IL-18 in autoimmunity: review

Diana Boraschi¹, Charles A. Dinarello²

¹ Laboratory of Cytokines, Unit of Immunobiology, Institute of Biomedical Technologies, National Research Council, CNR, Area della Ricerca di S. Cataldo, via G. Moruzzi 1, I-56124 Pisa, Italy
² Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver, CO 80262, USA

Correspondence: D. Boraschi <diana.boraschi@itb.cnr.it>

ABSTRACT. IL-18 is among the cytokines responsible for immune-mediated pathologies and is probably one of the factors that contribute to the pathogenesis of autoimmune diseases. Identification of the causes of uncontrolled IL-18 production and activity in autoimmunity would allow for novel therapeutic targets to effectively block autoimmune activation and inhibit concomitant tissue damage. IL-18 is produced mainly by monocytes/macrophages in response to stimuli of viral/bacterial origin, its production being therefore one of the effects of innate immunity initiated by host-pathogen interaction. In this review, we summarise the evidence supporting both the effector and the pathogenic role of IL-18 in autoimmunity, and propose that the disturbed mechanism of innate immunity, resulting from macrophage activation through innate immunity receptors (TLR/IL-1R family), may be the basis of pathologically high levels of IL-18 production and activation. Unravelling the mechanisms of IL-18 production and activity in autoimmune diseases will allow the identification of targets for more effective therapeutic intervention.

Keywords: IL-18, autoimmunity, inflammation, macrophage, Toll-like receptor, IL-18BP

MECHANISMS OF INNATE IMMUNITY IN THE INITIATION OF AUTOIMMUNITY

In autoimmune diseases, immune activation is inappropriately directed against self antigens. It is widely recognised that the autoimmune process is triggered by unknown events, possibly in combination with predisposing genetic tracts. It has also been hypothesized that traumatic, stressful and infectious events may take part in the initiation of autoimmune reactions in susceptible hosts, and that molecular similarity between viral/bacterial molecules and self antigens may be at the root of autoimmune recognition. Immune activation is initiated in response to invading/stressful events of different origin (both exogenous and endogenous). The innate immune system discriminates between self and alien/abnormal molecular patterns and mounts the first set of inflammatory and defence responses to eliminate the unrecognised element(s), and to initiate the slower and more specific adaptive response. The first interaction between the host and the stress elements (viruses, bacteria, foreign particles, trauma) occurs through the interaction with host cellular sensing structures, in the first place the receptors of the TLR/IL-1R family. This is an important class of receptors involved in the initiation of the inflammatory response and of the innate immune reactions. These receptors share a common signalling pathway and include the TLR receptor family (Toll-like receptors; at least ten different chains in humans), and the IL-1R/IL-18R superfamily. TLR are germline, encoded receptors that recognise different microbial structures of bacterial, viral, fungal, and protozoal origin (LPS, lipopeptide, dsRNA, flagellin, CpG, etc.) as well as endogenous, stress-related proteins (heat shock proteins 60 and 70, fibrinogen), thereby triggering responses and activating inflammatory reactions. TLR are mainly expressed by myeloid cells (macrophages), although their presence has been described in T lymphocytes and in several other cells and organs [1]. The IL-1R/IL-18R superfamily includes the receptors for the inflammatory/immunoenhancing cytokines IL-1 and IL-18 [2–4], for the regulatory cytokine IL-33 [5–7], and for a series of orphan receptors, including the putatively inhibitory receptors TIR8/SIGIRR [8–10], and RP105 [11, 12]. Macrophages are among the first cells which are recruited to the site of inflammation and come in contact with invading micro-organisms or foreign agents. Macrophages are versatile, plastic cells, which respond to environmental signals with diverse functions. Classical macrophage activation in response to microbial products (e.g., LPS) and interferon-γ (IFN-γ) has long been recognised and gives rise to potent effector macrophages (M1), which kill microorganisms and tumour cells and produce proinflammatory cytokines and chemokines (including IL-12, TNF-α, IL-1, IL-6, IL-8, MIP-1α). More recently, it has been shown that anti-inflammatory molecules, such as glucocorticoid hormones, IL-4, IL-13 and IL-10, are more than simple inhibitors of macrophage activation, in that they induce a distinct activation pathway (alternatively activated macrophages) [13, 14]. Alternative macrophage activation with IL-4 and IL-13 induces M2 macrophages, which can regulate inflammatory responses and adaptive...
Th1 immunity, scavenger debris, and promote angiogenesis, tissue remodelling and repair [15, 16]. Classically and alternatively activated (polarised) macrophages have been referred to as M1 and M2, in analogy with the Th1/Th2 dichotomy in T cell responses. M1 or M2 polarised macrophages differ in terms of receptor expression, cytokine and chemokine production, and effector function. Differential cytokine production characterises polarised macrophages. The M1 phenotype includes IL-12 and TNF-α, while M2 macrophages typically produce IL-10, the IL-1 receptor antagonist (IL-1Ra) and the type II IL-1 receptor (IL-1RII). Differential production of chemokines, which attract Th1 versus Th2 or T regulatory cells, integrates M1 and M2 macrophages in circuits of amplification and regulation of polarised T cell responses. The microenvironment thus influences macrophage activation and their subsequent functions.

In this light, genetic and environmental conditions that promote M1/Th1 polarisation and inhibit M2/Th2 regulatory activity may contribute to the establishment of a chronic inflammatory condition. This may develop into autoimmunity following triggering events (e.g., an infection or trauma) that would induce an autoimmune adaptive response, through mechanisms of molecular mimicry.

M1/Th1–M2/Th2–M17/Th17 NETWORK AND IL-18

Based on the above considerations, it would be important to analyse the regulatory circuits determining the activation of innate immunity cells for the production of IL-18, in particular the development of M1 versus M2 macrophages in autoimmune diseases, and their mutual role in initiating and maintaining the autoimmune reaction. In addition, recent data suggest the pivotal role of IL-17-producing T helper cells (Th17) in chronic inflammatory-autoimmune diseases [17, 18]. The production of the Th17-polarising cytokines, in particular TGF-β and IL-6, depends on macrophages/dendritic cells, in response to certain pathogenic stimuli. Indeed, “type 17” pathogens are represented by extracellular pathogenic bacteria, against which Th17 cells appear to have a key protective role [17], as opposed to the protective role of Th1 for intracellular pathogens, and of Th2 for multicellular parasites. Thus, it may be proposed that macrophages producing Th17-polarising cytokines in response to these pathogens be called M17 macrophages. The role of IL-18 in Th17 responses appears to be that of activating/amplifying IL-17 production in already polarised Th17 cells, in a TCR-independent manner in synergy with IL-23, similar to its role in TCR-independent activation of Th1 cells together with IL-12 [17, 18]. The expression pattern of different receptors of the TLR/IL-1R family, and their capacity to react to infectious/stress-related stimuli by producing IL-18 may allow identification of the possible role of triggering events such as infections or traumas in the initiation of the autoimmune dysregulation. Not to be forgotten is the analysis of polymorphisms in the genes coding for the relevant TLR/IL-1R and those coding for the IL-18 ligands, receptors, IL-18BP. This analysis may show whether there is a genetic basis for the development of IL-18-associated autoimmune. Indeed, enhanced production and activity of IL-18 appears to be at a fundamental level in autoimmune pathologies. The production of bioactive IL-18 is a multistep process involving synthesis of the precursor, synthesis and activation of the cleaving enzyme caspase-1, maturation and extracellular transport [3, 4]. IL-18 effects are further dependent on the expression of the two receptor chains IL-18Ra, the ligand binding chain, and IL-18Rβ, the accessory chain [3, 4], on regulation of the expression of the four IL-18BP isoforms (two able to bind and inhibit IL-18, two unable to bind and possibly inhibiting the activity of the binding isoforms) [19], on the presence of IL-1F7, a ligand of IL-18Ra which enhances the IL-18-inhibiting activity of IL-18BP [20], on the presence of active caspase-3, which cleaves and inactivates the IL-18 protein [21], and on the expression of TIR8/SIGIRR, which could act as downstream inhibitors of TLR/IL-1R signal transduction [8-10].

The unravelling of the molecular basis of dysregulated IL-18 overproduction and activity in autoimmune diseases will help to define novel therapeutic targets to limit pathological IL-18 excess. In particular, the correlation of the TLR/IL-1R expression pattern in macrophages with their activation state and IL-18 production capacity, and analysis of macrophage polisation or re-direct the activity of already polarised macrophages toward homeostatic control of IL-18 activation. Also, members of the TLR/IL-1R family may be identified as possible targets for novel therapeutic treatments aimed at inhibiting the excessive IL-18 production at the basis of autoimmune stimulation.

Cytokines in autoimmune diseases: pathological versus pathogenic role

Increasing evidence indicates that dysregulation of effector cytokines in the maintenance of immune and inflammatory activation is the basis of autoimmune reactions. The host response to infection initiates a brisk cytokine response, which facilitates mechanisms for eliminating the invading organism. Once danger is eliminated, cytokine production is turned off and tissue damage resolves. In contrast, persistent, dysregulated cytokine production results in progressive tissue damage. Since the autoimmune pathologies are based on the hyperactivation of the immune response, it is not surprising that immune-related factors are produced in excessive amounts as a consequence of dysregulated immune activation, and that they contribute to the effector phases of the disease, mediating tissue and organ damage. Thus, therapeutic approaches based on cytokine inhibition may have a beneficial effect in limiting the activity of the damage-inducing effector molecules. An example is the use of TNF-blocking therapies (with soluble receptors or with monoclonal antibodies) for rheumatoid arthritis and chronic inflammatory bowel disease (including Crohn’s disease). In addition,
blocking IL-1 activity with the IL-1 receptor antagonist (IL-1Ra) is entering clinical use for the same diseases with encouraging results [22]. However, of major importance for the efficacy of anti-cytokine therapies would be to identify the pathogenic role of these factors, i.e. their involvement in the first steps of dysregulated immunological triggering that initiates the disease.

