Signalling, inflammation and arthritis

Crossed signals: the role of interleukin-15 and -18 in autoimmunity

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Several cytokines are involved in the complex processes ultimately leading to autoimmune diseases. In a preceding review, we have already discussed the role of the IL-12 and -17 families of cytokines. This review is focused on IL-15 and -18. Both these molecules have pro-inflammatory activity and act on many cell types and because of their broad spectrum of activity they play an important role in autoimmunity and disease pathogenesis. Their biological activity is ultimately regulated by the signalling cascades set into motion within their target cells. In this second review, we will, once again, describe the signal transduction pathways activated by these two cytokines and focus on how this relates to the pathogenesis of autoimmune diseases. We will also describe some of the therapeutic approaches that are being investigated to curtail the pro-inflammatory activities of these two molecules.

Key words: Inflammation, Cytokines, Autoimmunity, Rheumatoid arthritis, Signal transduction, Janus Kinase–Signal Transducers and Activators of Transcription pathway, Nuclear factor-κB.

Introduction

The inflammatory reaction observed in autoimmune disease involves both cellular and soluble players. Pro-inflammatory cytokines such as the members of the IL-12 and -17 families, which we have discussed in our previous review [1], are undoubtedly at the centre of the processes which result in unregulated activation of immune cells. On the other hand, other immunoregulatory cytokines have been demonstrated to have a very important role. In particular, IL-15, which is critical for the regulation of lymphocyte homeostasis as well as NK cell development, and IL-18, a potent inducer of IFN-γ and TNF have also been shown to have a major role in the events leading to an excessive inflammatory response. In this review, we have focused our attention to IL-15 and -18 discussing their complex biological activity and their signalling cascades in the normal immune response and in diseases, as well as some of the current therapeutic approaches targeting these two cytokines.

IL-15

IL-15: structure and production

IL-15 is a 14–15 kDa member of the four α-helix cytokine family with structural similarities to IL-2 [2, 3]. Like IL-2, IL-15 stimulates the proliferation of CD4⁺ and CD8⁺ T cells, immunoglobulin M (IgM) or CD40L-treated B cells, as well as the generation and persistence of NK cells [4–9]. However, IL-2 and -15 exhibit major differences in the control of their synthesis and secretion [5, 10]. IL-2 production is tightly regulated during transcription, whereas, IL-15 mRNA is constitutively expressed by a wide variety of human tissues and cell types, including activated monocytes, macrophages, dendritic cells (DCs), osteoclasts and fibroblasts of the spleen, gingiva and skin [3, 5, 11–13]. Interestingly, the patterns of IL-15 protein and mRNA expression differ greatly, indicating tight control of protein production at both translational and post-translational levels. IL-15 mRNA exists as two alternatively spliced isoforms, which encode for two forms of IL-15 protein, a secreted form (48aa) [3] and an intracellular form (non-secretary form) (21aa) which is localized to both cytoplasmic and nuclear compartments [14].

IL-15 production is induced by a variety of exogenous factors, including double-stranded RNA, Type I IFN, lipopolysaccharide, UV irradiation, a number of viruses including, herpes simplex virus and respiratory syncytial virus, and fungal and bacterial agents such as Candida albicans, Escherichia coli and Staphylococcus aureus [15–18].

IL-15: receptor and signalling

IL-15 binds to a heterotrimeric receptor that comprises the same β chain (IL-2Rβ, -15Rβ and CD122) and the common γ chain (γc; CD132) as the IL-2 receptor. The IL-15 receptor also includes a cytokine-specific chain, IL-15Rα chain. IL-15R complex is expressed on monocytes, DCs, NK, T cells and fibroblasts [19, 20] (Fig. 1). IL-15Rα mRNA is expressed in a number of cell types and tissues, including T, B cells, macrophages, kidney and heart [21]. IL-15Rα binds IL-15 with high affinity [22]. It has a short cytoplasmic domain and an extracellular region containing one sushi domain (a highly conserved protein binding domain also known as a glycoprotein 1 motif), which is critical for IL-15 binding [23–25].

