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Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens

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Abstract Interleukin-18 (IL-18) is a proinflammatory cytokine that belongs to the IL-1 cytokine family, due to its structure, receptor family and signal transduction pathways. Similarly to IL-1 β , IL-18 is synthesized as a precursor requiring caspase-1 for cleavage into an active IL-18 molecule. However, with regard to its capacity to induce the production of Th1 cytokines and to enhance cell-mediated cytotoxicity, IL-18 is also related to IL-12. Produced mainly by antigen-presenting cells, IL-18 is a pleiotropic factor involved in the regulation of both innate and acquired immune responses, playing a key role in autoimmune, inflammatory, and infectious diseases. This review summarizes recent advances in the understanding of IL-18 structure, processing, receptor expression, and immunoregulatory functions and emphasizes the critical role of this cytokine in bacterial infections. It focuses on the participation of this cytokine in the defense against intracellular bacteria, including *Listeria*, *Shigella*, *Salmonella*, and *Mycobacterium tuberculosis*. Since this cytokine may be particularly useful in immunoprophylactic and immunotherapeutic interventions in which the cellular response is most desirable, the potential therapeutic aspects of IL-18 is also discussed.

Keywords IL-18 · Interleukin 18 receptor · Immunoregulation · Intracellular bacteria · Mycobacteria; Infection

Abbreviations APC: Antigen-presenting cell · BCG: Bacille Calmette-Guérin · *FIL1*: Family of interleukin 1 · *GM-CSF*: Granulocyte/macrophage colony stimulating factor · *ICE*: Interleukin 1 β converting enzyme · *IFN*: Interferon · *IKK*: I- κ B kinase · *IL*: Interleukin · *IL-18BP*: Interleukin 18 binding protein ·



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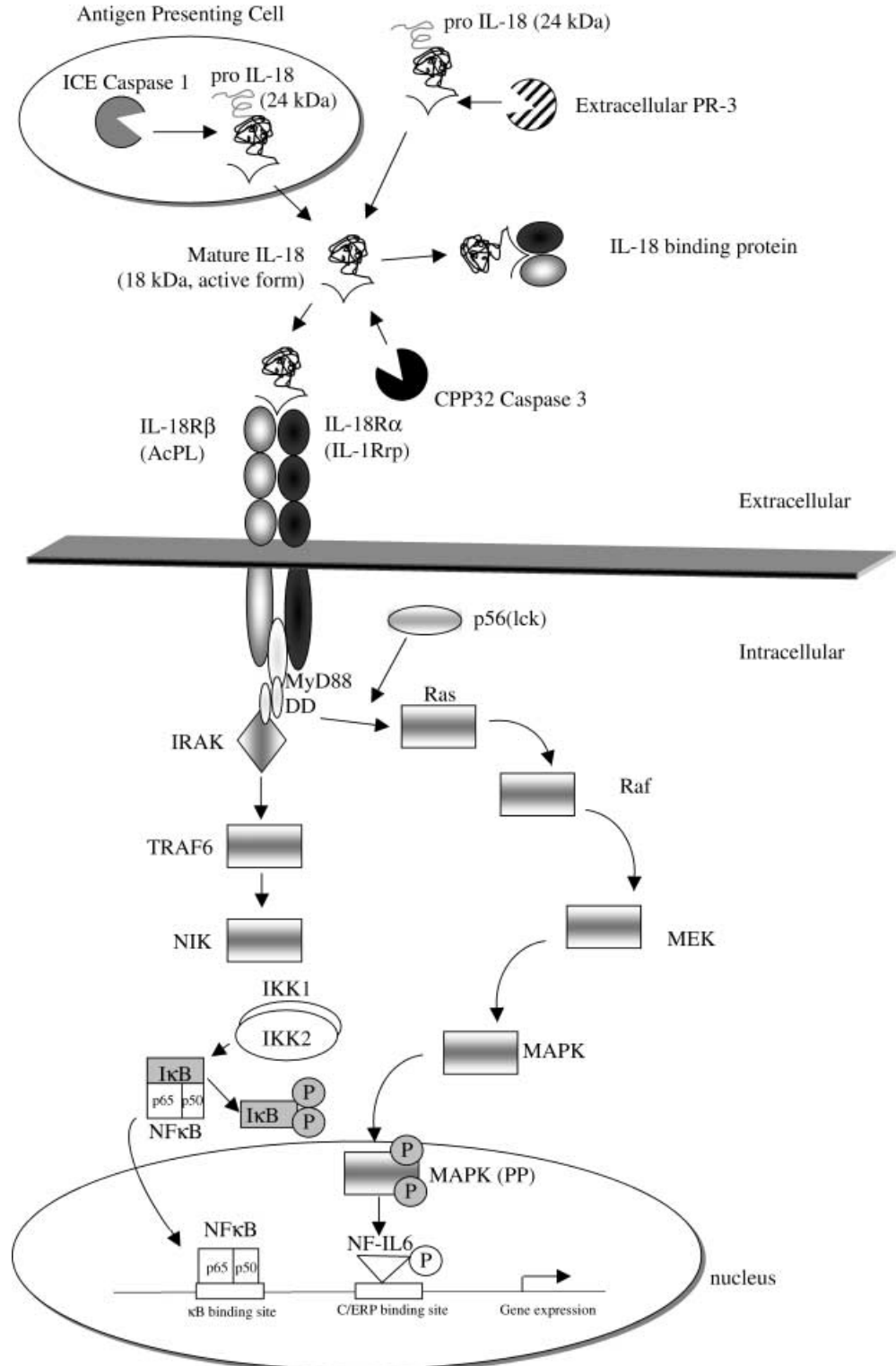
IL-18R: Interleukin 18 receptor · *IRAK*: Interleukin 1 receptor associated kinase · *LPS*: Lipopolysaccharide · *MAPK*: Mitogen-activated protein kinase · *MEK*: Mitogen-activated extracellular signal regulated kinase activating kinase · *NF*: Nuclear factor · *NIK*: NF- κ B inducing kinase · *NK*: Natural killer · *PBMC*: Peripheral blood mononuclear cell · *STAT*: Signal transducer and activator of transcription · *TIR*: Toll/interleukin 1 receptor · *TLR*: Toll-like receptor · *TNF*: Tumor necrosis factor · *TRAF6*: Tumor necrosis factor receptor associated factor 6

IL-18 and its relationship to the IL-1 family

Interleukin (IL) 18 is an IL-1 related, proinflammatory cytokine which plays a pivotal role in systemic and local inflammation. Initially designated as interferon (IFN) γ inducing factor, IL-18 was first isolated from the liver of mice primed with *Propionibacterium acnes* and chal-

lenged with lipopolysaccharide (LPS) [1]. However, IL-18 mRNA is produced in a wide range of cells, including T cells, B cells, macrophages, and Kupffer cells. The murine IL-18 gene encodes a precursor of 192 amino acids containing an unusual leader sequence of 35 amino acids. The precursor is cleaved into an active mature 18-kDa molecule by removal of this amino-terminal leader