In experimental animal models and in human disease, CD4+ T cells play a central role, and both Th1 and Th2-related cytokines are apparently involved in maintaining autoimmune disturbances [23]. More recent findings also indicate a major role in chronic and destructive inflammatory/autoimmune pathologies for Th17 cells [23, 18]. A major pathogenic role is attributed to Th1- and Th17-related cytokines in many autoimmune conditions, particularly those involving chronic inflammation. In addition, Th1 cytokines also sustain autoimmune activation by expanding the pathological T cell clones.

By examining the autoimmune role of Th1-related cytokines, a central pathological effect has been described for the inflammatory Th1-dependent cytokine IFN-γ. For instance, in murine lupus, the ratio of IFN-γ to IL-4 secreting cells (i.e. the Th1/Th2 ratio) increases with disease progression [24]. Administration of IFN-γ exacerbates the disease in humans and mice [25-28], whereas mice deficient in IFN-γ or IFN-γR develop a less acute disease and have slower disease progression [29-31]. The characteristic pathology-associated double negative (CD4- CD8-) T cells and autoantibodies are absent in IFN-γ-deficient mice [32]. In IFN-γR-deficient mice, the renal damage is dramatically reduced [29, 31]. Other studies indicate that IFN-γ accounts for the fatal kidney disease in lpr mice [33, 34]. In EAE, a severe experimental demyelinating disease in rats and mice resembling human multiple sclerosis, IFN-γ and Th1 activation apparently play a major role in the pathological progression [32, 35].

**IFN-γ-INDUCING CYTOKINE IL-18**

IFN-γ production is amplified by the cytokine IL-18, in true synergy with other Th1-related cytokines, IL-2, IL-15, IL-12 and IL-23 [36-39]. IL-18, the cytokine previously known as IFN-γ-inducing factor (IGIF), is a potent activator of polarised Th1 cells and induces IFN-γ production and lymphocyte proliferation [36, 40]. Similar to the closely related inflammatory cytokine IFN-1, IL-18 is synthesized as an inactive precursor molecule, which is cleaved by the IL-1β converting enzyme (caspase-1) resulting in active (mature) IL-18 [41, 42]. Figure 1 summarises the features of IL-18 production, processing and release.

**IL-18 RECEPTORS**

As shown in figure 2, two chains of the IL-18 receptor are required for initiation of signal transduction. The α chain

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**Figure 1**

**Cell sources of the IL-18 precursor.** As shown, the IL-18 precursor is found constitutively expressed in many cells [419]. Upon activation of caspase-1 by TLR triggering, macrophagic cells, dendritic cells, or Kupffer cells process the inactive IL-18 precursor into an active, mature form, which is released from the cell [41, 42]. The precursor piece remains inside the cells and undergoes ubiquitination. IL-18 alone does not induce IFN-γ but rather requires co-stimulation with IL-12, IL-15 or IL-2 [36-39]. As a result of true synergism, IFN-γ is produced by CD4+ T cells, CD8+ T cells, NK and NK T lymphocytes. The role of these co-stimulators for the production of IL-18-dependent IFN-γ is thought to be the upregulation of the IL-18 receptors, particularly the β chain [43, 50]. In turn, IFN-γ promotes expression of the caspase-1 gene and activation of caspase-1 from inactive precursor [377, 467]. Alternatively, in synergy with IL-23, IL-18 activates Th17 cells to produce IL-17 [18]. In the absence of known synergies, IL-18 can activate Th2 responses [397].
of IL-18R is required for ligand binding, whereas the β chain does not bind IL-18 but is required for signalling. IL-18Rα and IL-18Rβ are members of the IL-1R superfamily, with an extracellular domain comprising three Ig-like domains and with an intracellular segment responsible for signal transduction. The ligand-binding chain IL-18Rα is expressed on the surface of Th1 lymphocytes and NK cells, and on a variety of other cells including macrophages and B cells, neutrophils and basophils, endothelial cells, smooth muscle cells, synovial fibroblasts, chondrocytes, and epithelial cells [43-49]. After binding of IL-18 to the IL-18Rα, the accessory chain, IL-18Rβ, is recruited into a signalling complex [50, 51]. Signal transduction is initiated by the approximation of the Toll-IL-1 receptor (TIR) domains present in the intracellular segment of the receptor chains [52]. The signalling pathway, shared with other receptors of the TLR/IL-1R family, involves recruitment of the adapted molecule MyD88 and of the kinase IRAK, followed by interaction with TRAF6. Activation of IKK causes degradation of IκB and subsequent activation of NFκB [53, 54]. An additional signalling pathway has been described in IL-18-stimulated cells, which involves activation of MAPK p38 [55-58]. Expression of chains of the IL-18R complex is upregulated by cytokines such as IL-12 and IL-2, and inhibited by IL-4 [43, 60-65]. In particular, it has been shown that IL-12 can modulate the cell response to IL-18 by upregulating expression of the accessory chain IL-18Rβ. Indeed, true synergism of IL-12, IL-23, IL-21, IL-2, and IL-15 with IL-18 for IFN-γ production can be mainly attributed to upregulation of IL-18Rβ [63, 66-68]. The IL-18Rα chain can bind with low affinity another molecule of the IL-1 cytokine family, i.e. IL-1F7 [20], a binding that however does not recruit the accessory chain IL-18Rβ, and therefore which does not initiate IL-18Rβ-dependent signalling. That IL-18Rα could be activated by ligands other than IL-18, possibly using a different co-receptor, is suggested by recent data in an experimental model of autoimmune encephalitis, in which deletion of the IL-18 gene did not change susceptibility to disease induction, whereas deletion of the IL-18Rα gene made mice resistant [69]. Since mice do not express IL-1F7, another IL-18-like ligand able to activate IL-18Rα for initiation of autoimmune-related inflammation is currently being sought.

IL-18 BINDING PROTEIN

IL-18BP is a naturally occurring, secreted protein, which possesses high affinity binding to IL-18 (dissociation constant of 400 pM), and therefore neutralises the biological activity of IL-18 [19, 70]. IL-18BP is specific for mature IL-18 and does not bind the IL-18 precursor when assessed by ELISA [71] or BIAcore binding [19]. With the exception of IL-1F7 [20], IL-18BP does not bind to other members of the IL-1 family or several cytokines tested. IL-18BP is not a soluble form of the membrane-bound IL-18Rα, although it has many characteristics of a soluble receptor similar to the IL-1 type II decoy receptor [72]. Unlike all members of the IL-1 receptor family, which have three Ig-like domains in the extracellular receptor segment, IL-18BP has only one Ig-like domain. It seems that the transmembrane and the first two extracellular domains of the ancestral IL-18 receptor were deleted during evolution. The only amino acid identity with the IL-18Rα chain and IL-18BP is found in the third Ig domain of...
the α chain [73]. There is limited amino acid homology between IL-18BP and the IL-1 type II decoy receptor. In fact, IL-18BP is similar biologically to the IL-1 decoy receptor in that its function is primarily to bind and neutralise the ligand rather than act as a ligand passer.

The human IL-18BP gene is located on chromosome 11q13. Using Northern blot analysis, IL-18BP is highly expressed in spleen and the intestinal tract, both immunologically active tissues. There are four isotypes of human IL-18BP and two isotypes of murine IL-18BP [19]. These isotypes are formed by alternate mRNA splicing of the respective genes. A single copy of the IL-18BP gene exists for humans, mice and rats [74]. Only those isofoms that retain the intact Ig domain are biologically functional, by neutralising IL-18 [19]. For example, human IL-18BP has four isotypes termed IL-18BP α, β, γ and δ. Only IL-18BP α and IL-18BP γ have the intact Ig domain and neutralise IL-18 [19]. The other two isofoms, although they are produced in humans, do not bind and do not neutralise IL-18. However, the mRNA splicing that creates these isofoms is not a haphazard event in that the spliced mRNA has an open reading frame, which results in the same carboxyl terminal for all isofoms. It is possible that IL-18BP α isoform β and δ bind another member of the IL-1 family. The mouse has two isofoms, IL-18BP γ and IL-18BP δ. Murine IL-18BP γ and IL-18BP δ isoforms, possessing the identical Ig domain, also neutralise > 95% murine IL-18. However, murine IL-18BP δ, which shares a common C-terminal motif with human IL-18BP α, also neutralises human IL-18 [19].

The sites for binding of IL-1 to the IL-1 receptor type I were used to model the binding of IL-18 to IL-18BP [19]. Modelling predicted a large mixed electrostatic and hydrophobic binding site in the Ig domain of IL-18BP, which could account for its high affinity binding to the ligand. By mutational analysis, two residues in IL-18, glutamic acid at position 35 and lysine at position 89, were found to be important both for binding to IL-18Rα and subsequent biological activity [73, 75], and for binding to IL-18BP and subsequent neutralisation [73].

The regulation of IL-18BP gene expression appears to be via IFN-γ [76]. In a human colon carcinoma epithelial cell line, IFN-γ induced gene expression and release of IL-18BP. The increase in IL-18BP was also observed in a variety of intestinal cell lines and in a human keratinocyte cell line. The histone deacetylase inhibitor sodium butyrate suppressed IFN-γ-induced IL-18BP gene and protein expression [76]. The promoter for IL-18BP has been described as including two IFN-γ responsive elements [77]. Thus, like other genes encoding cytokine inhibitors (soluble receptors, receptors antagonists and binding proteins), the cytokine itself or a related cytokine induces its own negative regulator in a feed-back loop. Therefore, in an Th1 response, the production of IL-18-dependent IFN-γ contributes to the suppression of IFN-γ by increasing the production of IL-18BP.