IL-15Rα not only exists as a membrane-bound receptor but also in soluble form [26, 27]. The transmembrane IL-15Rα undergoes specific proteolytic cleavage to generate a soluble 26kDa protein in mouse and 42kDa protein in humans [26, 27]. In mice, the generation of soluble form of IL-15Rα (sIL-15Rα) is produced by cleavage of transmembrane IL-15Rα by TNF-converting enzyme [26]. Shedding of the soluble form of the receptor may act as a further checkpoint for IL-15 activity. IL-15 has only a single binding site for interaction with IL-15Rα. Therefore, the availability of free IL-15 will be reduced by the presence of this high-affinity, soluble receptor [28]. Competition with the membrane-bound receptor could result in reduced IL-15R signalling and therefore reduced biological activity of IL-15. This is supported by in vivo evidence which suggests that administration of sIL-15Rα results in the inhibition of NK cell proliferation [29] and antigen-specific T-cell responses [30]. Conversely, it has recently been reported that sIL-15Rα can convert IL-15 to a superagonist that enhances the IL-15 responsiveness in CD8⁺ and NK cells both in vivo and in vitro [28]. It is speculated that upon sIL-15Rα binding to IL-15 a conformational change
occurs in the cytokine, which enhances its interaction with the βc receptor.

Although IL-15 was originally identified as a soluble cytokine in tissue culture supernatant of a simian renal epithelial cell line CV-1/EBNA [3], it has proven difficult to detect in biological fluids from healthy individuals despite widespread mRNA expression. In fact, evidence now indicates that IL-15 is not normally secreted but forms stable high-affinity complexes with IL-15Rα on the cell surface of antigen presenting cells (APCs) [20]. IL-15, bound to cell surface IL-15Rα on APCs, is then presented in trans to T cells and NK cells expressing the βc receptor dimer [20]. IL-15 then induces signalling at the interface of the immunological synapse and contributes to co-stimulatory signals that modify the cell response [31].

Since the IL-2R and -15R contain the same β- and γ-subunits, subsequent signal transduction is mediated by the same molecules, Janus kinases 1 and 3 (JAK1/3) and Signal Transducer and Activator of Transcription 3 and 5 (STATs 3/5) [4, 32, 33]. JAK1 is recruited to the IL-2Rγ chain, whereas JAK3 is recruited to the IL-2Rγ chain (Fig. 1). Additionally, IL-15 triggers several other signalling pathways, including phosphorylation of the Src-family of protein tyrosine kinases Lck and Syk, stimulation of the phosphatidylinositol-3-kinase (PI3-K)/AKT pathway, and stimulation of the Ras/Raf/MAPK pathway leading to the activation of Fos- and Jun-containing transcription factor complexes [34]. IL-15-mediated T-cell proliferation is also regulated through FK506-binding protein 12 (FKBP12) activation of p70-S6 kinase. Moreover, FKBP12 is involved in the pathway leading to the activation of ERK MAPK downstream of IL-15 [35].

Notably, signalling pathways can also be triggered through IL-15Rα. Membrane-associated IL-15Rα has a short cytoplasmic tail with no obvious signalling motifs or docking sites, and therefore was thought to have no signalling capabilities. However, the cytoplasmic domain of IL-15Rα can recruit TNF receptor-associated factor (TRAF) 2 following IL-15 binding resulting in nuclear factor-κB (NF-κB) activation. NF-κB is one of the most important regulators of pro-inflammatory gene expression, resulting in the synthesis of cytokines, such as TNF-α, IL-1β, -6 and -8 [36]. Syk kinase has also been shown to be critical for IL-15Rα signalling [37, 38].

