TNF receptors, vascular cell adhesion molecule (VCAM)-1 and ICAM-1 on monocytes. Blocking the binding of TNF receptors, VCAM-1 or ICAM-1 on monocytes to their ligands on stimulated T cells each suppressed IL-18 enhanced TNF α and IL-1 β production in monocytes induced by contact with pre-stimulated T cells, which suggests that upregulation of the expression of the TNF receptors VCAM-1 and ICAM-1 is responsible for the IL-18 stimulated cell-cell interaction between monocytes and activated T cells.⁴⁶

IL-18 stimulation of peritoneal macrophages induces IL-6 production independent of the intermediate induction of endogenous cytokines such as TNF α or IL-1 β .⁴⁴ It has also been reported that IL-18 promotes IFN γ , granulocyte-macrophage colony stimulating factor (GM-CSF) and nitric oxide release from macrophages.^{44–47} However, IFN γ production may be attributed to minute numbers of contaminant T cells or NK cells in IL-12/IL-18-stimulated macrophage populations.⁴⁸ In purified CD14+ cells but not CD3+/CD4+ cells, IL-18 induced gene expression and synthesis of IL-8 and IL-1 β but did not induce the anti-inflammatory cytokines IL-1Ra or IL-10.³⁹ IL-18 also induced the production of MCP-1 in macrophages, which was independent of IL-12 and was not mediated by autocrine cytokines such as IFN γ or TNF α .⁴⁹ Recently, IL-18 together with IL-12—but not alone—was reported to prevent spontaneous apoptosis of cultured monocytes, to promote monocyte clustering and subsequent differentiation into macrophages. These morphological changes were accompanied by increased secretion of CXC chemokine ligands (CXCL9, CXCL10 (up to 100-fold) and CXCL8 (up to 10-fold)).⁵⁰

TLRs are involved in the uptake and processing of various exogenous and endogenous antigens and also mediate maturation of DCs and promote naive T cells toward a Th0, Th1 or Th2 phenotype. TLR-2 and TLR-4 are constitutively expressed on various cell members of the immune system including macrophages, neutrophils and DCs. In RA synovial tissue, enhanced expression of both TLR-2 and TLR-4 was demonstrated. TLR expression in both the lining and the sublining was associated with the staining of IL-18 but not with IL-1 β , IL-17 or TNF α .⁵¹ IL-18 promotes IFN γ secretion by T cells which, in turn, induces enhancement of TLR-2 and TLR-4 expression on monocytes.⁵¹ These data suggest that IL-18 results in TLR mediated activation of the innate and adaptive immune

responses which might lead to a vicious circle of deteriorating RA synovitis.

Neutrophils

Human peripheral blood derived neutrophils constitutively expressed IL-18R (α and β) commensurate with the capacity to rapidly respond to IL-18. IL-18 administration promoted neutrophil accumulation in vivo whereas IL-18 neutralisation suppressed the severity of footpad inflammation following carrageenan injection. Such findings suggest that IL-18 can induce inflammation by activating neutrophils. Upregulation of CD11b by IL-18 is partially responsible for in vivo neutrophil recruitment.⁵² Furthermore, IL-18 strongly induced leucotriene B₄ synthesis by human peripheral blood neutrophils. IL-18 induced neutrophil recruitment and leucotriene B₄ production could be blocked by TNF α neutralisation. In addition, IL-18 failed to induce neutrophil accumulation in vivo in TNFRp55^{-/-} mice.⁵³ IL-18 therefore activates and attracts neutrophils by inducing the production of TNF α which, in turn, induces the synthesis of leucotriene B₄. Leucotriene B₄, a well known chemoattractant of neutrophils, leads to neutrophil accumulation and subsequent local inflammation. This is further supported by the observation that inhibition of leucotriene B₄ synthesis attenuated IL-18 induced CIA.⁵³

In addition, IL-18 stimulates the ability of neutrophils to produce inflammatory mediators.⁵² Peripheral blood derived neutrophils produced high levels of IL-8 and low levels of IL-1 α and TNF α in response to IL-18. In contrast, neutrophils in RA synovial fluid released high levels of IL-8, IL-1 α and TNF α in response to IL-18. Thus, the potential of neutrophils for ex vivo cytokine release in response to IL-18 appears to be qualitatively and quantitatively altered by prior in vivo activation. IL-18 also stimulates neutrophil degranulation⁵² but has no effect on the rate of neutrophil apoptosis, suggesting that IL-18 does not shorten the survival of neutrophils at sites of inflammation. Systemic administration of IL-18 increased neutrophil counts in the circulation, which suggests that it induces haematopoietic cell proliferation causing neutrophilia.⁴⁷ Taken together, these data suggest that IL-18 induces neutrophils to accumulate in sites of synovitis and then activates them to cause deterioration in RA synovitis.