The serum levels of IL-18BPα in a cohort of healthy subjects as determined by a specific ELISA were 2.15 ± 0.15 ng/mL (range 0.5-7 ng/mL) [70]. In patients with sepsis and acute renal failure, the levels rose to 21.9 ± 1.44 ng/mL (range 4-132 ng/mL), due to increased production and not to renal retention. Using the law of mass action and knowing the dissociation constant of IL-18BP to IL-18, total IL-18 and free IL-18 were calculated. Total IL-18 in healthy individuals was 64 ± 17 pg/mL and approximately 85% was in the free form [70]. Total IL-18 and IL-18BPα were both elevated in sepsis patients upon admission (1.5 ± 0.4 ng/mL and 28.6 ± 4.5 ng/mL, respectively). At these levels, most of the IL-18 is bound to IL-18BPα, however the remaining free IL-18 in sepsis patients is still higher than in healthy individuals. One can conclude from these studies that IL-18BPα considerably inhibits circulating IL-18 in sepsis. Nevertheless, exogenous administration of IL-18BP may further reduce circulating IL-18 activity.

The relative gene expression of the IL-18-neutralising (α and γ) and non-neutralising (β and δ) isoforms of IL-18BP was studied in Crohn’s disease during active phases of the disease [78]. Intestinal endothelial cells and macrophages were the major source of IL-18BP within the submucosa, similar to what was observed in cultured human endothelial cells and peripheral blood monocytes. Gene expression, as measured by steady state mRNA levels for IL-18BP as well as the IL-18BP protein, were elevated in intestinal biopsies from patients with active disease [78]. Unbound IL-18BP α isoforms a and c and inactive isoform δ were present in specimens from patients with active disease as well as in tissues from control patients. The IL-18BP β isoform was not detected. Elevated IL-18BP has been described in several autoimmune diseases including rheumatoid arthritis [46, 79-81], in hepatitis C treated with IFN-α [82] and in patients with chronic liver diseases [83].

**IL-18Rα- AND IL-18BP-BINDING CYTOKINE IL-1F7**

The IL-1 homologue IL-1F7 has been discovered from expressed sequence tag database searches [84-87]. Among the several isoforms of IL-1F7, present exclusively in the human genome [88], the splice variant IL-1F7b can be matured by caspase-1 to give rise to a mature protein able to bind to IL-18Rα, although with low affinity (about 100-fold lower than that of IL-18) [89]. Possibly due to such low affinity, binding of IL-1F7b to IL-18Rα does not induce recruitment of the accessory chain IL-18Rβ, nor cell activation in terms of IFN-γ production [89]. Likewise, IL-1F7b does not antagonise the IFN-γ-inducing capacity of IL-18 [89]. A possible down-regulatory role in IL-18-induced activation has been suggested for IL-1F7 by the observation that IL-1F7 can bind to IL-18BP [20]. Indeed, binding of IL-1F7 to IL-18BP amplifies IL-18 inhibition by IL-18BP. This can be explained by the fact that, similarly to what occurs with IL-1 bound to soluble IL-1RII [90], the IL-1F7 bound to IL-18BP can form a complex with the accessory chain IL-18Rβ and prevent it from forming a functional receptor complex [20]. A possible agonist activity (IL-12- and IFN-γ-dependent anti-tumour activity) has been also proposed for IL-1F7, based on results of in vivo gene transfer in the mouse [91]. Overall, the role of IL-1F7 in health and disease still needs to be fully explored. IL-1F7 transcripts have been detected in several normal tissues and in carcinoma cells [86, 89]. Of particular relevance however, is the fact that IL-1F7 is significantly expressed in monocytes [20], suggesting a role for IL-1F7 as natural modulator of IL-18 in the initiation of innate defence responses.
INHIBITORY RECEPTORS TIR8/SIGIRR AND RP105

Recent evidence attributes a regulatory role to at least two receptors of the TLR/IL-1R family, namely TIR8/SIGIRR [8-10], and RP105 [11, 12]. TIR8/SIGIRR is a unique receptor of the IL-1R/IL-18R superfamily which, at variance with other members of the family, encompasses a single Ig-like domain in its extracellular portion [92]. The intracellular domain of TIR8/SIGIRR has the highest similarity to the intracellular adapter MyD88 among members of the TLR/IL-1R family [93]. TIR8/SIGIRR does not interact with IL-1α, IL-1β or IL-1Ra, and its intracellular domain is unable to transduce signals [92]. On the other hand, TIR8/SIGIRR apparently plays a central role in the down-regulation of inflammation mediated by TLR/IL-1R. Indeed, TIR8/SIGIRR-deficient mice and cells are more susceptible to stimulation with IL-1, IL-18 and TLR agonists (LPS, CpG oligonucleotides), whereas TIR8/SIGIRR-overexpressing cells were less susceptible to IL-1 and IL-18 stimulation [9, 10]. TIR8/SIGIRR expression is ubiquitous; however, it is preferentially expressed by epithelial cells (kidney, gut, liver) and is possibly involved in the control of intestinal inflammation [8, 10]. On the other hand, TIR8/SIGIRR is poorly expressed in leukocytes and cannot be induced by a series of inflammatory/anti-inflammatory stimuli [8]. Notably, expression of TIR8/SIGIRR in the mouse was significantly decreased in every organ/cell following in vivo administration of LPS [8]. The mechanism by which TIR8/SIGIRR down-regulates TLR/IL-1R-mediated activation is possibly based on the ability of the TIR-containing intracellular domain of TIR8/SIGIRR to compete for the adapter MyD88 and the signalling intermediate TRAF6, thus removing them from the signal transduction pathway of TLR/IL-1R [9]. More recently, with the use of different deletion mutants of TIR8/SIGIRR it has been shown that while for inhibition of TLR4 signalling only the intracellular TIR-containing domain of TIR8/SIGIRR was necessary, the presence of the extracellular Ig-like domain was also required for inhibiting IL-1R signalling, possibly through interference with the interaction between IL-1R and IL-1RAcP [94]. RP105 (CD180) is a transmembrane receptor protein first identified in murine B cells and involved in B cell proliferation and protection from apoptosis [95]. The extracellular portion of RP105 is a leucine-rich repeat domain structurally similar to that of TLR, implying a role in pathogen sensing, in particular in mediating LPS effects on B cells [96, 97] in concert with the accessory molecule MD-1 [98, 99]. A possible down-regulatory role of RP105 can be hypothesized on the basis of a series of experimental evidence. In humans, anti-inflammatory M2-biased macrophages (tumour-associated macrophages generated in vitro) showed up-regulation of mRNA and protein expression for both RP105 and MD-1 [100]. In autoimmune patients with lupus, Sjögren’s syndrome, and dermatomyositis, there is a significant increase in the population of RP105-negative B cells, which are responsible of autoantibody production [101-104]. Finally, recent data indicate that RP105, in concert with MD-1, can down-modulate TLR4/MD-2- and IL-1R-mediated activation by a still undefined mechanism [11, 12].

RECEPTOR T1/ST2 AND ITS LIGAND IL-33

T1/ST2 is a receptor of the IL-1R/IL-18R superfamily which, at variance with other receptors but similarly to TIR8/SIGIRR, does not induce an inflammatory response [105-108]. Initially, T1/ST2 was found to be unable to bind known members of the IL-1 family [109-111]. Two putative ligands of T1/ST2 have been identified; membrane and secreted protein which bound T1/ST2 with low affinity but which could not trigger TIR-dependent NFκB activation upon binding [109, 110]. Besides the membrane form of the receptor (ST2L), two alternatively spliced forms have been identified, the soluble ST2, corresponding to the extracellular domain of the membrane receptor [112], and the membrane-anchored ST2V protein [113]. T1/ST2 is preferentially expressed by fibroblasts, mast cells, and Th2 cells (as opposed to Th1 cells which selectively express the IL-18R) [114-116], and appeared to be involved in Th2 anti-inflammatory/allergic effector function [116-120]. It has been shown that T1/ST2 can down-regulate TLR2, TLR4, TLR9, and IL-1RI signalling, but not the MyD88-independent TLR3 signal transduction, based on the capacity of sequestering the adapters MyD88 and Mal through the intracellular TIR domain [7]. It was therefore hypothesized that all signalling pathways involving MyD88 and/or Mal may be regulated by T1/ST2. Recent data indicate that soluble ST2 could inhibit LPS-induced IL-6 production in THP-1 cells by inhibiting IkB degradation and the consequent binding of NFκB to the IL-6 promoter [121]. Recently, a novel member of the IL-1 family, IL-33, has been reported to be the specific ligand for T1/ST2 [5]. IL-33 is the eleventh identified member of the IL-1 family (IL-1F11), is synthesized as a pro-cytokine which should be cleaved by caspase-1 to generate the mature active form, and, within the IL-1 family, is most closely related to IL-18 [5]. Upon binding to T1/ST2, IL-33 recruits the IL-1RAcP as co-receptor (Michael U. Martin, personal communication), activates NFκB and MAP kinases, and induces IL-4, IL-5, and IL-13, but not IFN-γ, as expected for a Th2-type cytokine [5, 122]. In vivo administration of IL-33 in mice induces pathological changes such as eosinophilic infiltration and mucus secretion in airways and intestine, and increase in IgA and IgE, all effects consistent with classical Th2 diseases, such as asthma [5]. Mice deficient in T1/ST2 do not develop a Th2 response to Schistosoma egg antigen [118]. Thus, many of the inflammation-inhibitory effects of T1/ST2 activation may be due to activation of Th2 regulatory responses. Physiologically, T1/ST2 is induced by inflammatory stimuli, and appears to be involved in the late control of the inflammatory response (presumably by activating anti-inflammatory Th2 cells), including endotoxin tolerance [7, 107, 123]. In clinical situations, expression of T1/ST2 in human breast tumours is predictive of relapse-free survival [124]. Soluble T1/ST2 was found at increased levels in serum of patients with pulmonary inflammation [125], heart failure [126], and autoimmune lupus [127], and in the CSF of patients with subarachnoid hemorrhage. In patients with asthma, increased expression of T1/ST2 has been correlated with improved pulmonary function [128].
INTRACELLULAR NOD-LIKE RECEPTORS