**IL-15: biological activity**

IL-15 is a broad spectrum immune-regulatory cytokine that supports the survival, proliferation and functional activity of a number of immune cell types (Fig. 2 and Table 1). Indeed, mice deficient in IL-15 exhibit depleted NK, NKT, Tγδ, CD8+ and...
memory phenotype T-cell numbers, emphasizing the important role of IL-15 in lymphocyte subset development [39].

Numerous reports have shown that IL-15 is critical for the maintenance of long-lasting, high-avidity T-cell responses by sustaining the survival of CD8\(^+\)/CD44hi memory T cells [6, 40–44]. A recent study has identified a subset of effector memory CD8\(^+\) T cells (IL-7R\(^A\)lowCCR7\(^+\)), which proliferate weakly in response to TCR stimulation but proliferate in response to IL-15 resulting in the non-specific expansion of a polyclonal population of effector memory cells [45]. IL-15-induced proliferation and expansion of autoantigen-specific CD8\(^+\) effector memory T cells could promote autoimmune responses leading to enhanced pathogenesis of the disease. Indeed, patients with SLE show increased IL-15 levels and an expanded CD8\(^+\) effector memory T-cell population correlating with disease severity [46]. IL-15 also enhances the TCR-dependent proliferation of IL-17-secreting T cells (Th17) and Th17/Th1 (IL-17 and IFN-\(\gamma\)-producing) cells [47], which are known to play a role in the development of a number of autoimmune diseases [1, 48]. Therefore, IL-15-induced expansion of IL-17-producing T-cell populations may result in enhanced autoimmune activity.

As already mentioned earlier, gene-targeting studies in mice have demonstrated that IL-15 is essential for the survival, activation, and functional activity of NK cells. Mice over-expressing IL-15 have an expanded NK cell population, which results in enhanced innate immune reactions [42]. Furthermore, efficient NK cell killing has recently been shown to be mediated through the formation of a regulatory synapse with DCs to which IL-15R\(\alpha\) on the NK cells accumulates [49].

IL-15 is also critical for the functional maturation of both macrophages and DCs [50]. IL-15 enhances the phagocytic activity of monocytes and macrophages and induces the production of pro-inflammatory factors such as IL-8 and MCP-1 [51]. In DCs, IL-15 up-regulates the expression of co-stimulatory molecules and IFN-\(\gamma\), enhancing the ability of DCs to activate CD8\(^+\) cells and NK cells [15, 52]. In addition, reduced numbers of peripheral DCs have been observed in IL-15\(^-/-\) and IL-15R\(\alpha\)-/- mice suggesting a role for IL-15 in DC survival [53].

Interestingly, IL-15\(^-/-\) and IL-15R\(\alpha\)-/- mice do not present with any defects in B-cell responses [54, 55]. However, activated B-cell functions are greatly influenced by IL-15. Activated B cells proliferate in response to IL-15 and in combination with CD40L produce IgA, IgG1 and IgM [56]. IL-15 also acts as a survival factor for activated B cells [57].

In neutrophils, IL-15 is critical for their influx into inflammatory foci during antigen-induced inflammation. In vivo administration of IL-15 can induce the production of IL-18 within 90 min, resulting in neutrophil influx that is independent of IFN-\(\gamma\) [58]. IL-15 also inhibits apoptosis of neutrophils by decreasing expression of pro-apoptotic protein Bax [59] and increasing expression of anti-apoptotic myeloid cell differentiation factor-1 [59, 60].