Fig. 1 Pathways of IL-18 signal transduction. Activation of pro-IL-18 involves caspase-1 (also known as interleukin-1 β converting enzyme, ICE) and the extracellular 29-kDa serine esterase PR-3. Processed, active IL-18 mediates IL-18R aggregation, and the heterodimeric complex recruits the adaptor molecule MyD88 as well as IL-1 receptor associated kinase (IRAK). After phosphorylation IRAK dissociates from the receptor complex and associates with TNF receptor associated factor 6 (TRAF6), which then leads to the sequential activation of NF- κ B binding kinase (NIK), I- κ B kinases (IKK-1, IKK-2) and NF- κ B. Alternatively, the binding of IL-18 triggers the activation of Ras resulting in an activation cascade of Raf, mitogen-activated extracellular signal regulated kinase activating kinase (MEK), and mitogen-activated protein kinase (MAPK). This induces phosphorylation of MAPK. After phosphorylation MAPK is translocated into the nucleus and phosphorylates NF-IL6, which allows NF-IL6 to associate to the C/ERP binding site



sequence. Maturation requires the aspartate-specific protease caspase-1 or IL-1 β converting enzyme (ICE) that cleaves pro-IL-18 at Asp-35 [2, 3]. The activation of IL-18 by ICE demonstrates the first parallel to IL-1 β , which is also activated by ICE. Caspase-3 (CPP32), a protease shown to be involved in apoptosis, cleaves both the precursor and the mature form of IL-18 after Asp-71 and Asp-76, thus generating inactive degraded products. Therefore CPP32 may constitute a potential down-regulator of IL-18 [4]. In addition to caspases, the 29-kDa serine esterase PR-3, released by activated neutrophils, monocytes, and endothelial and mast cells, is also able to cleave pro-IL18 into the active form [5]. These observations suggest that pro-IL-18 can be processed both intracellularly and extracellularly (Fig. 1).

Cloning of the human IL-18 encoding cDNA has also been reported [6]. The human IL-18 gene is located on chromosome 11q22 [7]. Human pro-IL-18 contains 193 amino acids and shares 64% identity with its mouse homologue. Two isoforms of rat IL-18 [8] and porcine IL-18 [9] have also been identified recently (for a general review see [10, 11]).

Although the IL-1 family members IL-1 α , IL-1 β , and IL-1 receptor antagonist (IL-1Ra) share less than 30% primary amino acid sequence homology, they display structural similarities. Based on structural predictions, Bazan et al. [12] have suggested that murine IL-18 shares a 12 β sheet structure in common with that of the IL-1 family, prompting IL-18 to be termed IL-1 γ . It should be noted that the discovery and characterization of novel IL-1 related molecules have recently enlarged the IL-1-like family. Four members of the "family of IL-1" (FIL1 δ , FIL1 ϵ , FIL1 ζ , and FIL1 η) show significant similarity to IL-1 α , IL-1 β , IL receptor antagonist, and IL-18 [13]. The relationships between the different members of the IL-1 family suggest that IL-1 α , IL-1 β , and IL-18 form a single subfamily; FIL1 ϵ , FIL1 ζ , and FIL1 η form a second subfamily, and IL-1Ra and FIL1 δ a third [13]. Consistent with evolution by gene duplication, the new IL-1 superfamily members are clustered in the same region of human chromosome 2q that contains IL-1 α , IL-1 β , and IL receptor antagonist. IL-18 is the only member of this superfamily that does not map to this region. Although IL-1 α , IL-1 β , and IL-18 promote inflammation and enhance immune responses, the biological activities of the novel FILs remain to be characterized. Another IL-1 related cytokine, designated IL-1H, which is related mostly to IL-1Ra (36% of similarity), has also been described recently [14].

The IL-18 receptor and the TIR superfamily

The receptor for human IL-18 has been isolated from the Hodgkin's disease derived cell line L428 [15]. As with most cytokine receptors, the IL-18 receptor (IL-18R) is a heterodimer consisting of a constitutive ligand binding chain (the α chain), originally identified as IL-1 receptor related protein (IL-1Rrp), and an inducible accessory

chain (the β chain), designated accessory protein like (AcPL) [15, 16]. IL-1 receptor related protein binds IL-18 with low affinity, whereas AcPL does not bind IL-18 but increases the affinity of the receptor and participates in signal transduction [17]. IL-1 α , IL-1 β , and IL-1Ra are unable to bind to IL-18R [16]. However, it has recently been suggested that IL-1H binds to IL-18R but not to IL-1R, suggesting that IL-1H is another ligand for IL-18R and a new factor in the inflammatory and immune responses mediated by IL-18R [14]. The presence of IL-18R mRNA has been demonstrated in various organs including the spleen, thymus, liver, lung, heart, small and large intestine, prostate, placenta, skeletal muscle, kidney, pancreas, and brain [16, 18]. IL-18R is selectively expressed on Th1 but not on Th2 cells and can therefore be considered as a cell surface marker to distinguish Th1 from Th2 cells [16].

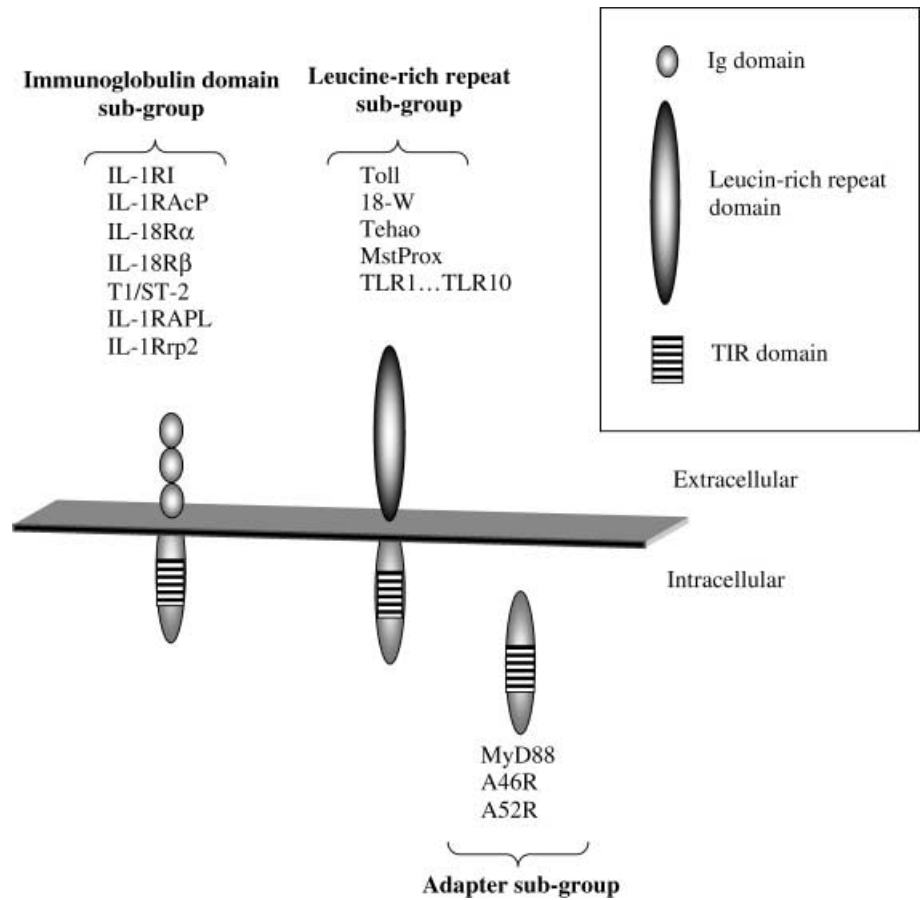
IL-18R shares the structural organization of the IL-1R/Toll-like receptor (TLR) superfamily. This family shares a conserved region termed a Toll/IL-1R (TIR) domain and appears to play a central role in inflammation and host defense against infection [19]. The TIR domain is a conserved sequence of around 200 amino acids and has three particular regions that are strongly conserved. This family can be divided into three groups based on similarities other than the TIR domain (Fig. 2).