Dendritic cells (DCs)

Several recent reports have reported a significant role for IL-18 in the biology of DCs. Monocyte derived DCs (DC1) preferentially induce a Th1 response and plasmacytoid-derived DCs (DC2) have been linked to a Th2 response. Consistent with its role in modulating Th1/Th2 responses, IL-18 also regulates DC1 and DC2 responses. IL-18R α and IL-18R β are constitutively expressed only on DC2s and enhance their chemotaxis and allostimulatory capacity.⁵⁴ In DC1s, both IL-12 and IFN γ upregulate the expression of IL-18R, and co-stimulation with IL-12 and IL-18 induces high levels of IFN γ production and upregulated CD40 expression.⁵⁵ Together with IL-12, IL-18 plays a critical role in the reciprocal maintenance and expansion of DCs and NK cells, respectively.⁵⁶ For example, IL-18 is constitutively released from DC1s²⁴ and production of IFN γ by NK cells relies mainly on DC derived IL-18.⁵⁷ Thus, IL-18 might have a critical role during the initiation phase of an immune response by recruiting and modulating the function of DCs, but a clear in vivo functional relevance of the effects of IL-18 on DCs is yet to be demonstrated.

Endothelial cells

Consistent with the expression of functional IL-18R on human endothelial cells, IL-18 upregulates chemokine IL-8 and adhesion molecules ICAM-1, VCAM-1 and E-selectin expression on

endothelial cells and further promotes endothelial cell-leucocyte adhesion.⁵⁸⁻⁵⁹

Angiogenesis is a key process in the development of synovial inflammation in RA. IL-18 induces human microvascular endothelial cell proliferation, migration and endothelial cell tube formation on Matrigel matrix. It also induces blood vessel growth in the Matrigel plugs and the sponge granuloma models implanted in mice.⁶⁰ Moreover, IL-18 injected intradermally into murine skin induced a significant neovascular reaction.⁶¹ It may also stimulate angiogenesis indirectly by induction of vascular endothelial growth factor (VEGF) from RA synovio-cytes.⁶² IL-18 therefore appears to be angiogenic both in vitro and in vivo. However, some reports have suggested that IL-18 might be anti-angiogenic. IL-18 specifically inhibits fibroblast growth factor-2 stimulated proliferation of endothelial cells in vitro and suppresses fibroblast growth factor induced corneal neovascularisation by systemic administration in mice. It also inhibits embryonic angiogenesis in the chick chorioallantoic membrane.⁶³ Other findings support the notion that IL-18 induces apoptosis in endothelial cells via a novel signalling pathway involving NF- κ B dependent phosphatase and tensin homologue deleted on chromosome 10 (PTEN) activation and has an anti-angiogenic role.⁶⁴⁻⁶⁶ However, synovial fluid from patients with RA can induce tube formation of endothelial cells which is inhibited by anti-IL-18 antibody,⁶⁰ suggesting an essential role for IL-18 in the induction of synovial angiogenesis in RA. IL-18 might therefore also contribute to the development of RA by induction of neovascularisation.

Synovial fibroblasts

IL-18 also accelerates inflammatory responses by inducing various types of chemokines and adhesion molecules in fibroblast-like synovio-cytes. IL-18 upregulates the production of CXC chemokines including IL-8, epithelial-neutrophil activating protein (ENA-78) and growth-regulated oncogene (gro α), and CC chemokines such as MIP-3 α in RA synovial fibroblasts,⁶⁷⁻⁶⁸ presumably leading to the recruitment of neutrophils and memory lymphocytes into the injured joint with expansion of the inflammatory responses. IL-18 also promotes RA synovial fibroblasts to express adhesion molecules ICAM-1 and VCAM-1, which might contribute to the recruitment of inflammatory cells into the joint.⁵⁹⁻⁶⁹ IL-18 requires phosphatidylinositol 3-kinase pathways as well as NF- κ B to induce ICAM-1/VCAM-1 expression on RA synovial fibroblasts. In addition, IL-18 may also induce the production of VEGF and serum amyloid A protein in RA synovial fibroblasts.¹⁷⁻⁶² We observed that IL-18 slightly increased the production of RANKL and osteoprotegerin in RA synovial fibroblasts (unpublished data). It is not yet known whether osteoclast differentiation is facilitated or inhibited by IL-18 stimulated RA synovial fibroblasts.