IL-15 can act as a growth factor for mast cells, supporting their proliferation and preventing their apoptosis through the up-regulation of Bcl-x\(_L\) expression [61]. Mast cells use a unique high-affinity receptor for IL-15 known as IL-15R\(\alpha\) (Fig. 1) and the signalling events following IL-15 binding to this receptor are different to other cell types. Upon IL-15 binding to IL-15R\(\alpha\) JAK2/STAT5 or Tyk2/STAT6 are activated and this signalling cascade does not require the presence of the IL-2R\(\alpha\)- or \(\gamma\)-chains [61–63]. Interestingly, mast cells not only express IL-15R\(\alpha\) but also a number of functional isoforms of IL-15R\(\alpha\); however, the relevance of these two high-affinity receptors on mast cells is still unclear. IL-15 is also a major modulator of non-immune target cells inducing angiogenesis in endothelial cells and conferring protection from apoptosis in endothelial cells, fibroblasts, epithelial cells, as well as oesteoclasts (Table 1).

### IL-15: role in autoimmunity

IL-15 is a potent pro-inflammatory cytokine and it has been demonstrated to play a role in the pathogenesis of a number of autoimmune diseases. IL-15 is detectable in the serum of patients with ulcerative colitis [64]. The inflamed mucosa from patients with IBD expresses increased levels of IL-15 mRNA [65] and T cells from the lamina propria are more responsive to IL-15 than control T cells leading to enhanced production of the pro-inflammatory cytokines TNF-\(\alpha\) and IFN-\(\gamma\) [66].

Psoriatic lesions express high levels of IL-15 [67]. In a human psoriasis xenograft model, the administration of an antibody that inhibited IL-15 binding to IL-15R resulted in reduced severity of signs of the disease [68]. Indeed, Zhang et al. [69] report a genetic association between IL-15 and psoriasis. A haplotype of the 3’UTR region of IL-15 is associated with an increased risk of psoriasis and increased activity of IL-15.

Patients with multiple sclerosis (MS) have increased numbers of peripheral blood mononuclear cells (PBMCs) expressing IL-15 mRNA compared with healthy controls [70] and enhanced expression of IL-15 mRNA has been found in MS-active plaques [71]. In addition, monocytes from relapsing–remitting patients have an increased level of membrane-bound IL-15 and their T cells express high levels of IL-15R, which may contribute to enhanced IFN-\(\gamma\)-release during MS [72].

Studies have shown that IL-15 can be detected in the serum and synovial membrane of patients with RA [73–76]. In association with IL-18, -12 and IFN-\(\gamma\), IL-15 has also been detected in synovial membrane derived from patients with JRA [77]. In addition, IL-15 expressed by fibroblast-like synoviocytes and endothelial cells, has been shown to cause a marked increase in transendothelial migration of both CD4\(^+\) and CD8\(^+\) T cells [78]. These data were supported by the observation that the expression of IL-15 leads to the accumulation of T cells in RA synovial tissues engrafted into mice with severe combined immunodeficiency [78].

IL-15 enhances synovial T-cell proliferation and cytokine release. Synovial neutrophils are also activated by IL-15 [5, 79]. Indeed, upon stimulation with IL-15, neutrophils from patients with RA, but not from healthy donors, produce significant amounts of IL-18 and other chemotactic factors that may help to promote chronic inflammation [58].

As mentioned earlier, IL-15 is a potent growth and survival factor for mast cells. Mast cells make a major contribution to the pathogenesis of arthritis and are found in abundance in synovial sections from patients with RA [80]. Studies in mast cell-deficient mice have highlighted their importance for disease onset as these mice do not develop joint inflammation and are resistant to arthritis [81]. It has been proposed that mast cells may be a cellular link between autoantibodies, the complement system and the
onset of inflammatory arthritis [81]. Interestingly, mouse mast cells have been shown to express constitutive and LPS-inducible IL-15 that is stored intracellularly [82]. Intracellular IL-15 has been shown to suppress the activity of chymase in mast cells resulting in decreased production of neutrophil-recruiting chemokines [82]. It is therefore possible that extracellular and intracellular sources of IL-15 play distinct roles in mast cell function and consequently in disease pathology.

Targeting IL-15

Several approaches have been used to antagonize the activity of IL-15 in vivo. These include the administration of soluble murine IL-15Rα, neutralizing antibodies and mutated forms of IL-15, all of which have been successful for the prevention and treatment of CIA in mice [83, 84].