NOD-like receptors (NLR) are a family of intracellular proteins that share some structural features with TLR/IL-1R and are involved in the regulation of apoptosis, inflammation, and host defence [130-133]. Structurally, NLR proteins usually include three distinct domains: the N-terminal effector-binding domain (e.g., CARD, caspase-recruitment domain), the nucleotide-binding oligomerisation domain (NOD), the C-terminal ligand-recognition domain. The latter is mostly a leucine-rich repeat similar to the binding domain of TLR receptors and of RIP105. The increasingly growing family of NLR encompasses members of animal, plant, fungal and bacterial origin, including over 20 human proteins. The ability of NLR to activate or inhibit caspases (either directly or through adapter proteins, by homotypic CARD-CARD interaction, in an intracellular complex called inflammasome) is at the basis of their involvement in regulation of apoptosis (activation of apoptotic caspases) and inflammation (activation of caspase-1 and subsequent maturation of IL-1α, IL-1β and IL-18, activation of caspase-3 and subsequent degradation of IL-18) [134-139]. It has been suggested that NLR, in particular NOD1 (CARD4; ubiquitously expressed) and NOD2 (CARD15; expressed mainly by monocytes and antigen-presenting cells), are involved in intracellular pathogen sensing, as they can recognise different moieties of Gram-negative peptidoglycans and initiate the inflammatory defence response [140-147]. Of particular interest is the recent notion that different mutations in the gene coding for NOD2/CARD15 underlie the intestinal inflammatory disease of a significant proportion of Crohn’s disease patients [144, 145, 148-155], and is at the basis of the auto-inflammatory disease Blau syndrome (or familial juvenile systemic granulomatosis, a rare form of uveitis, arthritis, and dermatitis) [151, 156-158]. Based on these observations, it was proposed that NOD2 acts physiologically as an activator of the TLR2-dependent anti-inflammatory response and IL-10 production, thus its loss-of-function mutations result in excessive TLR2-dependent activation of Th1 responses and persistent inflammation [159, 160].

Mutations in another protein of the NLR family, NALP3, are apparently involved in systemic inflammatory diseases, a series of autosomal dominant, severe pathologies characterised by persistent and generalised inflammation. These diseases include the Muckle-Wells syndrome (characterised by recurrent fevers, leukocytosis, cold-urticaria, painful arthropathies, serum amyloid A and CRP elevations, chronic conjunctivitis, uveitis, rashes, death due to renal amyloidosis), the chronic infantile neurological cutaneous and articular (CINCA) syndrome and the neonatal onset multisystem inflammatory disease (NOMID) (both characterised by intermittent fevers, chronic sterile meningitis, uveitis, sensorineural hearing loss, urticarial skin rash, deforming arthritis), and the familial cold autoinflammatory syndrome (FCAS) (urticarial skin rash, arthropathies, fever, leukocytosis, serositis). These diseases are all due to gain-of-function/loss-of-inhibition mutations in the NACHT nucleotide-binding site domain of NALP3 [136, 161-163]. NALP3 (cryopyrin) displays an N-terminal PYR effector binding domain, which interacts with the PYR domain of the adapter protein ASC, which in turn activates caspase-1 maturation after homotypic CARD-CARD interaction. Disease-related mutations in NALP3 cause de-regulation of NALP3 signalling which induces persistent activation of caspase-1, with subsequent increase in the production of caspase-1-dependent inflammatory cytokines (IL-1, and possibly IL-18) and establishment of the chronic inflammatory disease [130, 131, 136, 162, 164]. Very interestingly, the clinical symptoms of diseases due to increased or poor control of caspase-1 activity (CIAS, Muckle-Wells syndrome, adult onset Still’s disease, systemic onset juvenile idiopathic arthritis) often include skin rashes and/or pruritic urticaria. This is likely due to the fact that caspase-1 is also responsible for the maturation of IL-33, which through T1/T2, activates Th2 responses, IgE production, and mast cell degranulation. A schematic representation of the NALP3 inflammasome is shown in figure 3.

IL-18 IN AUTOIMMUNITY

Increased levels of IL-18 often correlate with the severity of autoimmune pathologies in experimental models of autoimmunity and also in clinical situations. That IL-18 may have a pathologic/pathogenic role is been suggested by the fact that blocking IL-18 has a beneficial effect in several models of autoimmune/inflammatory diseases (table 1).

Multiple sclerosis

The possible pathological role of IL-18 in EAE (experimental autoimmune encephalitis, the experimental model of multiple sclerosis) has been suggested by the correlation of the level of steady state mRNA for IL-18 and caspase-1 with the severity of the disease stage [165, 166]. Moreover, IL-18-treated antigen-specific T cells successfully transferred the disease to normal recipients [167]. On the other hand, IL-18-deficient mice were reportedly resistant to disease induction [168], whereas caspase-1 deficient mice exhibit decreased disease severity [169]. Recent data however claim that deletion of the IL-18 gene does not affect susceptibility to disease induction, whereas deletion of the IL-18Ra gene does [69]. The evidence is that accessory cells of IL-18Ra-deficient mice do not support the development of IL-17-producing T helper cells (Th17), responsible for the pathology [69]. That IL-18 is anyway involved in EAE (either by Th1 activation or possibly by amplifying the Th17 response) is suggested by the use of neutralising anti-IL-18 antibodies, which inhibited autoantigen-induced IFN-γ production by T cells in vitro and cerebral lesions in vivo [166], and by the targeted overexpression of IL-18BP in the CNS that increases Th2 polarisation and suppresses ongoing EAE [170]. In human multiple sclerosis, there are reports that disease activity correlates with elevated circulating and CSF levels of IL-18 and caspase-1. In addition, IL-18 positive cells have been observed in demyelinating lesions [171-181].

Myasthenia gravis

In experimental myasthenia gravis in the rat, blockade of IL-18 with specific antibodies suppresses disease develop-
In a murine model of myasthenia gravis, IL-18 deficiency (IL-18-/- mice) suppressed the generation of anti-acetylcholine receptor autoantibodies [184]. Elevated circulating IL-18 occurs in humans with myasthenia gravis [185, 186].

Rheumatoid arthritis

In experimental models of arthritis, administration of IL-18 exacerbated the disease. Initial studies were carried out using streptococcal cell wall (SCW)-induced arthritis [187]. A neutralising rabbit anti-murine IL-18 antibody was injected shortly before induction of arthritis by intra-articular injection of SCW fragments. Significant suppression of joint swelling (> 60%) was noted on days 1 and 2 of SCW arthritis after blockade of endogenous IL-18, and joint TNF-α and IL-1 levels were also decreased. Severe reduction of chondrocyte proteoglycan synthesis is a prominent component of SCW-induced arthritis but a near

Table 1

Reduction in autoimmune and/or inflammatory disease severity upon neutralisation of endogenous IL-18