Members of the first subgroup all contain three extracellular immunoglobulin like domains and are defined by the IL-1 type I receptor. This receptor can bind to both IL-1 α and IL-1 β as well as to IL-1Ra. This subgroup also includes the chains of IL-18R, IL-1 receptor related protein, and AcPL, as well as orphan receptors T1/ST-2, IL-1 receptor related protein 2, and IL-1 receptor accessory protein like. Interestingly, T1/ST2 and IL-18R present opposite effects in terms of T cell function since T1/ST2 plays a role in Th2 development [20], and IL-18 is a key regulator of Th1 cells. This is an unusual situation in that opposite immune responses are elicited by stimulating the same receptor family.

The *Drosophila melanogaster* Toll protein was the first member described belonging to the second subgroup of the TIR superfamily. Toll was demonstrated to play a role both in the development and in antifungal defense in the adult fly [21]. Apart from Toll, *D. melanogaster* also contains other TLRs, such as 18-Wheeler, required for antibacterial defenses [22]. Of the ten human TLRs that have been described, only two have a known function. TLR2 is involved in responses to microbial products from Gram-positive bacteria such as peptidoglycan and lipoteichoic acid [23], whereas TLR4 is required for responses to LPS [24].

The last subgroup comprises MyD88, and the two viral proteins A46R and A52R. All lack an extracellular domain and are involved in signaling rather than being a receptor. MyD88 is a crucial protein for signaling by IL-1 type I receptor, IL-18R, TLR2, and TLR4. Lack of MyD88 results in impaired responses to IL-1, IL-18, and LPS [25]. This cytoplasmic protein appears to function as the adaptor for the superfamily. It is thought to be re-

Fig. 2 The Toll/IL-1R (*TIR*) superfamily. The *TIR* superfamily is defined by a homologous *TIR* domain and can be subdivided into three main groups. Members of the first group contain immunoglobulin like domains and include IL-18R α and IL-18R β . The second group is defined by the presence of leucine-rich repeats and includes the Toll-like receptors (*TLRs*). The third group consists of nonreceptor cytosolic proteins that include MyD88 and two viral proteins, A46R and A52R



cruited to the receptor complex in response to IL-1 and connects the TIR domain to downstream signaling proteins via a homotypic interaction [26].

Recently a secreted IL-18 binding protein (IL-18BP) has been purified from human urine [27] and from sera of mice under endotoxin shock [28]. IL-18BP has been shown to bind IL-18 with high affinity. The most abundant form of IL-18BP bears a single immunoglobulin domain and belongs to a novel secreted protein family, distinct from both the IL-1 and IL-18 receptor families [27]. The binding of IL-18BP to IL-18 results in neutralization of the IL-18 effector functions, ultimately leading to the suppression of IFN- γ production [27]. This suggests that IL-18BP acts as a soluble decoy receptor for IL-18 to inhibit its biological functions [27]. Thus it may act as a natural anti-inflammatory and immunosuppressive molecule. Two isoforms of murine IL-18BP and three isoforms of human IL-18BP have been reported [29]. Interestingly, some poxviruses encode putative IL-18BPs that appear to participate in the inhibition of IFN- γ production [30]. Recent studies have introduced site-specific alterations into the human mature IL-18 polypeptide, which enhanced the biological activities of IL-18 but decreased neutralization by IL-18BP [31].

IL-18 signal transduction pathway

The downstream effectors of the IL-18 transduction pathway are closely related to those of IL-1 signaling [32]. The nuclear factor (NF) κ B activation cascade involves IL-18 mediated IL-18R α aggregation in association with the signal transducing subunit AcPL (Fig. 1). The activated IL-18R complex recruits the IL-1 receptor associated kinase (IRAK) via the adaptor protein MyD88 [33]. After phosphorylation IRAK dissociates from the N-terminus of MyD88 and interacts with tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6), which then relays the signal via NF- κ B binding kinase (NIK) to two I- κ B kinases (IKK-1 and IKK-2). This finally leads to the release of the NF- κ B subunits p65/p50 from I- κ B and its translocation into the nucleus to bind to κ B-binding sites [34, 35]. The generation of IRAK-deficient mice has confirmed the crucial role of this factor in IL-18 induced signaling and function in Th1 and natural killer (NK) cells [36]. Recently a Toll-interacting protein (Tollip) has been identified and shown to regulate the recruitment of IRAK to the receptor complex, allowing it to interact with MyD88 [37].

An alternative pathway involves activation of Ras with recruitment of nonreceptor protein tyrosine kinases p56 (lck), leading to the activation of mitogen-activated extracellular signal regulated kinase activating kinase

(MEK) through activation of Raf. MEK phosphorylates mitogen-activated protein kinase (MAPK), inducing its nuclear translocation (Fig. 1). Translocated MAPK phosphorylates NF-IL6 allowing its association with the C/ERP binding site [33, 34, 38, 39].

Biological properties of IL-18 and IFN- γ production

As mentioned above, IL-18 and IL-1 β are related to the same family in terms of structure, processing, receptor family, and signal transduction pathways. As with IL-1 β , IL-18 exerts proinflammatory properties (Table 1), but this cytokine is also related to IL-12, with regard to its capacity to induce the production of Th1 cytokines and to enhance cell-mediated immune cytotoxicity.

IL-18 acts in synergism with IL-12

IL-12 is a pivotal cytokine representing the link between the cellular and humoral branches of an effective host immune defense. It is a heterodimeric cytokine that is secreted mainly by antigen-presenting cells (APCs) and plays a key role in the induction of T cell dependent and independent activation of macrophages, generation of Th1 and cytotoxic T cells, suppression of IgG1 and IgE production, and resistance to bacterial and parasitic infections [40]. IL-12 is comprised of two disulfide-linked protein subunits designated IL-12p35 and IL-12p40. Both subunits must be produced within the same cell to obtain the biologically active dimer IL-12p70. The production of IL-12p40 exceeds the production of IL-12p35 by from 40-fold to more than 500-fold [41]. Some 5–40% of IL-12p40 is secreted as a homodimer IL-12p(40)₂, which has been shown to exert antagonistic activity on the IL-12 receptor both in vitro [42] and in vivo [43]. On the other hand, the IL-12p(40)₂ stimulates the differentiation of CD8⁺ T cells, indicating agonistic properties. The IL-12p35 subunit lacks any biological activity.