Two antibodies that inhibit IL-15 activity have been developed and tested in clinical trials. Milkiβ is a mAb raised against IL-2/IL-15Rβ subunit that blocks trans-presentation of IL-15 by APC to target NK and CD8+ T cells [85]. It prolongs primate cardiac allograft survival [86] and it has also been tested in Phase I clinical trials in patients with MS, RA, refractory coeliac disease and HTLV-1-associated tropical spastic paraparesis. However, it has been argued whether the administration of this antibody will be effective since its effects are mediated via IL-15 and partial blockade. Indeed, the possibility of inducing autoimmune disorders will be effective since its effects are mediated via IL-15 and partial blockade of IL-15 activity in the pre-clinical studies.

AMG14 (also known as HuMax-IL-15), a human IgG1 mAb that binds and neutralizes the activity of both soluble and membrane-bound IL-15 in vitro has also been tested in humans [88]. Preliminary results have shown that IL-15 neutralization was well tolerated and resulted in marked improvements in disease activity [88]. However, this initial study was not placebo controlled and consequently another multi-centre, randomized, double-blind, placebo trial was conducted, which reported no significant alterations in the levels of NK and CD8+ memory T cells [88].

The two major signalling pathways activated by IL-15 are the NF-κB and the JAK–STAT pathways and they have been the subject of intense research in the search for possible therapeutic interventions. Activation of NF-κB is the major event in the signalling cascade downstream of the IL-18 receptor and will be discussed subsequently.

In conclusion, IL-15 is an immunoregulatory cytokine which exhibits pro-inflammatory activity by acting on a wide variety of cell types. Some of these effects are direct and include Th1 and Th17 polarization as well as activation of effector cells such as B, NK, mast cells and neutrophils. Other effects are indirect and involve the production of other pro-inflammatory cytokines such as IFN-γ and IL-17 as well as IL-18. In particular, the induction of IL-18 in neutrophils is of primary importance for the initial stages of the inflammatory process and we will now discuss in detail the effects of this other immunoregulatory cytokine.

IL-18

IL-18: structure and production

IL-18 is a member of the IL-1 superfamily of cytokines and was initially discovered as an IFN-γ-inducing factor produced by macrophages stimulated with microbes or microbial products [93]. IL-18 is produced as an inactive 23kDa precursor that is cleaved by caspase 1 to form the biologically active 18kDa species [94]. Proteinase-3, caspase-3, cathepsins and elastase can also cleave the precursor polypeptide, but this can result in the production of inactive forms of IL-18 [95–97].

The IL-18 promoter does not contain a TATA sequence and is constitutively active upstream of exon 2, resulting in the constitutive expression of IL-18 mRNA [98]. Control of IL-18 expression occurs primarily through processing of the pro-cytokine into the active form and its subsequent release. Not only is IL-18 controlled by cleavage of the pro-cytokine to the active form, the biological activity of IL-18 is also controlled by IL-18 binding protein (IL-18BP). IL-18BP has a high affinity for IL-18 and neutralizes its biological activity [99, 100]. There are four human isoforms of IL-18BP, which are specific for the mature active form of IL-18 and their expression is localized to the spleen and intestinal tract [101]. Interestingly, IL-18BP expression in keratinocytes and intestinal cell lines is up-regulated in response to IFN-γ [102]. Therefore, a negative feedback loop exists for IL-18-induced IFN-γ production by Th1 cells. The IL-1 homologue, IL-1F7, also binds to IL-18BP and it has been proposed that it plays a role as a negative regulator of IL-18 activity. When bound to IL-18BP, IL-1F7 can form a complex with IL-18Rβ chain preventing the formation of a functional receptor complex [101].