Although IL-18R α mRNA was constitutively expressed by RA fibroblast-like synovio-cytes, there is still controversy concerning the expression of IL-18R β on fibroblasts. Möller *et al*⁷⁰ found that the simultaneous presence of RT-PCR products of both IL-18R α and IL-18R β was seen in 5 of 20 RA synovial fibroblast cultures and in no cultures derived from OA. When I κ B- α was assayed in IL-18R α ⁺ β ⁺, IL-18R α ⁺ β ⁻ and IL-18R α ⁻ β ⁻ synovial fibroblasts, I κ B- α activation by IL-18 occurred only in IL-18R α ⁺ β ⁺ synovial fibroblasts, indicating that the presence of both IL-18R chains is a prerequisite for IL-18 signal transduction. These workers also observed some ICAM-1 induction by IL-18 in 5 of 33 cultures, but in most of the RA synovial fibroblast cultures there was no response. In addition, stimulation of the cultures with IL-18 neither increased cell proliferation nor upregulated the production of VCAM-1, metalloproteinases (MMP)-1 and -3, GM-CSF, prostaglandin E₂ or nitric oxide. Kawashima and Miossec⁷¹ reported that IL-18R β in fibroblast-like

Figure 1 Osteoclast formation induced from precursor cells co-cultured with rheumatoid arthritis synovial T cells in the presence of macrophage colony stimulating factor (M-CSF). After 7 days of culture, cells were fixed and stained for tartrate-resistant acid phosphatase. M-CSF, 33 ng/ml; interleukin-18, 10 ng/ml. Scale bar 100 µm.

synoviocytes could not be detected by RT-PCR for amplification of 30 cycles, and IL-18 failed to induce production of IL-6 in RA synovial fibroblasts. Total RA synovium cells containing T cells showed a strong expression of both IL-18R α and IL-18R β mRNA. They therefore postulated that cellular contamination might explain IL-18R β mRNA expression by fibroblast-like synoviocytes in the work of Möller *et al.*⁷⁰ However, in response, Möller *et al.*⁷² excluded T cell contamination by checking IFN γ mRNA in the cDNA of previously studied cultures. We think it is possible that the difference in cycles of amplification in RT-PCR might be responsible for the different results described in the above two studies. To detect IL-18R β mRNA, Möller *et al.*⁷⁰ amplified the cDNA by PCR for 30 cycles in the first step and then amplified the PCR products by nested RT-PCR for 20 additional cycles. Consistent with the mRNA expression levels, only IL-18R α protein but not IL-18R β protein in RA synovial fibroblasts was detected by western blotting. The data from the two studies are therefore not conflicting in nature. Since IL-18R β expression in synovial fibroblast cultures from most cases of RA was marginal, the authors of both studies concluded that the presence of macrophages or T cells that can respond directly to IL-18 is essential for the proinflammatory effects of IL-18 in synovitis in RA.^{70 71}

Chondrocytes

It was reported that IL-18 inhibited TGF- β induced proliferation, enhanced nitric oxide production and stimulated the expression of several genes in normal human articular chondrocytes including inducible nitric oxide synthase, inducible cyclooxygenase, IL-6 and stromelysin.²³ In our own studies,²² constitutive expression of IL-18R α in chondrocytes was found in only 4/8 cases (50%) of normal cartilage, 9/14 cases (64%) of OA cartilage and 6/10 cases (60%) of RA cartilage. After IL-1 β stimulation the expression of IL-18R α was significantly upregulated in all cases. The constitutive expression of IL-18R β was barely detectable by RT-PCR for amplification of 35 cycles in all cases so nested RT-PCR had to be carried out; IL-1 β upregulated the expression of IL-18R β by about fourfold. Chondrocytes isolated from only about 30% of cases were responsive to IL-18, and 100 ng/ml IL-18 had only a modest effect on MMP-1, MMP-3 and MMP-13, increasing production nearly twofold. The positive control IL-1 β showed a much more potent effect on the production of MMPs than IL-18. We failed to demonstrate a synergistic effect on MMP production between IL-1 β and IL-18. It may be that, compared with IL-1 β , IL-18 is too weak to induce MMPs from chondrocytes. The crucial role of IL-1 in IL-18 induced cartilage destruction was further supported by *in vivo* and *in vitro* experiments. As discussed in this context, IL-18 gene transfer into the mouse knee joint could induce destruction of joint cartilage.²⁸ Of particular interest was the fact that IL-18 gene transfer in IL-1 deficient mice did not show cartilage damage although joint inflammation was similar to that in wild-type animals. Blocking of IL-1 with IL-1Ra or an IL-1 β converting enzyme inhibitor resulted in complete protection against IL-18 mediated cartilage degradation *in vitro*. These data therefore show that IL-18 does not induce cartilage destruction directly but appears to induce cartilage destruction indirectly via IL-1 β .