Naive T cells do not respond to IL-12 or IL-18 on anti-CD3 coated plates, whereas the combination of IL-12 and IL-18 induces them to proliferate and to produce IFN- γ in a synergistic manner [1, 44]. This synergistic effect has also been shown to be important for enhancement of NK cell cytotoxicity and Th1 cell differentiation. However, the two cytokines use distinct signaling pathways. IL-12 activates signal transducer and activator of transcription (STAT) 4 in Th1 cell, whereas IL-18 induces nuclear translocation of the p65/p50 NF- κ B complex through the serine-threonine kinase IRAK pathway [34] (Fig. 1). IL-18 alone does not trigger IFN- γ production by naive T cells because of their lack of IL-18R expression. IL-12 is required to induce IL-18R production on the surface of naive T cells [44, 45]. Reciprocally, IL-18 induces the expression of the IL-12 receptor [46]. The two cytokines may also synergize at the transcriptional level of the IFN- γ gene [47]. IL-18 activates the IFN- γ promoter via activator protein 1 and NF κ B-binding

Table 1 Comparison of the biological activities of IL-18 and IL-1 β

Properties	IL-18	IL-1 β
IRAK activation	+	+
NF- κ B translocation	+	+
Precursor form	+	+
Expression in NOD mice	+	+
Induction of IL-8/IL-1 β /TNF- α	+	+
Activation of Th1 responses	+	\pm
Activation of Th2 responses	-	-
Synergy with IL-12 for IFN- γ production	+	+
Protection against infection	+	+
Binding to IL-1R	-	+
Binding to IL-18R	+	-

sites, whereas IL-12 stimulates IFN- γ production by binding to the STAT4-binding site in the IFN- γ promoter [34]. The combination of the two cytokines activates activator protein 1 and STAT4 binding sites, as demonstrated in human CD4⁺ T cells [47, 48].

Immunomodulatory functions of IL-18

High levels of IL-18 were first described in activated macrophages and Kupffer cells [1]. However, other APCs such as dendritic cells [49], Langerhans cells [50], myelomonocytic cell lines [4], and B cell lines [4] also represent major sources of IL-18. Nonhematopoietic cells such as keratinocyte [50], astrocytes, microglial cells, osteoblasts, and intestinal epithelial cells produce IL-18 (Fig. 3). Many of the biological properties of this pleiotropic cytokine have been recently reviewed [10, 11, 51, 52, 53] and are summarized in Table 2 and Fig. 3.

IL-18 modulates the activity of Th1 cells, cytotoxic T lymphocytes, NK cells, macrophages, dendritic cells, and B cells. IL-18 also upregulates inducible nitric oxide synthase production and the production of adhesion molecules. It degrades cartilage by reducing chondrocyte proliferation, increases cyclo-oxygenase 2 expression, induces glycosaminoglycan release, and activates matrix metalloproteinases. It has also been demonstrated to inhibit osteoclasts via granulocyte/macrophage colony stimulating factor (GM-CSF) and to inhibit angiogenesis. IL-18 also shows chemoattractant properties on polymorphonuclear cells by stimulating IL-8 production. The production of chemokines involved in the recruitment of monocytes/macrophages, macrophage inflammatory protein-1 α , and monocyte chemoattractant protein 1 is also induced in IL-18 activated peripheral blood mononuclear cells (PBMC) [54].

Of course, the best-described function of IL-18 consists in promoting expression of cytokines such as IFN- γ , TNF- α , and GM-CSF. In mice deficient for IL-18 production [55] or for ICE [3, 5] little or no IFN- γ is produced. These in vivo observations confirm the role of IL-18 initially described as a dominant IFN- γ inducing factor, a feature shared with the heterodimeric cytokine IL-12.

Fig. 3 Cellular sources and the targets of IL-18. Both APCs and nonhematopoietic cells have been reported to produce IL-18. IL-18 and IL-12 synergize to induce the differentiation of CD4⁺ Th0 cells to enhance the cytotoxic response of CD8⁺ T cells, NK, and NK-T cells, and to induce IFN- γ production by T cells, NK cells, and macrophages. The inflammatory response is mainly mediated by activated macrophages that produce inflammatory cytokines, nitric oxide (NO), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 α (MIP-1 α), and prostaglandin (PG) molecules. IL-18 has a potential to induce Th2 cells in an IL-4 dependent manner. In response to stimulation of IL-18 and IL-3 mast cells and basophils, which are major inducers and effectors of allergic inflammation, produce large amounts of IL-4 and IL-13. Administration of a mixture of IL-12 and IL-18 has a potential to inhibit IgE production