IL-18: receptor and signalling

IL-18 signals through the IL-18 receptor complex (IL-18R), which is a heterodimer consisting of IL-18Rα and -18Rβ subunits. The extracellular domains of both IL-18R chains contain three Ig-like domains and an intracellular region comprising a Toll/IL-1 receptor (TIR) domain (Fig. 1). The IL-18Rα subunit is responsible for ligand binding, whereas the IL-18Rβ subunit is a non-binding signalling chain. The IL-18R complex is expressed on lymphocytes and other cells such as plasmacytoid DCs [103] and is up-regulated by IL-12, -2, [104, 105] and inhibited by IL-4 [106].

Following binding of IL-18 to the low-affinity IL-18Rα, the IL-18Rβ chain is recruited forming the high-affinity heterodimeric receptor complex that brings the TIR domains within the intracellular regions of each receptor chain into close proximity. Once the TIR domains of the IL-18Rα and β-chains are engaged the adaptor protein myeloid differentiation factor 88 (MyD88) then docks to the IL-18R complex resulting in the phosphorylation of IL-1 receptor-associated kinases (IRAKs) [107]. IRAK1 and IRAK4 have both been implicated in IL-18 signalling in NK and Th1 cells, since IRAK1–/– and in particular IRAK4–/–, mice display impaired responses to IL-18 stimulation [108, 109]. IRAK activation results in the recruitment of TRAF6, IκB kinase (IKK) is then activated and degrades IκB, allowing NF-κB to translocate to the nucleus activating gene transcription [36].

IL-18 signal transduction is thought to be primarily mediated through this MyD88-dependent pathway. Indeed, MyD88 is an essential component of IL-18R signalling, since Th1 cells from MyD88−/− mice are unresponsive to IL-18 [110]. In addition, the inhibitory receptors Toll IL-1R 8/single Ig IL-1-related receptor (TIR8/SIGIRR), which contain TIR domains in their intracellular region, compete with the IL-18R complex for MyD88 and TRAF6 inhibiting IL-18 activity. Moreover, TIR8/SIGIRR-deficient mice are more susceptible to IL-18 stimulation, whereas, cells over-expressing TIR8/SIGIRR are less susceptible to IL-18 stimulation [111]. However, IL-18 has also been shown to induce the production of MCP-1 through the PI3K/AKT and MEK/ERK1/2 pathways.
IL-18: biological activity

IL-18 acts synergistically with IL-12 to induce IFN-γ production by several types of cells. IL-18 can induce IFN-γ production from T cells independently of TCR activation, a property unique to IL-18 [104] (Fig. 3 and Table 2). Considering the importance of IFN-γ for pathogen clearance, IL-18 has been shown, using animal models, to be required for the IFN-γ-dependent eradication of several microbial infections. However, its biological role extends beyond IFN-γ production and Th1 polarization. IL-18 induces both T- and NK-cell maturation and potentiates cytotoxicity. In fact, IL-18-deficient mice show reduced NK cytolytic activity [113]. IL-18 is also involved in Th17 cell responses and, in synergy with IL-23, can amplify IL-17 production by Th17-polarized cells in a TCR-independent manner [115].

IL-18 can also induce TNF-α production both in vitro and in vivo, an effect that links this cytokine to the development of the inflammatory response and the pathogenesis of several autoimmune diseases. Chemokines such as CXCL8, CXCL5 and CCL20 as well as adhesion molecule (ICAM-1, VCAM-1) pathways. Inhibition of both of these pathways results in the complete abrogation of IL-18-induced MCP-1 production [112, 113].

A soluble form of IL-18Rα (sIL-18Rα) has recently been identified, which is produced by differential splicing [114]. In vitro studies reveal that the novel soluble receptor can inhibit IL-18-induced IFN-γ production. A number of spliced variants of sIL-18Rα have been identified. Indeed, it has been previously observed that IL-18Rα exists as heterogenous molecules ranging from 60 to 110 kDa [93]. It is therefore possible that IL-18Rα-spliced variants may each have distinct functions in the regulation of IL-18 activity.