<table>
<thead>
<tr>
<th>Disease Model</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(DSS) dextran sodium sulfate-induced colitis</td>
<td>[331, 333, 334]</td>
</tr>
<tr>
<td>(TNBS) trimnobrezone sulfonic acid-induced colitis</td>
<td>[330, 334]</td>
</tr>
<tr>
<td>CD62L transfer-induced colitis</td>
<td>[337]</td>
</tr>
<tr>
<td>(SCW) Streptococcal Cell Wall-induced arthritis</td>
<td>[187]</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>[193-195]</td>
</tr>
<tr>
<td>Streptozotocin-induced diabetes</td>
<td>[395]</td>
</tr>
<tr>
<td>EAE in rats</td>
<td>[166]</td>
</tr>
<tr>
<td>Experimental myasthenia gravis in rats</td>
<td>[182, 183]</td>
</tr>
<tr>
<td>Diabetes in NOD mice</td>
<td>[396]</td>
</tr>
<tr>
<td>ConA-induced hepatitis</td>
<td>[427, 461]</td>
</tr>
<tr>
<td>Endotoxin-induced pulmonary neutrophils</td>
<td>[462]</td>
</tr>
<tr>
<td>Ischemia-induced acute renal failure</td>
<td>[463]</td>
</tr>
<tr>
<td>Ischemia-induced myocardial dysfunction</td>
<td>[464, 465]</td>
</tr>
<tr>
<td>Endotoxin-induced myocardial dysfunction</td>
<td>[466]</td>
</tr>
<tr>
<td>Nephritis and lymphadenopathy in murine lupus</td>
<td>[294, 295]</td>
</tr>
</tbody>
</table>
complete reversal of the depressed chondrocyte proteoglycan synthesis was observed in the anti-IL-18-treated animals. These studies clearly established the pathological role for endogenous IL-18 in this model. The effect of IL-18 is apparently independent of IFN-γ since anti-IL-18 antibodies could equally inhibit SCW arthritis in mice deficient in IFN-γ [184]. That IL-18 is pathogenic in experimental group B streptococcal arthritis was confirmed by amelioration of the disease upon administration of anti-IL-18 antibodies and exacerbation following administration of exogenous IL-18 in mice [188]. IL-18 also plays a role in collagen-induced arthritis (CIA) [189-191]. IL-18 was injected into DBA-1 mice immunised with collagen in incomplete Freund’s adjuvant. There was an increase in the erosive and inflammatory component of the condition [192]. Using mice deficient in IL-18, CIA was less severe compared to wild-type controls [190]. Histological evidence of decreased joint inflammation and destruction was observed. Levels of bovine collagen-induced IFN-γ, TNF-α, IL-6 and IL-12 from spleen cell cultures were decreased in IL-18-deficient mice. Thus, there is likely a pathological role for IL-18 in CIA. However, it should be noted that IL-18 can also have a beneficial effect, as serum anti-collagen antibody levels are significantly reduced in the IL-18-deficient mice. Blocking of IL-18 was used in CIA models [193-195]. Wild-type DBA-1 mice were treated with either neutralising antibodies to IL-18 or the IL-18BP after chronic onset of disease. The therapeutic efficacy of neutralising endogenous IL-18 was assessed using different pathological parameters of disease progression. The clinical severity in mice undergoing CIA was significantly reduced after treatment with either IL-18 inhibitor [195]. Attenuation of the disease was associated with histological evidence of reduced cartilage erosion. The decreased cartilage degradation was further documented by a significant reduction in the levels of circulating cartilage oligomeric matrix protein (an indicator of cartilage turnover). Both IL-18 inhibitory strategies efficiently slowed disease progression, but only anti-IL-18 antibody treatment significantly decreased an established synovitis. Serum levels of IL-6 were significantly reduced with both neutralising strategies. In vitro, neutralising IL-18 resulted in a significant inhibition of TNF-α, IL-6, and IFN-γ secretion by macrophages [195]. Mice with established CIA (21 days after the primary immunisation with collagen) were treated for 3 weeks with murine IL-18BP as a fusion protein with the Fc portion of murine IgG1 [194]. Both the clinical disease activity scores and the histological scores of joint damage were reduced by 50%. Proliferation of collagen-stimulated spleen and lymph node cells, as well as the change in serum levels of IgG1 and IgG2a antibodies to collagen, were also decreased. Cell sorting analysis showed a decrease in spleen NK cells and an increase in CD4+ T cells. The production of IFN-γ, TNF-α, and IL-1β in cultured spleen cells was reduced. The steady state mRNA levels of IFN-γ, TNF-α, and IL-1β in isolated joints were likewise decreased. Thus, the mechanisms of IL-18BP inhibition of CIA include reduction in cell-mediated and humoral immunity to collagen as well as a decrease in production of proinflammatory cytokines in the spleen and joints. In CIA of BB rats, administration of IL-18 exacerbated arthritic symptoms, whereas anti-IL-18 neutralising antibodies attenuated CIA [196].

Employing an adenoviral vector containing the murine IL-18BP, intra-articular over-expression of IL-18BP significantly reduced the incidence of CIA in treated knee joints [193]. Affected knee joints of IL-18BP-treated mice showed less severe arthritis, with reduced cellular infiltration and less bone erosion. Reduction in cartilage loss was also observed in treated mice. IgG1 anti-collagen type II antibodies were similar to those in the control vector group.

In experiments with mice deficient in IL-18 or in IL-12p40 (lacking both IL-12 and IL-23), antigen-induced arthritis could develop in IL-18-deficient mice as well as in wild type animals, whereas no disease symptoms or antibodies to methylated BSA were evident in mice lacking IL-12p40 [197]. Additional studies with IL-12p35-deficient mice (lacking IL-12 only) and with IL-23p19-deficient animals (lacking IL-23 only) showed that only IL-23-deficient mice were resistant to disease induction [198]. Thus, the role of IL-18 (as well as IL-12) in experimental arthritis appears to be redundant, as compared to the key role for the IL-12p40 cytokine IL-23.

In human rheumatoid arthritis (including juvenile RA, adult-onset Still’s disease, and psoriatic arthritis), IL-18 is present at increased levels in serum and in the rheumatoid synovium, as well as in the bone marrow [79-81, 192, 199-220]. Rheumatoid subcutaneous nodules have the features of Th1 granulomas, with abundant expression of inflammatory cytokines including IFN-γ and IL-18 [221]. Polymorphisms in the promoter region of the IL-18 gene have been found associated with adult onset Still’s disease, RA, and juvenile arthritis [219-226]. IL-18 bioactivity in affected joints of patients with rheumatoid arthritis has been reported to correlate with disease activity [202, 227, 228]. IL-18 is mainly present as precursor protein, but the mature active form is also abundantly detected in macrophages and synovial fibroblasts. IL-18Rα and β receptor chains are found expressed on patients’ T lymphocytes, macrophages, and synovioctyes [79, 192, 202, 229, 230]. Elevated levels of IL-18BP are also present [46, 79, 80]. Synovial tissue from 29 patients with rheumatoid arthritis has been studied using specific staining for IL-18. IL-18 was detectable in 80% of the patients, in both the lining and sublining of synovial tissues from knees. There was a strong correlation between IL-18 and IL-1β expression but less with TNF-α staining [210]. Moreover, IL-18 expression correlated with macrophage infiltration and local inflammation scores. IL-18 staining also correlated with the erythrocyte sedimentation rate, a biomarker of systemic inflammation. Of considerable interest was the observation that in patients with co-expression of IL-18 and IL-12, there were also greater levels of IL-17 [231]. Blocking of endogenous IL-17 with specific inhibitors resulted in a protective inhibition of bone destruction [231-233]. Furthermore, IL-18 can inhibit chondrocyte proliferation and induce production of stromelysin and the inflammatory enzymes iNOS and COX-2, and promote cartilage degradation and glycosaminoglycan release [234]. IL-18 was also found to be able to induce production of serum amyloid A in RA synoviocytes, and to be responsible for acute liver failure in adult onset Still’s disease [235, 236]. More-
over, IL-18 participates in anomalous macrophage activation in hemophagocytic syndrome, a clinicopathological entity associated with adult onset Still’s disease, juvenile chronic arthritis, and possibly lupus erythematosus [237]. It is therefore conceivable to hypothesize a role for IL-18 in the maintenance of the destructive autoimmune and inflammatory processes of rheumatoid diseases, perhaps also by regulating the activities of IL-1β, TNF-α, IFN-γ, and IL-17. Indeed, treatments that ameliorate rheumatoid pathology concomitantly decrease IL-18 production [238]. The possibility of targeting IL-18 in the treatment of arthritis and more generally of chronic inflammatory diseases, is therefore receiving increasing attention [192, 239-249].

**Autoimmune uveitis and Behcet’s disease**

IL-18 is constitutively expressed in the epithelial cells of iris, ciliary body, and retina of normal mice, and is found at elevated levels after induction of experimental uveoretinitis (EAU) in susceptible rats and mice [250-252]. However, IL-18-deficient DBA1 mice were fully susceptible to induction of EAU, suggesting that the role of IL-18 is not essential to disease development [251]. Indeed, the non-essential, redundant role of IL-18 in the pathogenesis of EAU was confirmed in mice deficient in MyD88 or IL-1R, which were resistant to CFA-induced disease, whereas mice deficient in IL-18, TLR9, TLR4, or TLR2 were fully susceptible [252]. In human Behcet’s disease, a strong Th1-skewed response is apparently associated with disease severity. Elevated expression of IL-18 is found in serum, skin lesions, and pulmonary lavage fluid, and increased IL-18 production was observed in bronchoalveolar cells upon in vitro stimulation [210, 253-256]. Although no particular polymorphism in the IL-18 promoter gene could be associated with disease susceptibility, a clear correlation was found between a particular genotype and the risk of developing ocular lesions [257].

**Psoriasis**

In psoriasis, serum IL-18 levels correlate with disease severity and skin lesions, and increased IL-18 expression can be found in psoriatic keratinocytes [258-262]. Normal keratinocytes produce IL-18 but do not process it, conceivably because of the lack of the cleaving enzyme caspase-1 [262]. However, inflammatory and toxic agents (LPS, dinitrochlorobenzene, UVB) can induce production of biologically active IL-18 in keratinocytes, while immunosuppressive agents such as 1,25-dihydroxyvitamin D3 decrease it [263-267]. While caspase-1 induction and activation can be hypothesized in inflamed keratinocytes, potent caspase-1 activity and IL-18 production is present in activated Langerhans and dendritic cells in the skin [268-270]. In psoriasis and atopic dermatitis, the IL-18Rα is upregulated in keratinocytes with consequent inflammatory activation in response to IL-18 [271]. High levels of IL-18 have been detected in serum and scales of psoriatic patients [272-275], which correlate with disease severity, and are possibly relevant to the pathogenesis of skin lesions [276]. In mice overexpressing mature IL-18 in the skin, a stronger and persistent inflammatory skin reaction could be achieved in response to topical irritants as compared to controls [277]. Approaches to inhibit IL-18 in autoimmune skin diseases are underway [272, 278, 279].

**Autoimmune thyroiditis**

Analysis of inflammatory cytokine expression in the obese strain of chickens (a model of spontaneous autoimmune thyroiditis) showed upregulation of IL-18 expression, along with other cytokines, in the thyroid of autoimmune chicken as compared to normal birds [280]. In experimental autoimmune thyroiditis, IL-18 showed the same capacity as LPS in inducing TNF-α and autoreactivity in IL-12 p40-deficient mice [281]. Increased IL-18 can be found in serum and thyrocytes of patients with autoimmune Hashimoto’s thyroiditis and Graves’ ophthalmology [282-285]. In corticosteroid-responsive patients, treatment with methylprednisolone could induce a significant decrease in the circulating IL-18 levels paralleling an amelioration of disease symptoms [284]. However, in another study, no increase in IL-18 levels in serum or orbital tissue of Graves’ ophthalmology patients could be found when compared to normal individuals [286, 287]. A closer analysis of the serum of patients as compared to healthy controls apparently showed that an IL-18 increase is evident in Hashimoto’s thyroiditis, which is mainly a Th1 disease, whereas Grave’s ophthalmology is mostly characterized by an increase in Th2 cytokines (IL-4, IL-5) with no clear-cut enhancement of IL-18 levels [288]. No association between autoimmune thyroid disease with or without diabetes and a particular polymorphism of the IL-18 gene could be found [289].