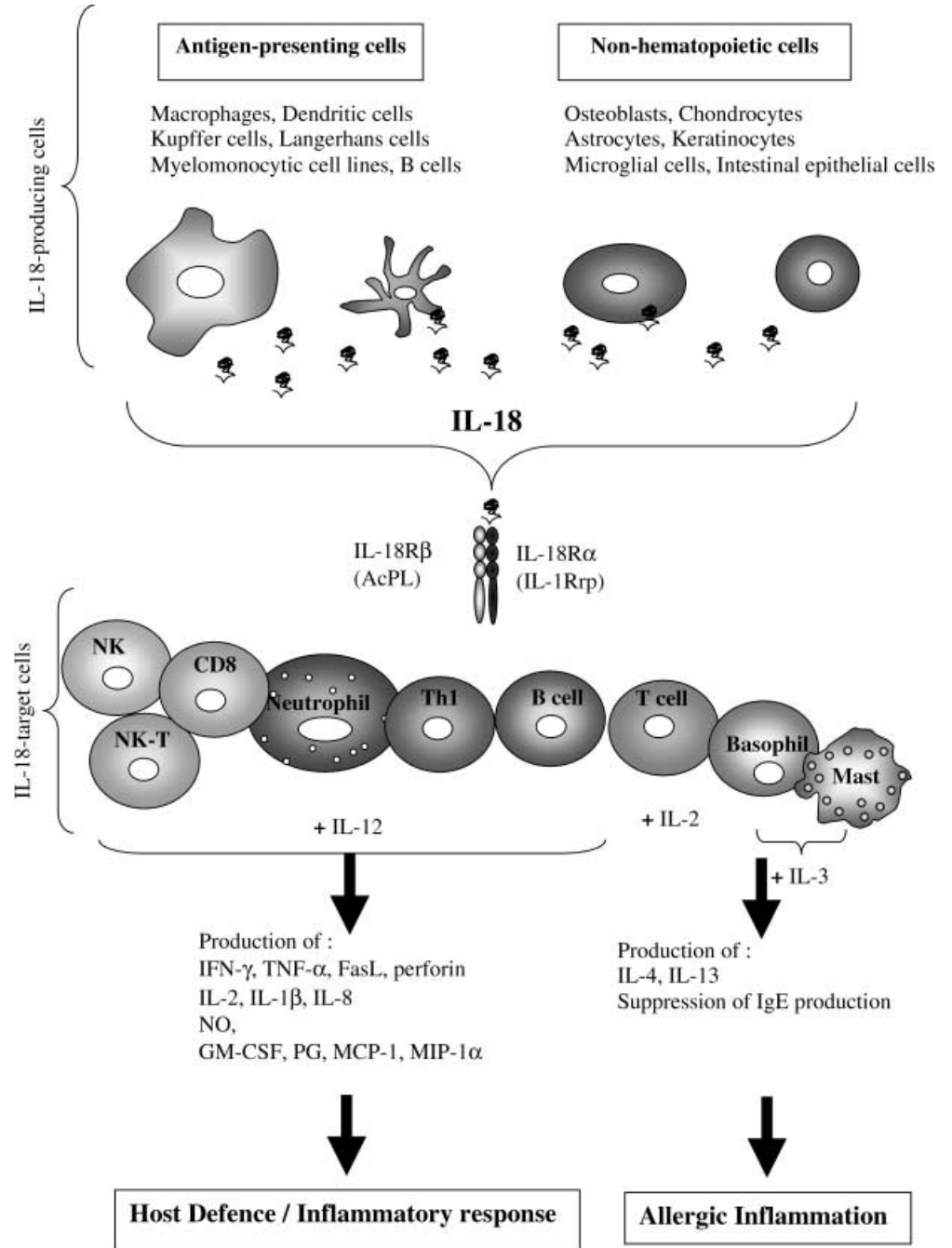


Table 2 Biological functions of IL-18

Biological functions	Cellular effectors	References
IFN γ production	T lymphocytes or NK cells	[77, 128]
Th1 activation	CD4 ⁺ T lymphocytes	[27, 129]
Inhibition of IgE production	Activated B cells	[130]
Cytotoxic response	Cytotoxic T cells, NK cells, NK-T cells	[55, 131, 132]
Expression of Fas ligand	NK cells, cytotoxic T cells	[133]
Proinflammatory activity	Macrophages, monocytic cell lines, osteoclasts	[59, 66]
Response in autoimmune and inflammatory diseases	Th1 and cytotoxic T cells, macrophages dendritic cells, B cells, chondrocytes	[49, 134]
Response in infectious diseases	Th1 cells	[65, 87, 97, 114, 135, 136]
Response in tumors	NK cells, cytotoxic T cells, macrophages	[56]
HIV-1 expression	Macrophages	[137]

Since IL-18 plays a crucial role in immunomodulation by acting on cell-mediated cytotoxicity, Th1 differentiation, and inflammation, antitumor actions of IL-18 have been investigated in vivo [56, 57]. IL-18 mediated anti-tumor action is T cell dependent and relies on upregulation of FasL-mediated tumoricidal activity [58]. Recent work also suggests that IL-18 is involved in the innate defense against tumors and invading micro-organisms [59]. In autoimmune diseases such as insulin-dependent diabetes mellitus exogenous IL-18 inhibits diabetes development in nonobese diabetic mice [60]. In contrast, IL-18 is involved in the development of multiple sclerosis and rheumatoid arthritis. In chronic inflammation such as Crohn's disease IL-18 also appears to promote the development of the inflammation [61, 62]. IL-18 plays distinct roles in atopic asthma, depending on its immunological environment and the route of administration [63, 64]. Biological properties and role of IL-18 in modulation of tumors and autoimmune and inflammatory disease have been reviewed previously [52, 65, 66].

Role of IL-18 induced by bacterial infections

The proinflammatory cytokines TNF- α , IL-1 β , and IL-6 as well as IFN- γ are among the first cytokines produced in response to infection by pathogenic bacteria [67]. Cytokines produced later during infection direct the immune responses toward either Th1 or Th2 immunity [68, 69]. Development of naive T cells into Th1 cells has been shown to be driven by IL-12, while Th2 maturation depends on IL-4 [70]. Activated Th1 cells participate in cell-mediated immunity against intracellular pathogens and produce IFN- γ , IL-2, TNF- α , TNF- β and GM-CSF. In contrast, activated Th2 cells are involved in humoral and allergic immune responses and produce mainly IL-4, IL-5, IL-6, IL-10, and IL-13 [65, 71]. It has also been shown that IFN- γ acts synergistically with IL-12 to mediate optimal development of the Th1 phenotype [72].

Although exposure to IL-18 enhances host defenses that may participate in resistance to parasitic, fungal, and bacterial pathogens (Table 3), an excess of IL-18 production may also be detrimental by inducing injury in the host [65].

Extracellular bacteria

Streptococcus pyogenes, an important human pathogen that causes a wide range of diseases, induces IL-12, IL-18, and IFN- γ secretion by human PBMC [73]. The kinetics of IL-18 production are fast and resemble those of proinflammatory cytokines, reflecting the suggested proinflammatory nature assigned to IL-18 [74]. The production of IL-4 and IL-10 has been found to be limited, demonstrating that this bacterium elicits a Th1 type immune response. However, a direct role of IL-18 in protection against *S. pyogenes* infection remains to be elucidated.

Table 3 IL-18 inducing infectious agents

Infectious organism	Host system	Reference
Parasite		
<i>Leishmania major</i>	Mouse	[81, 138]
<i>Trypanosoma cruzi</i>	Mouse	[139]
<i>Shistosoma mansoni</i>	Mouse	[126]
<i>Plasmodium berghei</i>	Mouse	[65]
<i>Toxoplasma gondii</i>	Mouse	[140, 141]
Fungus		
<i>Cryptococcus neoformans</i>	Mouse	[136, 142]
Bacteria		
Extracellular		
<i>Propionibacterium acnes</i>	Mouse	[77, 79]
<i>Yersinia enterocolitica</i>	Mouse	[87]
<i>Streptococcus pyogenes</i>	Mouse	[73]
<i>Staphylococcus aureus</i>	Mouse	[81]
Intracellular		
<i>Burkholderia pseudomallei</i>	Human	[92, 143]
	Mouse	[91]
<i>Salmonella typhimurium</i>	Mouse	[97, 98]
<i>Salmonella dublin</i>	Mouse	[99]
<i>Salmonella cholerae suis</i>	Porcine	[100]
<i>Chlamydia trachomatis</i>	Human epithelial cells	[94]
	Mouse	[135]
<i>Listeria monocytogenes</i>	Mouse	[103]
<i>Mycobacterium leprae</i>	Mouse	[117]
	Human	[118]
<i>Mycobacterium avium</i>	Mouse	[117]
<i>Mycobacterium tuberculosis</i>	Mouse	[114]
	Human	[115, 144]
<i>Mycobacterium bovis BCG</i>	Mouse	[53, 55]
<i>Shigella flexneri</i>	Mouse	[102]
<i>Legionella pneumophila</i>	Mouse	[96]

The presence of IL-18 has also been detected in patients with bacterial meningitis [75]. The causative organisms in these patients were *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Escherichia coli*, *Hemophilus influenzae*, *Corynebacteria*, *Pseudomonas aeruginosa*, or *Morganella morganii*. IL-18 was detected in the cerebrospinal fluid in only 16% of the healthy controls and was detectable in 94% of the patients. However, serum concentrations of IL-18 were not significantly increased in patients with bacterial meningitis. IL-18 has been detected in cultures of murine astrocytes and microglia [76] and IL-18, IL-18R, AcPL and IL-18BP mRNA expression was found in the brain of normal adults [18]. This suggests that IL-18 is a mediator of inflammatory responses within the brain, similar to its reported role in the periphery where it induces release of IFN- γ and activates immune cells. Whether IL-18 participates in clearance of invading pathogens responsible for bacterial meningitis remains uncertain. However, overwhelming IL-18 release may, in addition to its possible beneficial actions, also enhance injury of host tissue.

Mice are relatively resistant to LPS, but pretreatment with *P. acnes* sensitizes them to LPS-induced shock that leads to acute liver injury due to overproduction of IL-18

[1, 77]. Overexpression of IL-18 in the liver of mice is followed by induction of IFN- γ , TNF- α , and FasL [78, 79]. Abrogation of IL-18 production by in vivo administration of neutralizing anti-IL-18 antibodies or using IL-18 deficient mice not only down-regulated IFN- γ and TNF- α production but also prevented complete FasL-mediated liver injury [55, 77, 80].

Along the same lines, IL-18^{-/-} mice infected with *S. aureus* developed markedly fewer septicemia than similarly infected wild-type mice [81]. The reduced septicemia may be attributed to a decreased TNF- α production in the IL-18^{-/-} mice because TNF- α has been shown to have a direct effector role in the pathogenesis of Gram-positive bacterial shock [82]. The observation that the knockout mice produce less TNF- α is also consistent with the fact that IL-18 up-regulates TNF- α synthesis by human PBMC [74].