IL-18: role in autoimmunity and allergy

Like IL-15, IL-18 exerts its effects on a wide array of cell types. Consequently, this has implications for the pathogenicity of several inflammatory diseases. Patients with Crohn’s disease have high serum levels of IL-18 [124]. Accordingly, a correlation between the level of IL-18 and severity of the disease has been shown in animal models, whereas IL-18-deficient mice or mice treated with IL-18BP can escape this disease. IL-18 also promotes the onset of insulin-dependent diabetes mellitus, through the Th1-mediated destruction of pancreatic β-cells [125]. Transgenic expression of IL-18 in murine keratinocytes results in the development of spontaneous atopic dermatitis. Moreover, in several studies, patients have shown a correlation between serum IL-18 and IgE levels and severity of atopic dermatitis. A role for IL-18 has also been postulated in bronchial asthma with elevated serum levels of IL-18, which correlate with the severity of the disease [126]. In mice, IL-18 exacerbates airway inflammation and infiltration possibly through the induction of chemokine release [126]. Increased serum IL-18 levels have also been observed in allergic rhinitis and, interestingly, a single nucleotide polymorphism in the IL-18 gene has recently been associated with this disease [127, 128].

IL-18 has also been shown to be up-regulated in RA [129]. Elevated levels of IL-18 are found in the synovial tissue, synovial fluid and sera of patients with RA. The administration of IL-18 to mice with CIA significantly increases the severity of the disease that is reduced following IL-18 blockade [130, 131].

Furthermore, the expression of IL-18 in rheumatoid synovium has been found to correlate with IL-1β expression, macrophage infiltration and inflammation. In addition, the bioactivity of IL-18 from affected joints of patients with RA correlates with disease severity. Notably, elevated IL-18BP levels have also been described in RA [132].

Joint inflammation induced by IL-18 is partly TNF-α dependent. Mice deficient in TNF-α display reduced lymphocyte infiltration and joint inflammation in response to IL-18 [133]. Indeed, RA patients receiving the anti-TNF-α antibody infliximab show reduced IL-18 production and disease symptoms were ameliorated [134]. As mentioned earlier, IL-18, in synergy with IL-23, amplifies IL-17 production, which has been shown to play a pathogenic role in autoimmune diseases, in particular RA, by promoting cartilage destruction and osteoclastogenesis [135].

Targeting IL-18

Antagonizing the biological activity of pro-inflammatory cytokines such as IL-18 has the potential to improve symptoms and
the pathological features associated with the inflammatory processes in which they are involved. To this end several therapeutic approaches have been developed and are currently under investigation. The naturally occurring IL-18BP was first investigated as a therapeutic approach in animals, with very promising results [136–138] and is currently being tested in humans. As with all naturally occurring inhibitors there are no problems related to their possible immunogenicity; however, issues regarding their pharmacokinetics still need to be addressed. From this point of view, the use of mAbs specific for IL-18 and -18R may offer a better solution. Indeed, animal models testing the efficacy of mAbs against IL-18 and -18R have given results that warrant further exploration in humans [139]. Considering the role of IL-18 in the normal immune response, it is possible that anti-IL-18 therapy may result in increased mycobacterial infections that have previously been observed as a side-effect of anti-TNF therapy. Furthermore, interfering with Th1 responses may alter the normal anti-tumour immune response [140]. These and other possible problems need to be taken into account in any therapeutic approach directed towards IL-18 or its receptor.

IL-18 activity could be prevented by blocking caspase-1-mediated cleavage of the cytokine into its mature form. This approach would also prevent the formation of the mature form of the pro-inflammatory cytokine IL-1. Agents capable of blocking caspase-1 have already showed promising results in humans and clinical trials are currently ongoing for the treatment of RA [141].