**Systemic lupus erythematosus, Sjögren syndrome, and autoimmune myopathies**

In different models of murine lupus, including the MRL/lpr/lpr mouse (a model of the lupus autoimmune lymphoproliferative syndrome ALPS, and of Sjögren syndrome), elevated IL-18 levels were found in serum and organs, including kidney [290-294]. Inhibition of IL-18 effects in lpr/lpr mice resulted in a delay in disease development and a decrease in its severity [293-296]. Elevated plasma levels of IL-18 can be detected and correlate with disease activity and renal damage in human SLE [291, 297-314], so that therapeutic strategies targeting IL-18 are envisaged [315-319]. Increased expression of the IL-18 protein can be detected in serum, salivary gland and lacrimal duct tissues of Sjögren’s syndrome patients and of affected mice [212, 320-322]. IL-18 has been found to cause atrophy of the lacrimal and salivary glands through elevation of NO production [322]. In autoimmune inflammatory myopathies (dermatomyositis and polymyositis), high levels of IL-18 could be found both in serum and in muscle biopsies of patients [323]. IL-18 was produced by macrophages and dendritic cells in the affected tissue, while the IL-18 receptor was upregulated in CD8+ T cells and endothelial cells.

**Inflammatory bowel disease (IBD) and Crohn’s disease**

Several reports support the possible involvement of IL-18 in the pathogenesis of Crohn’s disease, an autoimmune...
inflammatory bowel disease characterised by a pathogenic role of Th1 cells. The role of IL-18 in inducing/perpetuating intestinal inflammation is also evident in non-autoimmune conditions, e.g. in shigellosis or by direct administration [324-327], with a strict correlation between the presence of IL-18 and the severity of inflammation. In experimental models of colitis in the mouse and rat, the inflammatory pathology correlates with increased levels of tissue and serum IL-18, whereas anti-IL-18 antibodies, IL-18 antisense mRNA, and the inhibitory IL-18BP can decrease the pathological signs [328-342]. In addition, induction of experimental colitis could not be achieved in IL-18-deficient mice [332], whereas mice overexpressing IL-18 are significantly more susceptible to induction of experimental colitis [343]. Drugs that inhibit development of experimental colitis inhibit, in parallel, IL-18 expression [344, 345]. Mice deficient in caspase-1, the enzyme that matures both IL-1 and IL-18, were also resistant to induction of experimental colitis, which was paralleled by a significant decrease in the spontaneous production of IL-1, IL-18 and IFN-γ [336, 346]. Similar inhibition of colitis was obtained by treatment with inhibitors of caspase-1 [336, 347]. Mice deficient in the interferon regulatory factor-1 develop a significantly more severe experimental colitis. The increased severity does not correlate with changes in the expression of tissue IL-18, but levels of colonic IL-18BP were drastically reduced. Administration of exogenous IL-18BP could reverse the severity of the disease [348]. The ability of IL-18 to prevent immunological tolerance to orally administered antigens may contribute to the establishment of autoimmune gut disease [349].

In human Crohn’s disease, ulcerative colitis, and coeliac disease, increased levels of IL-18 are found in serum, and IL-18, IL-18R chains, and active caspase-1 are increased in chronically inflamed mucosa as compared to early lesions or to normal colonic mucosa [350-362]. Increased IL-18 is evident in mucosal epithelial cells, but is also due to the increase in IL-18-producing DC-SIGN+ mucosal DC in Crohn’s patients [363]. Moreover, IL-18 is a potent proliferative stimulus for intestinal mucosal lymphocytes of Crohn’s patients, which, at variance with normal mucosal lymphocytes, show upregulation of the IL-18R chains and are therefore constitutively susceptible to IL-18 activation [339, 364, 365]. Polymorphisms in the promoter and/or coding region of the IL-18 gene have been found to be associated with either increased susceptibility to Crohn’s disease and ulcerative colitis, or to disease severity to varying degrees depending on the population cohort [366-370]. Levels of the IL-18 natural inhibitor IL-18BP are also increased in the plasma of patients with Crohn’s disease and ulcerative colitis [78, 360]. This mechanism of control of IL-18 activity however, does not appear to be effective, as the overall levels of free active IL-18 (i.e. not bound and inhibited by IL-18BP) are still significantly higher in Crohn’s patients [360]. That IL-18BP is an important player in controlling intestinal damage is suggested by data from experimental models. The increased severity of experimental colitis in IRF-1-deficient mice is associated with decreased levels of colon IL-18BP [348], while the gut lesions in experimental colitis are decreased by administration of IL-18BP [333].

Intestinal inflammation and damage in experimental colitis is associated with an increase in active caspase-1 in the tissue [340, 371, 372]. It is interesting to note that intestinal parasites such as Entamoeba histolytica cause intestinal damage through proteases with caspase-1 activity [373]. Agents that induce apoptosis of colonic epithelial cells concomitantly increase active caspase-1, and inhibition of caspase-1 activity concomitantly inhibits mucosal cell death [374, 375]. Association of Crohn’s disease with loss-of-function mutations in the gene of NOD2/CARD15, an intracellular protein involved in the caspase-1-activating inflammasome in macrophages, further underlines the importance of caspase-1 products such as IL-18 in the pathogenesis of the disease [143, 144, 148-155, 376, 377]. Treatments that provide therapeutic benefits in Crohn’s patients (e.g. anti-TNF-α antibodies) re-establish appropriate apoptosis of mucosal lymphocytes by increasing the pro-apoptotic caspase pathway [378]. It is notable that therapy-induced increase of caspase-3, which has a major role in lymphocyte apoptosis, is concomitantly expected to decrease the levels of active IL-18, as caspase-3 is the main IL-18 degrading enzyme [379]. Biological therapies targeting IL-18 and other inflammatory cytokines mainly produced by macrophages, are being developed for more effective treatment of IBD [380-383].

Autoimmune type 1 diabetes (IDDM)

In murine models of insulin-dependent diabetes (IDDM) (NOD mice, streptozotocin, reovirus type 2), IL-18 correlated with disease progression and severity [384-392]. Islets from a non-obese, diabetic mouse strain exhibited IL-18 expression prior to T cell invasion [393], and IL-18 administration promoted disease development [394]. In the genetically diabetic NOD mouse, the IL-18 gene co-localizes to the same region of chromosome 9 as the diabetes susceptibility gene idda2 [385]. NOD mice overexpressing a diabeticogenic TcR have increased production of IL-18, IL-12 and TNF-α [388]. On the other hand, experimental IDDM induction could be prevented in IL-18-deficient mice or with administration of IL-18BP [394-396]. However, there is evidence that IL-18, when administered exogenously to adult NOD mice, can delay the disease onset [387]. On the other hand, in a different study, systemic administration of IL-18 by means of IL-18-expressing plasmid delivery did promote diabetes development in young NOD mice [393]. This discrepancy may be explained in light of the dual role of IL-18 as modulator of either Th1 or Th2 responses depending on the cytokine micro-environment [397]. In addition, in response to inflammatory stimuli, islet β cells can produce IL-18 but do not express the IL-18R chains, suggesting that IL-18 does not have a direct effect on them [398]. In fact, in vitro treatment of pancreatic islets with IL-18 does not have a significant effect on insulin accumulation, or on glucose-stimulated insulin release [399]. Activation of the IL-1- and IL-18-converting enzyme caspase-1, increases in pancreatic and retinal tissues during diabetes development in mice and correlates with increased apoptosis [400-402]. Inflammatory stimuli such as IFN-γ and TNF-α increase both caspase-1 expression and activation, and apoptosis in
pancreatic β cells [403], while inhibitors of caspase-1 could block β cell apoptosis [404]. However, caspase-1 processing of IL-18 or IL-1β does not appear to be an absolute requirement in the pathogenesis of autoimmune diabetes, since NOD mice deficient for expression of caspase-1 fully develop both the spontaneous and the chemically-induced (streptozotocin) disease [405]. In human IDDM, elevated IL-18 levels can be detected in high-risk individuals, before the development of the disease [406], whereas in patients there is a correlation between IL-18 levels and autoantibody status [407]. As in mice, the human gene for IL-18 maps to an interval, on chromosome 9, where the diabetes susceptibility locus Idd2 resides [408].