However, IL-18^{-/-} mice infected with *S. aureus* developed significantly more severe septic arthritis than the control mice. This can be explained by the fact that the mutant mice developed an impaired Th1 response, which has frequently been associated with septic arthritis [83] and increased IL-4 production. Indeed, IL-4 and IL-4 dependent Th2 responses are known to promote *S. aureus* induced septic arthritis [84]. IL-18 appears thus, on one hand, to protect from *S. aureus* induced septic arthritis and, on the other, to promote septicemia.

Yersinia enterocolitica is an extracellular, rod-shaped pathogen that causes enteritis and enterocolitis in humans and rodents. Systemic infection including abscesses and granulomatous lesions can occur in spleen and liver, particularly in immunocompromised individuals [85]. IL-12 induced IFN- γ production is essential for clearance of *Y. enterocolitica* infection in mice [86]. Although predominantly IL-12 dependent, the production of *Yersinia*-specific IFN- γ also partially depends on IL-18, as evidenced by the assessment of IFN- γ production in the supernatants of spleen cells from *Yersinia*-infected C57BL/6 mice stimulated with heat-killed *Yersinia* in the presence of anti-IL-12 or anti-IL-18 antibodies [87]. Modulation of yersiniosis by IL-18 was demonstrated by in vivo administration of anti-IL-18 antibodies before *Y. enterocolitica* infection. Neutralization of IL-18 antibodies caused a 100- to 1000-fold increase in bacterial counts in the spleen of infected mice. These results suggest that IL-18 is involved in clearance of *Yersinia* infection. However, no significant difference in IFN- γ production was observed between anti-IL-18 treated C57BL/6 mice and control mice, suggesting that IL-18 mediates resistance through an IFN- γ independent mechanism [87]. In vivo treatment with recombinant IL-18 did not improve clearance of *Y. enterocolitica* in mice.

Intracellular bacteria

Melioidosis is an important cause of illness and death in Southeast Asia and is due to infection by the Gram-negative bacillus *Burkholderia* (formerly *Pseudomonas*)

pseudomallei. The clinical features of melioidosis vary widely and range from acute sepsis to chronic infections [88]. *B. pseudomallei* is a facultative intracellular pathogen that survives and replicates in human phagocytic and nonphagocytic cells in vitro [89]. In a murine model of melioidosis IFN- γ has been shown to play an essential role in the host defense [90]. NK cells and CD8⁺ cells are the dominant sources of IFN- γ in mice infected with *B. pseudomallei* and IFN- γ production by both cell populations was inhibited by coinubation with neutralizing monoclonal antibodies to IL-12 or IL-18 [91]. Furthermore, supernatants from spleen cells stimulated with *B. pseudomallei* contained high levels of IL-18 induced in a dose-dependent manner by the micro-organism, which were correlated with the induction of IFN- γ . Markedly elevated plasma levels of IFN- γ , IL-18, IL-12p40, and IL-15 have been found in patients with melioidosis [92]. IL-18 concentrations were especially high and showed the strongest correlation with IFN- γ levels. Neutralization of IL-12 strongly inhibited IFN- γ production during whole-blood stimulation with heat-killed *B. pseudomallei*, which was further inhibited by the addition of neutralizing anti-IL-18 antibodies [92]. This suggests that during melioidosis IL-18 is an important IFN- γ inducing cytokine in the presence of other costimulatory signals, especially IL-12.

The obligate intracellular bacterium *Chlamydia trachomatis* is the cause of major genital and ocular infections in humans throughout the world. The development of Th1-mediated immune responses is necessary for protective immunity against *Chlamydia* [93], suggesting that IL-18 plays a role in protection. The host mucosa represent the initial sites of *Chlamydia* infection. Therefore the ability of *Chlamydia* to induce the secretion of IL-18 by epithelial cells has been investigated [94]. Human epithelial cell lines constitutively produce pro-IL-18, but the secretion of mature IL-18 is enhanced after *Chlamydia* infection. IL-18 release could be prevented by inhibitors of chlamydial but not of host cell protein synthesis, and depends on the activation of caspase-1, indicating a role for *Chlamydia*-derived product(s) in the activation of caspase-1 and subsequent secretion of active IL-18.

The causative agent of Legionnaire's disease, *Legionella pneumophila*, is an intracellular pathogen of phagocytic cells, primarily of alveolar macrophages. Cytokines participating in cellular immunity, especially IFN- γ , are responsible for resistance to primary *L. pneumophila* lung infection [95]. IFN- γ limits the iron concentration in phagocytic cells, thus creating an intracellular environment that is nonpermissive for *L. pneumophila* replication. Intratracheal inoculation of mice with virulent *L. pneumophila* induced the production of IL-18 in bronchoalveolar lavage fluids [96]. However, blocking intrapulmonary IL-18 activity with the anti-IL-18R antibodies did not alter the resolution of *L. pneumophila* lung infection, although it resulted in the inhibition of intrapulmonary IFN- γ production. Surprisingly, blocking endogenous systemic IL-12 with anti-IL-12 antibodies, which

also inhibits intrapulmonary IFN- γ production, resulted in enhanced intrapulmonary growth of the bacteria within 5 days after infection [96].

The role of IL-18 in host resistance to virulent *Salmonella* in the mouse typhoid model has been investigated by several groups [97, 98, 99]. When mice were treated in vivo with anti-IL-18 antibodies, resistance to infection was impaired, and the bacterial loads in the liver and spleen were increased [97], indicating that IL-18 is required to control bacterial growth in mice infected with virulent *S. typhimurium*. Administration of recombinant IL-18 protected mice from challenge with a lethal dose of virulent *Salmonella*, which is accompanied by an important reduction in bacterial numbers in the tissues. This effect was not observed in IFN- γ receptor^{-/-} mice, suggesting that IFN- γ is required for the protective effects of recombinant IL-18 administration during salmonellosis. Elhofy and Bost [99] found that *S. dublin* reduces the production of IL-18 mRNA and the secretion of IL-18 by murine macrophages. Mice fed orally with *S. dublin* displayed a reduced IL-18 mRNA production at mucosal sites within hours after inoculation. *Salmonella* has thus evolved mechanisms to limit the host response, especially an optimal IFN- γ mediated Th1 response. On the other hand, in vivo or ex vivo infection of intestinal porcine mucosa with *S. cholerae suis* resulted in activation of IL-18, as evidenced by a decrease in size of IL-18, consistent with the cleavage of pro-IL-18 into the mature form by caspase-1 [100].

The etiological agent of bacillary dysentery, *Shigella flexneri*, has been shown to induce macrophage apoptosis, through the activation of caspase-1 [101]. Although *casp-1*^{-/-} mice infected with *S. flexneri* do not develop early acute inflammation [102], intense inflammation develops much later in these mice and is more severe than the acute inflammation in wild-type mice. *Casp-1*^{-/-} mice are unable to control the infection, and the inflammation continues to worsen after an extended period of time. When recombinant IL-18 is administered to *casp-1*^{-/-} mice inflammation decreases, and the presence of the bacteria is restricted to a degree similar to that observed in wild-type mice. Therefore recombinant IL-18 reconstitutes a wild-type inflammatory response in *casp-1*^{-/-} mice. Consistent with this observation, IL-18^{-/-} mice infected with *S. flexneri* also cannot regulate the inflammation, nor control *Shigella* infection. This indicates that IL-18 is required to mount an inflammatory response capable of efficiently eliminating *Shigella* infection.