As mentioned earlier, the main downstream event in IL-18 signalling is the activation of NF-κB. NF-κB target genes are involved in cellular events ranging from proliferation to inflammation and include genes for cytokines and chemokines as well as cyclins and anti-apoptotic genes and it is through this transcription factor that IL-18 exerts its pro-inflammatory activity. Historically, inhibition of NF-κB has been found to be one of the key mechanisms by which steroids, NSAIDs as well as some natural and synthetic compounds act. Despite the prediction that blocking NF-κB may result in immunosuppression and increased susceptibility to tumour development, several therapeutic strategies that target this pathway have been pursued. These include inhibition of IκB phosphorylation and degradation as well as inhibition of NF-κB nuclear translocation. Compounds targeting IKK molecules such as IMD-0560, SC-514, TPCA-1, BMS-345541 and ML120B have shown promising activity in vitro and in pre-clinical studies with block of the secretion and release of metalloproteinase as well as cytokines and prevention of bone erosion [142–146].

Among the drugs known to affect IκB degradation, the boronic acid peptide bortezomib, which has already been approved for the treatment of human malignancies, is possibly the most interesting. In pre-clinical studies in rats, bortezomib has been shown to effectively reduce the severity of experimental autoimmune encephalomyelitis (EAE) reducing the incidence of clinical relapses, central nervous system (CNS) histopathology, and T-cell responses [147]. A plant-derived compound with a mechanism of action similar to glucocorticoids has been recently described to be able to block the inflammatory response [148]. This transcription repressor activity is also shared by some ligands of peroxisome proliferator-activated receptors (PPAR) and PPAR agonists have shown efficacy in several pre-clinical models [110, 149–151].

Conclusions
In the past 15 yrs, our understanding of the complex cytokine network, which is central to the pathogenesis of autoimmune disease, is increased dramatically. In these two reviews, we have aimed at highlighting the basic biology of a number of cytokines, which we believe play a key role in the events that lead to chronic inflammation and eventually, in the case of RA, articular inflammation and bone erosion. We have focused on the signalling cascades and we have pointed out the therapeutic approaches that are currently being developed to target such pathways. In the case of IL-15 and especially IL-18, some of these approaches have already moved from in vitro and pre-clinical studies to clinical trials suggesting that this is indeed a way forward. It is now time to move even further forward and an integration of the different approaches, such as targeting multiple cytokines and multiple pathways simultaneously, should result in greater therapeutic efficacy with a reduction in side-effects as some of the in vitro and in vivo work seems to suggest.

Rheumatology key messages

- Among the complex network of pro-inflammatory cytokines involved in the development of autoimmune diseases, IL-15 and -18 have both been shown to be of great importance.
- The targeting of IL-15 and -18 for therapeutic purposes is already a reality with a novel approach focussed on blocking their receptor binding.
- The intracellular pathways that these two cytokines activate appear to be an exciting area of research which may result in novel drugs for the treatment of inflammatory diseases.

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Clinical Vignette
Hypertrophic osteoarthropathy associated with a left ventricular tumour

The patient, a 36-yr-old Polynesian man, presented with a 3-month history of swelling of his extremities with clubbing of the fingers and toes and polyarthritis affecting his hands, feet and knees. Plain radiographs revealed extensive periosteal reaction along the shafts of the metacarpals (arrow in A). Axial proton density fat saturated sequence on MRI shows periosteal new bone (short arrow in B) involving the shafts of the metacarpals giving a ‘halo’ appearance. There is also associated periosteal oedema showing high signal (long arrow). The patient was eventually diagnosed with a high-grade undifferentiated pleomorphic sarcoma of his left ventricle (C, D). The hematoxylin and eosin (H&E) section taken from the resected tumour (C) shows pleomorphic spindle cell proliferation, bizarre nuclei and atypical mitoses.

Vascular endothelial growth factor (VEGF), a cytokine normally inactivated in the lungs, has been implicated in the pathogenesis of hypertrophic osteoarthropathy [1].

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