Although many of the autoimmune effects of IL-18 in different models and clinical situations are mediated through IFN-γ, and are amplified by IL-12, in some experimental models a direct pathological role for IL-18 has been described. However, IL-18 cannot be considered as an exclusively Th1 cytokine, as its presence may be unrelated to that of IFN-γ. Indeed, IL-18 takes part in the Th17 responses by amplifying the IL-17 production of polarised Th17 cells in synergy with IL-23, thus contributing to the destructive inflammation in several chronic inflammatory, autoimmune diseases [18]. On the other hand, IL-18 can act as a Th2 cytokine in some circumstances [397]. Indeed, in the presence of IL-4 and IL-2, IL-18 can induce significant production of the Th2 cytokines IL-4 and IL-13 by Ag-stimulated T cells, whereas IFN-γ is produced in the absence of IL-4 [409, 410]. Moreover, IL-18R is present on mast cells and basophils, which produce large amounts of IL-4 and IL-13 in response to IL-18 and IL-3 [411]. Thus, IL-18 has been found to play a dual role in Th2-dependent responses such as in helminthic infections and allergic asthma in the mouse. Together with IL-12, IL-18 can suppress IgE production, Th2 cell development, lung hyper-reactivity and eosinophil infiltration, by biasing the immune response to allergic towards Th1 [397, 412, 413]. Moreover, IL-18 deletion could enhance pulmonary eosinophil infiltration [414, 415]. On the other hand, administration of IL-18 induced eotaxin production and subsequently provoked enhanced eosinophil accumulation in pulmonary lesions, and also induced increased synthesis of Th2 cytokines and IgE [410, 415-418]. Thus, although IL-18 has a major role in immune responses, it can become an anti-inflammatory, Th2-biasing factor depending on the cytokine micro-environment (e.g., presence or absence of IL-12).

**IL-18 IN METABOLIC SYNDROME**

As mentioned above, several properties of IL-18 are independent of IFN-γ, e.g., induction of joint inflammation and induction of septic shock. In contrast to other proinflammatory cytokines such as IL-1β, TNF-α or IL-12, there is a constitutive intracellular pool of the IL-18 precursor [419], and exposure to inflammatory stimuli has little effect on IL-18 gene transcription. The increase in mature IL-18 observed under these circumstances is largely due to caspase-1-mediated cleavage of pro-IL-18. In addition, it is thought that the bioactivity of IL-18 is kept in balance by high concentrations of IL-18BP in blood and tissues [71]. In humans, IL-18 concentrations are reported to be increased in patients with type 2 diabetes mellitus, in obese individuals with the metabolic syndrome [71]. Infusion of glucose into normal volunteers and patients with impaired glucose tolerance induces an acute increase in serum IL-18 concentrations. It has been therefore hypothesized that the increased IL-18 concentrations have a pathophysiological role in insulin resistance and lipid deposition.

Unexpectedly, we observed that IL-18-deficient mice have a markedly increased body weight compared to the wild-type littermates of the same age, and have found that in the absence of IL-18, mice develop several features characteristic of the metabolic syndrome: obesity, insulin resistance and hyperglycemia, lipid abnormalities, atherosclerosis [420]. We reported these pathological conditions associated with the metabolic syndrome in mice deficient in IL-18 or the IL-18Rα chain, as well as in mice transgenic for expression of IL-18BP [420]. These mice, in contrast to wild-type mice, eat several times a day and the increased food intake accounts for the obesity. IL-18 deficiency results in a loss of the circadian regulation of food intake and appetite suppression [420]. Because of the large, constitutively-expressed intracellular pool of the IL-18 precursor, especially in liver cells, additional roles for the cytokine other than those played in innate immunity are suspected, and the loss of appetite control appears to a manifestation of the non-immune mechanisms of IL-18.

Obesity in IL-18-deficient mice was due to accumulation of fat tissue based on increased food intake. IL-18-deficient mice also displayed hyperinsulinemia, consistent with insulin-resistance and hyperglycemia. Further analysis of the glucose metabolism in IL-18-deficient mice showed insulin resistance at the hepatic level causing hyperglycemia in these mice, with enhanced expression of gluconeogenesis genes in the liver. In addition, the molecular mechanisms responsible for the hepatic insulin resistance in the IL-18-deficient mice likely involved defective phosphorylation of STAT3, one of the intracellular pathways activated by IL-18. In contrast, MyD88, which mediates a second pathway of activation by IL-18 receptor, was not involved in this process. Recombinant IL-18 reversed hyperglycemia in IL-18-deficient mice through activation of STAT3 phosphorylation. These findings demonstrate a new role of the cytokine IL-18 in the homeostasis of energy intake and insulin sensitivity.

**Role of IL-18 in the loss of insulin-producing β-cells**

Several studies report that the levels of IL-18 in various transplant models, as well as in kidney transplant patients, correlate with graft failure. Using mice that overproduce IL-18BP as diabetic islet graft recipients, it was reported that IL-18 indeed plays a role in the damage inflicted upon transplanted islets. In view of the wide distribution of IL-18-producing cells, it was essential to identify the cellular sources of the damaging IL-18. To address this issue directly, islets from IL-18-deficient mice were transplanted into wild type mice recipients resulting in a greater survival compared to wild-type islets transplanted into wild-type mice [421]. This finding supports the concept of local endogenous islet IL-18 being sufficient to promote β-cell injury during islet transplantation. In fact, lack of islet-derived IL-18 from grafted islets resulted in a similar outcome to that obtained by reduced activity of IL-18 in
mice transgenic for IL-18BP, suggesting that host-derived IL-18 plays a negligible role in islet graft failure [421].

**Deficiency in IL-18 and IL-18Rα reveal distinctly opposing phenotypes**

An unexpected finding emerged upon transplantation of islets from IL-18Rα-deficient mice into wild-type recipient mice. It was anticipated that implantation of islets that lack IL-18Rα would result in a similar protected phenotype as that of IL-18-deficient islets. However, graft failure in IL-18Rα-deficient islets was accelerated compared to islets from wild-type donors [421]. Remarkably, the median survival time of IL-18Rα-deficient islets grafted into a wild-type diabetic host was 9 days whereas the median survival time of IL-18-deficient islets in a wild-type host was 14.5 days [421]. One explanation is that excess IL-18 from IL-18Rα-deficient islets exits into the surrounding host tissue where it triggers the production of IL-18-induced injurious mediators. In fact, IL-18Rα-deficient islets spontaneously produce 2-fold greater IL-18 levels. IL-18Rα-deficient splenocytes also produced more IL-18 than wild-type cells. However, isolated IL-18Rα-deficient macrophages, although unresponsive to IL-18, produced more TNF-α than wild-type macrophages in vitro. This finding challenged the underlying assumption that the IL-18Rα is specific to the IL-18 pathway, and prompted further examination of the differences between IL-18 and IL-18Rα-deficient cells.

**Alternate signaling of IL-18Rα**

The unexpected increase in islet failure, observed in wild-type mice transplanted with islets from IL-18Rα-deficient mice, was associated with increased spontaneous production of IL-18 from IL-18Rα-deficient islets and splenocytes, and of TNF-α from macrophages. Splenocytes from IL-18Rα-deficient mice stimulated in vitro by ConA, TLR2 engagement or by anti-CD3 antibodies consistently produced more proinflammatory cytokines compared to wild-type cells, whereas IL-18-deficient cells produced less than wild-type [421]. For example, IL-18Rα-deficient splenocytes released nearly three-fold greater TNF-α and MIP-1α than IL-18-deficient cells upon stimulation by Staphylococcus epidermidis.

The divergence of responses between IL-18Rα- and IL-18-deficient cells is unexplained. More likely, the data suggest the existence of an IL-18-independent inhibitory pathway that converges with the IL-18 pathway at the IL-18Rα. Accordingly, in cells deficient in the receptor, a putative inhibitory signal, along with the pro-inflammatory IL-18 signal pathway, is absent. Gutcher et al. also provided clear evidence that an IL-18-independent engagement of IL-18Rα exists [69]. In murine experimental autoimmune encephalomyelitis (EAE), IL-18-deficient mice are susceptible to disease progression whereas IL-18Rα-deficient mice are protected. As such, these investigators concluded that there are two distinct pathways converging on the IL-18R: one signal requires IL-18 and the other involves an unknown ligand.

The study comparing islet survival in IL-18- and 18Rα-deficient mice differs from the EAE model in several aspects. Islets from mice deficient in IL-18 or IL-18Rα implanted into a wild-type animal allow for responses of an intact immune system to the genetically altered cells. In the EAE, altered immune system responses are inherent to Macrophage

**Figure 4**  
IL-18 in autoimmune activation of lpr mice. IFN-γ-stimulated macrophages process inactive caspase-1 into the active enzyme. This, in turn, cleaves and activates precursor IL-18 and allows its release. Defective lpr T cells constitutively express the IL-18 receptor complex (IL-18Rα and IL-18Rβ) necessary for IL-18-dependent cell activation. Active IL-18 binds to the IL-18 receptor complex and induces IFN-γ production.
the knock-out gene. Additionally, in the transplanted islet, early responding cells, such as macrophages, most probably mediate damage; the EAE model provides insights into mechanisms of cell-mediated, autoimmune-processes. Nevertheless, with striking similarity to the islet transplantation data, deficiency in IL-18R α chain confers an opposite phenotype to that observed in IL-18 deficiency.

Whether the convergence upon the IL-18R α chain involves a second ligand or a novel receptor accessory chain is presently unknown. IL-1F7, a member of IL-1 family with significant sequence homology with IL-18, binds to IL-18BP and IL-18R α chain [20, 86]. Upon binding to IL-18R α, IL-1F7 does not induce IFN-γ production and exhibits no apparent competition with IL-18 [20]. The combination of IL-18BP and IL-1F7 results in greater inhibition of IL-18 activity compared to IL-18BP alone, conferring on IL-1F7 the property of a naturally occurring modulator of IL-18 activity [20]. For any signal in the IL-1 family of receptors to occur, an accessory chain is recruited, the binding of which in this case would result in inhibition of IL-18 activity. Whether the accessory chain is the established IL-18R β chain or a novel receptor chain is yet undetermined. However, it was reported that mixing IL-1F7 with soluble IL-18R α and IL-18R β chains did not result in a ternary complex, as formed in the presence of IL-18 and the same receptor subunits. Therefore, we speculate that the accessory receptor chain recruited for IL-1F7 is novel. This model provides a mechanism by which lack of the IL-18R α chain results in the loss of a negative signal, accompanied by the appearance of a heightened inflammatory response.