A critical role for IL-18 has also recently been described in *Listeria monocytogenes* infected mice. The findings of Neighbors et al. [103] established that IL-18 plays an important role in cell-mediated immunity via its effects on both the innate and adaptive immune responses. IL-18 appeared to be even more potent against this pathogen than either IL-12 or IFN- γ . Because infection was greatly exacerbated by anti-IL18R monoclonal antibody even in IFN- γ ^{-/-} mice, and because administration of recombinant IL-18 enhanced bacterial clearance in the complete absence of IFN- γ , it was concluded that IL-18

was dominant over IFN- γ in controlling bacterial load after infection. The authors also demonstrated that IL-18 is comparable to TNF- α in its capacity to resolve the infection, and that IL-18 mediates protection to a large extent through TNF- α action in vivo. Moreover, IL-18 was required for the macrophage's subsequent release of nitric oxide in response to a *Listeria* infection in vivo, and since NO production is a critical mediator of the macrophage's listericidal effects [104], IL-18 dependent NO production may be a major mechanism of bacterial clearance. Another interesting issue arising from this study is that for the first time it was shown that in addition to its protective role in primary immunity to *Listeria*, IL-18 is as important in memory effector responses to reinfection with this bacteria. Therefore IL-18 provided significant protection in both primary and secondary responses to this intracellular pathogen.

Mycobacterial infections

Tuberculosis, a granulomatous disorder caused by *Mycobacterium tuberculosis* remains the leading cause of death worldwide. Successful elimination of *M. tuberculosis* from the host depends mainly on the efficient interaction between antigen-specific T cells and infected macrophages (Fig. 4). Patients with defective T cell function, such as those with acquired immunodeficiency syndrome [105, 106] and those undergoing immunosuppressive therapy [107], present an increased risk of developing tuberculosis. In contrast, no increased predisposition to tuberculosis has been reported in persons with impaired humoral immunity, indicating a crucial contribution of T cell mediated immunity against tuberculosis.

Critical role of IFN- γ and IL-12 in mycobacterial infections

CD4⁺ T cells are thought to contribute to protection against mycobacteria by antigen-specific production of cytokines, which activate infected macrophages to kill the intracellular bacteria. Among these cytokines, IFN- γ is the crucial effector molecule in mice [108, 109] as well as in humans [110, 111]. In mice IFN- γ mediates its protective effect predominantly by the induction of reactive nitrogen intermediates, which are necessary for the killing of the intracellular mycobacteria (Fig. 4). However, the contribution of reactive nitrogen intermediates to immunity against mycobacteria in humans still remains a matter of debate. Mice lacking IFN- γ present a markedly increased susceptibility to infection with *M. tuberculosis* [108, 109]. Humans with a defective IFN- γ receptor develop severe mycobacterial disease [110, 111]. Persons lacking either the α - or β -chain of the IFN- γ receptor were discovered because they died either from disseminated bacille Calmette-Guérin (BCG) infection or from infection with *M. avium* or *M. fortuitum*.

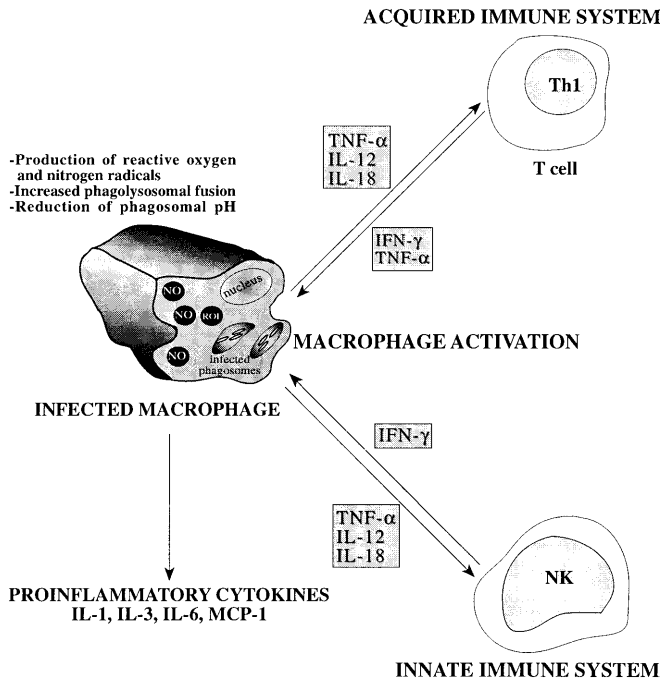


Fig. 4 Basic pathway of immune responses to mycobacterial infections. Infected macrophages become activated, leading to the release of TNF- α , IL-12, and IL-18 that are required to activate NK cells and to establish a Th1 response early in the infectious process. In response to these cytokines Th1 cells produce TNF- α and IFN- γ , which activate macrophages to kill infected mycobacteria by various mechanisms, including the production of reactive oxygen and nitrogen radicals. Phagocytosis of *M. tuberculosis* also induces the release of the proinflammatory cytokines IL-1, IL-3, and IL-6 and the chemokine monocyte chemoattractant protein (*MCP-1*)

IL-12 also plays a critical role in immunity to mycobacteria through its role of IL-12 is to increase the production of IFN- γ . Mice and humans lacking the p40 chain of the IL-12 heterodimer or the IL-12-receptor subunits secrete less IFN- γ than normal individuals and are susceptible to mycobacterial infection [112, 113]. IFN- γ has been successfully used to treat patients lacking IL-12 when administered in combination with antitubercular drugs [113], confirming that the major role of IL-12 is to enhance IFN- γ production and to promote a Th1 immune response.

Role of IL-18 in tuberculosis

IL-18 deficient mice display a phenotype similar to that of IL-12 deficient mice concerning to the reduction in NK cell activity, Th1 response, and IFN- γ secretion [55]. IL-12 and IL-18 double-deficient mice exhibited an even more severe defect in NK cell activity and Th1 response than that seen in single-knockout mice [55]. When infected by aerosol with *M. tuberculosis*, higher bacterial loads were found in the lung tissues of IL-18 deficient mice than in those of control mice [114]. When *M. tuberculosis* was administered intravenously, larger gran-

ulomas were found in the lungs and spleens of IL-18 deficient mice than in those of their wild-type littermates, and numerous bacteria were present in the granulomatous lesions. When exogenous recombinant IL-18 was administered, the sizes of the granulomas were significantly reduced, and virtually no bacteria were detected in the lesions, indicating that this cytokine plays an important role in immunity against *M. tuberculosis*. IL-18 deficient mice showed lower levels of splenic IFN- γ than did wild-type mice, although their IL-12 levels were normal, demonstrating that IL-18 is crucial for the generation of protective immunity to mycobacteria by inducing IFN- γ production. However, the production of NO by peritoneal macrophages was similar in IL-18 deficient and wild-type mice. Vankayalapati et al. [115] found that *M. tuberculosis* stimulated PBMC from tuberculosis patients secrete less IL-18 and IFN- γ than do those from healthy tuberculin reactors. Furthermore, the *M. tuberculosis* induced IFN- γ production was inhibited by anti-IL-18, but enhanced by the addition of recombinant IL-18. Surprisingly, alveolar macrophages secreted IL-18 in response to *M. tuberculosis*, suggesting that IL-18 is produced in the lung during tuberculosis infection. Finally, both IL-18 and IFN- γ concentrations were higher in pleural fluid of tuberculosis patients than in pleural fluid of patients with nontuberculous diseases.