Osteoclasts

Animal model studies highlight the central importance of IL-18 among several cytokines that participate in erosive inflammatory arthritis.^{11 27–33} However, IL-18 appeared to inhibit osteoclast formation in the co-culture of mouse osteoblasts and haemopoietic cells of spleen or bone marrow origin in the absence of M-CSF. The inhibitory effect of IL-18 was limited to the early phase of the co-culture which coincides with the proliferation of haemopoietic precursors.⁷³ Neutralising antibodies to GM-CSF were able to rescue IL-18 inhibition of osteoclast formation whereas neutralising antibodies to IFN γ did not.⁷³ It was further demonstrated that IL-18 inhibited osteoclast formation via T cell production of GM-CSF by using GM-CSF deficient mice.⁷⁴ In contrast, GM-CSF is known to stimulate osteoclast formation *in vitro*⁷⁵ and *in vivo*.⁷⁶ Moreover, there is accumulating evidence that activated T cells have the ability to support osteoclast formation.⁷⁷ Our data show that IL-18 indirectly stimulates osteoclastogenesis through upregulation of RANKL production from RA synovial T cells (as shown in fig 1) or phytohaemagglutinin activated human peripheral blood T cells in the presence of M-CSF.⁴⁰ IL-18 fails to induce IFN γ , GM-CSF, M-CSF or osteoprotegerin production from T cells or RA synovial T cells pre-stimulated with phytohaemagglutinin. These data are consistent with the findings of IL-18 induced bone destruction in experimental arthritis. IL-18 may also promote bone resorption by accelerating osteoclast development via induction of TNF α , IL-1 β and IL-6. IL-18 might therefore have a role in bone loss via induction of various osteoclastogenic factors in inflammatory arthritis.

CONCLUDING REMARKS

High levels of IL-18 have been reported in the serum, synovial fluid and synovial membrane tissue of patients with RA. IL-18

Figure 2 Schematic representation of interleukin (IL)-18 induced joint inflammation and joint destruction in rheumatoid arthritis (RA). DC, dendritic cells; Th1 type 1, T helper cell; Th0, precursor of type 1 and type 2 helper T cells; LTB4, leucotriene B4; MMP, matrix metalloproteinase; ECM, extracellular matrix; IFN γ , interferon γ ; TNF α , tumour necrosis factor α ; RANKL, receptor activator of NF κ B ligand. In RA, IL-18, which is mainly produced by macrophages, activates T cells and macrophages to produce proinflammatory cytokines, chemokines, adhesion molecules and RANKL which, in turn, induce the synthesis of leukotriene B4 to induce neutrophil migration into inflammatory sites, stimulate production of MMPs from synovial fibroblasts and chondrocytes and induce osteoclast formation.

is produced by macrophages, DCs and perhaps non-immune cells, and its actions are regulated by the need for proteinase cleavage as well as by the expression of its receptor by a number of potential targets. It is acceptable that IL-18 promotes inflammatory responses via the induction of effector proinflammatory cytokines such as TNF α and IL-1 β and chemokines/adherent molecules in RA. Intriguingly, TNF α and IL-1 β induce the release of IL-18 in RA joints.^{11 15 19} A positive circuit between IL-18 and IL-1 β /TNF α might therefore trigger and promote the inflammatory joint disorder. Although there are controversial reports on the action of IL-18 on synovial fibroblasts, chondrocytes and osteoclastogenesis, the net effect of IL-18 in RA is to perpetuate inflammation and induce bone erosion and cartilage destruction, either directly or indirectly. With regard to the level of IL-18R expression, the T cells and macrophages invading the synovium or in the synovial fluid are the chief cellular targets of IL-18. The direct effect of IL-18 on fibroblast-like synoviocytes or chondrocytes is not likely to be essential or important. The possible mechanism of IL-18 in RA inflammation and joint destruction is schematically represented in fig 2. The future success of anti-IL-18 therapy will provide valuable benefits for patients to slow the progression of RA.

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