PATHOGENIC ROLE OF IL-18 IN AUTOIMMUNITY

There is evidence that IL-18 is involved in autoimmune pathogenesis, and is essential for the first steps of autoimmune hyperactivation. Moreover, it appears that IL-18 also contributes to down-stream autoimmune amplification and detrimental effects. The pathogenic role of IL-18 was demonstrated during an experimental study in one murine model of lupus, the lymphoproliferative syndrome caused by the lpr gene [290, 293, 294]. The lpr/lpr homozygous mice exhibit each of the signs of the lymphoproliferative syndrome by 10 weeks of age, and develop progressive multiorgan damage culminating in early death from renal failure. In these mice, well before the first pathological signs are apparent, the elevated level of IL-18 (both as steady state mRNA and as protein) was consistently observed in all organs. Likewise, the expression of both IL-18 receptor chains was observed, a situation that sensitises cells to activation by IL-18. In healthy mice, the IL-18R β chain is usually not expressed unless in a “danger” situation when both chains of the IL-18 receptor are transiently upregulated. Thus, in lpr/lpr mice the IL-18 activity is abnormally regulated at two levels, hyperproduction of IL-18 and hyperexpression of IL-18 receptors. This latter event is possibly due to up-regulation by IL-12, which is also produced at excessive levels in models of autoimmune disease, including lpr/lpr mice. In a preliminary study in lymph nodes of young lpr/lpr mice (i.e. before appearance of the lupus-like disease symptoms), mRNA expression of IL-18 was examined and compared to that of the IL-18 inhibitor IL-18BP and of the IL-18 cleaving enzyme caspase-1 (Martinelli, Neumann, Quattroni and Boraschi, in preparation). As compared to healthy controls, lpr lymph nodes express higher levels of IL-18 and of caspase-1, and lower levels of IL-18BP, suggesting that increased production of free active IL-18 precedes disease development. The putative pathway of IL-18-dependent cell activation in the lpr mouse is summarised in figure 4. Defective lpr T lymphocytes spontaneously produce high levels of IFN-γ, which induces expression and activation of caspase-1 in macrophages and other cells [50, 422]. Active caspase-1 cleaves and activates precursor IL-18 and promotes its release. Soluble, mature IL-18, in the absence of high levels of IL-18BP, binds to the IL-18 receptor complex (upregulated in defective lpr T cells) and induces abundant production of IFN-γ in a self-sustaining amplification loop.

The confirmation of the pathogenic role of IL-18 in the development of the autoimmune syndrome was obtained using a vaccination approach to inhibit the excess production of endogenous IL-18. A cDNA vaccination strategy was designed, with an expression plasmid carrying the gene for the IL-18 precursor. Vaccination was performed in young lpr/lpr mice, which at the time had no signs of the disease, but which already showed increased IL-18 levels in various organs. After vaccination in the muscle tissue, the IL-18 plasmid synthesized the IL-18 protein, and consequently an anti-IL-18 response was generated. Why an antibody response against an autologous protein occurs after cDNA vaccination is not completely clear. One cannot rule out that the autoimmune-prone background of the recipient plays a role. The anti-IL-18 response triggered by vaccination significantly reduced the activity of IL-18 in the organs of lpr/lpr mice, and thus disease development was significantly delayed and was less severe, as judged by the pathological parameters of lymphadenopathy, renal damage, proteinuria, and early death [293]. Decrease in disease progression can also be detected when vaccination is performed in older mice with overt disease (P Bossù and D Neumann, unpublished observations). On the basis of the results obtained with anti-IL-18 vaccination, and following the notion that IL-18 acts in synergy with IL-12 in inducing Th1 inflammatory responses having a central role in several autoimmune syndromes, another study was carried out in lpr/lpr mice, by vaccinating animals with cDNA for both IL-12 and IL-18 [423]. Concomitant administration of cDNA coding for the two cytokines induced a potent inhibition of lymphadenopathy and splenomegaly (the most striking characteristics of the disease), and practically abolished the kidney damage and proteinuria. In addition, the strong inflammatory infiltrate evident in lungs of lpr/lpr mice was absent in IL-18/IL-12-vaccinated animals.

To confirm the pivotal role of IL-18 in the autoimmune pathogenesis, IL-18R α-deficient lpr/lpr mice were shown to survive longer and have significant reduction in renal pathology. Skin lesions, lymphadenopathy, and lung pathology were also diminished in IL-18R α-deficient lpr/lpr mice [424]. In agreement with results obtained with cDNA vaccination and with analysis of gene expression in kidney (Martinelli et al., in preparation), IL-18R α-deficient lpr/lpr mice did not show a decrease in autoantibody production or in end-organ disease, stressing the hypoth-
their drawbacks. Repeated administration of recombinant
Although very promising, each of these approaches have
due to a mutation in the criopyrin gene [432].
from FCAS patients, who have excessive caspase-1 acti-
treated animals, VX-765 is in trials for the treatment of
pended after detection of long-term liver abnormalities in
a rheumatoid arthritis trial with pralnacasan was sus-
drugs (such as VX-740, pralnacasan, and VX-765). While
pursued [428-431]. Orally active caspase-1 inhibitors went
mosomes is a therapeutic option that is being actively
inhibition of IL-18 [333, 396].
reduced in these mice. Because of the long plasma
defective in IFN-$\gamma$ when administered 6 days be-
before the endotoxin challenge. IL-18BP:Fc reduced hepatic
injury as well as expression of Fas [426]. IL-18BP:Fc also
decreased granuloma formation and production of the
chemokines MIP-1$\alpha$ and MIP-2. As shown previously
when administered 6 days before
in host reaction to mycobacterial infec-
tion and developed non-necrotic granulomas [459, 460].

**CONCLUSIONS AND FUTURE PERSPECTIVES**

Current information suggests that IL-18 may have a patho-
genic role in autoimmunity. Based of this information, future
studies should aim firstly, at verifying this hypoth-
thesis, and secondly at investigating the possibility that the
pathogenic excess of IL-18 in autoimmunity could be due
to dysregulated TLR/IL-1R-mediated activation of M1
macrophages and imbalance with M2 macrophages. In
fact, macrophages are the major producers of IL-18 in the
body, and its production is stimulated by agents of infec-
tious origin. IL-18 production is one of the first events of
the defensive innate immune reaction, and is very impor-
tant in initiating both the Th1- and the Th17-dependent,
inflammatory type immune response necessary to resolve
the infectious/stressful event and to re-establish homeosta-
sis. The role of innate immune cells (e.g. M1 or “M17”
inflammatory effector macrophages) and receptors (e.g.
TLR/IL-1R receptors) in the initiating events of autoim-
munity thus needs to be investigated. The initiating causes
of autoimmune pathologies are elusive, although they are
clearly multiple and can include both a genetic predis-
sposition and one or more triggering events. TLR/IL-1R acti-
vation in M1 and M17 macrophages can be one of these
events and the cause of excessive IL-18 production/ activity, in association with defective down-regulation of

**THE THERAPEUTIC APPROACHES TO INHIBIT IL-18 IN AUTOIMMUNITY**

Besides vaccination against IL-18, other therapeutic ap-
proaches under investigation to inhibit IL-18 in experi-
mental models and in clinical trials for autoimmune dis-
eases include the use of neutralising antibodies to IL-18, IL-
18 receptor blocking antibodies, the IL-18 binding pro-
tein IL-18BP, and caspase-1 inhibitors. IL-18BP is in clinical trials for a variety of autoimmune
diseases including rheumatoid arthritis. A divalent fusion protein of human IL-18BP linked human IgG1 Fc (IL-
18BP:Fc) binds and neutralises human, mouse, and rat
IL-18 with a dissociation constant of 0.3-5 nM. Using E.
coli-derived endotoxin, with a lethal dose of 90%, IL-
18BP:Fc administered 10 minutes prior to the endotoxin,
significantly reduced mortality [426]. IFN-$\gamma$ levels were
do not inducing an obvious immunosuppressed phe-
notypic role in the anti-mycobacterial response likely depends
on IFN-$\gamma$ induction [447, 448]. In patients with active
tuberculosis infections, the levels of IL-18 and IFN-$\gamma$ in
serum, pleural effusions and alveolar macrophages are sig-
nificantly increased [445, 449-453]. Administration of
IL-18 DNA or transfected cells in mice caused enhanced
IFN-$\gamma$ production and a potent anti-mycobacterial re-
ponse [454-456]. Likewise, IL-18 transgenic mice show
increased resistance to mycobacterial infection [457].
Conversely, mice susceptible to mycobacterial infection
produced significantly less IL-18 as compared to geneti-
cally resistant mice [458]; IL-18 knock-out mice were
defective in IFN-$\gamma$ production in response to mycobacte-
rial infection and developed non-necrotic granulomas [459, 460].
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this activation (e.g. at the level of equilibrium with M2 macrophages). Analysis of genetic polymorphisms in the TLR/IL-1R genes and of the genes coding for IL-18, its receptors and inhibitors, may shed some light on the reasons for this defective down-regulation. Understanding the role of innate immune mechanisms in the initial events of autoimmune will allow the design of better targeted therapeutic strategies, aimed at blocking the initial stages of autoimmune pathogenesis, and consequently its chronisation. A therapeutic approach based on the re-balancing of the dysregulated reaction is bound to be more effective and prone to fewer side-effects than any therapy based on inhibition of a single downstream effector molecule (particularly because these have a vital defence role and cannot be eliminated without consequence). For the future, if indeed TLR/IL-1R were to be identified as the molecules responsible for autoimmune pathogenesis, the study of their regulation by environmental, toxic, and infectious agents may allow us to identify possible pathogenic causes and to design preventive strategies.

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