Other mycobacterial infections

BALB/c mice that are genetically susceptible to *M. avium* exhibit less expression of IFN- γ and the IFN- γ -inducing cytokines IL-12 and IL-18 than do resistant mice [116]. Similarly, early expression of IL-12p40 and IL-18 was found in mice resistant to *M. leprae* 3–72 h after infection, but not in susceptible mice [117]. The deficient expression of IL-12 and IL-18 may result in a diminished level of cell-mediated immune responses against the infection and therefore to uncontrolled replication of *M. leprae* in susceptible hosts. In human leprosy patients Th1 responses are generally associated with resistance to *M. leprae* infection (tuberculoid type), whereas Th2 responses are associated with progressive disease (lepromatous type). Recently Garcia et al. [118] have shown that in vivo IL-18 mRNA levels are higher in lesions of tuberculoid patients than in those of lepromatous patients. When PBMC from tuberculoid patients were stimulated with *M. leprae* in the presence of neutralizing anti-IL-18 antibodies, IFN- γ levels induced in response to the antigens were inhibited by 80–99%, demonstrating that endogenous IL-18 contributes to *M. leprae* induced IFN- γ secretion. The addition of exogenous recombinant IL-18 increased *M. leprae* induced IFN- γ in tuberculoid patients but not in lepromatous patients. In contrast, the addition of IL-18 had no effect on IL-4 production either from tuberculoid or from lepromatous patients. When IL-12 and IL-18 were added together to PBMC from tuberculoid patients cultured in the presence of *M. leprae*, a significant increase in the IFN- γ production was ob-

served. IFN- γ secretion was rapidly induced by NK and T cells after 1 and 5 days of stimulation, respectively. In addition, IL-18 directly induced IFN- γ production from mycobacteria-reactive T cell clones. These observations suggest that IL-18 induces type 1 cytokine responses against *M. leprae* in tuberculoid leprosy patients to promote cell-mediated immune responses allowing them to control *M. leprae*. In contrast, the absence of type 1 cytokine responses in lepromatous leprosy patients results in weak cell-mediated immunity to the pathogen and therefore in progressive infection.

Therapeutic aspects of IL-18 against infection

Protection against pathogenic micro-organisms is mediated by both innate and acquired immunity. Phagocytic cells and NK cells are constituents of the innate immune system, whereas B and T cells are required for acquired immunity. Opsonization of bacteria coated with specific antibodies and activation of the complement represent the main protective immune mechanisms against extracellular bacteria. The defense against intracellular micro-organisms is mediated principally by phagocytic cells, and acquired immunity that amplifies innate immunity is required to kill these organisms. This can be achieved by activation of macrophages with IFN- γ and subsequent killing of the phagocytosed micro-organisms or by lysis of infected cells by cytotoxic CD8⁺ T cells. Therefore effective immune therapy against intracellular bacteria, including mycobacteria, might be enhanced by supplying IFN- γ inducers such as IL-12 and IL-18. It has been shown that combined administration of recombinant IL-12 and IL-18 significantly prolongs the survival time of *Cryptococcus neoformans* infected mice and reduces the lung and brain loads [119]. With the emergence of multidrug-resistant strains of *M. tuberculosis*, there is increased need to develop potential immunotherapies against mycobacterial infections. Several cytokine therapies have been tried with varying success [120]. IFN- γ has been tested in patients with multidrug-resistant tuberculosis with promising results [121]. However, because of its toxicity IFN- γ treatment is difficult to implement. So far there has been no attempt to treat tuberculosis patients with IL-12, although this cytokine has been tested in clinical trials in cancer patients and found to be reasonably well tolerated [122]. IL-2 is also well tolerated and appears to give rise to significant clinical improvement in multidrug-resistant tuberculosis patients, including human immunodeficiency virus positive patients [123]. We have recently developed a plasmid encoding IL-18 in order to evaluate the immunomodulating effect of IL-18 in mice after intradermal administration and mycobacterial infection [124]. Mice treated with the plasmid encoding IL-18 and subsequently infected with BCG produced lower levels of anti-BCG antibodies than did control animals. In contrast, these mice produced greater amounts of antigen-specific IFN- γ after in vitro restimulation than did controls. When coadministered to-

gether with a human immunodeficiency virus 1 Nef-encoding plasmid, the plasmid encoding IL-18 was found to modulate the specific immune response towards a Th1 type [125]. These findings suggest that the administration of IL-18 encoding DNA may be useful for the development of new immunotherapeutic or immunoprotective approaches against infections with intracellular parasites, such as mycobacteria, and viruses.

The immunostimulatory effect of IL-18 encoding plasmid DNA has also been shown to be efficient in vaccination against *Schistosoma mansoni* [126]. Percutaneous infection of mice with *S. mansoni* strongly induced the production of IL-18 mRNA in the skin. Intradermal coinjection of the IL-18 encoding plasmid with a *S. mansoni* glutathione S-transferase (Sm28GST) encoding plasmid generated a 30-fold increase in antigen-specific IFN- γ by spleen cells in comparison to splenocytes from mice that had received only the Sm28GST-encoding plasmid. In addition, the immunostimulatory effect was related to significant protection against schistosomiasis attributed to cooperativity between the two plasmids [126]. If safety issues can be satisfactorily resolved, the use of DNA encoding IL-18 presents the advantage of a more sustained presence of the cytokine at the site of injection than with the administration of the purified protein. This would also circumvent the short half-life of recombinant IL-18 and the side effects due to the administration of repetitive high doses of the cytokine.

As an alternative approach, recombinant BCG producing IL-18 has been constructed (BCG/IL-18; Biet et al. submitted). This strain was found strongly to augment the long-term production of IFN- γ . Mycobacterial antigen-specific secretion of GM-CSF was also greater in mice infected with BCG/IL-18 in those infected with nonrecombinant BCG. In contrast, a down-regulation of Th2-type cytokines, such as IL-5 and IL-13, was observed in splenocytes from mice immunized with BCG/IL-18 compared to mice immunized with nonrecombinant BCG, as judged by reverse transcriptase polymerase chain reaction. In contrast, the production of antibodies to BCG antigens was decreased in both mycobacterial-susceptible and mycobacterial-resistant mouse strains. These results indicate that the production of IL-18 by BCG enhances the ability of BCG to polarize the immune response into the Th1 direction. The ability of IL-18 produced by recombinant BCG to strengthen the natural capacity of BCG to induce Th1-type immune responses may have interesting therapeutic or immunoprophylactic potential. Whether it may have a positive impact on vaccination strategies, especially against intracellular pathogens such as *M. tuberculosis* is currently under investigation. IL-18 may also increase the therapeutic effect of BCG in the treatment of bladder cancer. Given that IL-18 displays antitumor activity in vivo [56, 127], production of IL-18 by a recombinant BCG strain may be particularly valuable to treat bladder cancer patients.

In addition, due to its capacity to down-regulate the Th2 immune response, recombinant BCG producing IL-

18 may perhaps also be valuable in the treatment of atopic disorders caused by the exacerbation of the Th2 response.

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