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REVIEW

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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-1β, 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 1β converting enzyme · *IFN:* Interferon · *IKK:* I-κB kinase · *IL:* Interleukin · *IL-18BP:* Interleukin 18 binding protein · *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-κB 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

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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 challenged 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

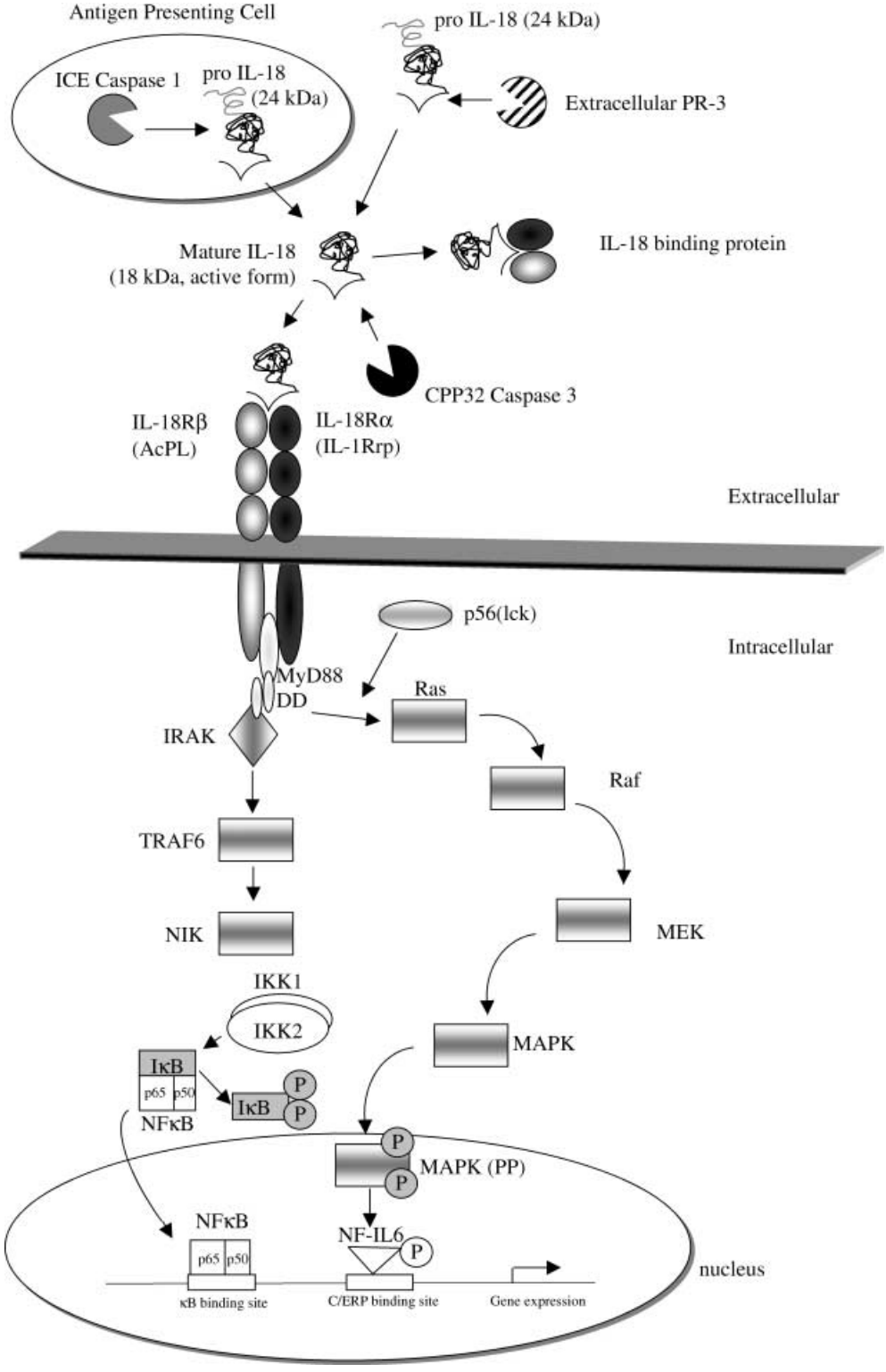

Fig. 1 Pathways of IL-18 signal transduction. Activation of pro-IL-18 involves caspase-1 (also known as interleukin-1β converting enzyme, ICE) and the extracellular 29-kDa serine esterase PR-3. Processed, active IL-18 mediates IL-18R aggregation, and the heterodimeric complex recruits the adaptor molecule 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-κB binding kinase (*NIK*), I-κB kinases (*IKK-1*, *IKK-2*) and NF-κB. 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-1β 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-1β, 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-1α, IL-1β, 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 12β sheet structure in common with that of the IL-1 family, prompting IL-18 to be termed IL-1γ. 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" (FIL1δ, FIL1ε, FIL1ζ, and FIL1η) show significant similarity to IL-1α, IL-1β, IL receptor antagonist, and IL-18 [13]. The relationships between the different members of the IL-1 family suggest that IL-1α, IL-1β, and IL-18 form a single subfamily; FIL1ε, FIL1ζ, and FIL1η form a second subfamily, and IL-1Ra and FIL1δ 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-1α, IL-1β, and IL receptor antagonist. IL-18 is the only member of this superfamily that does not map to this region. Although IL-1α, IL-1β, 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-1α, IL-1β, 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-1α and IL-1β 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-

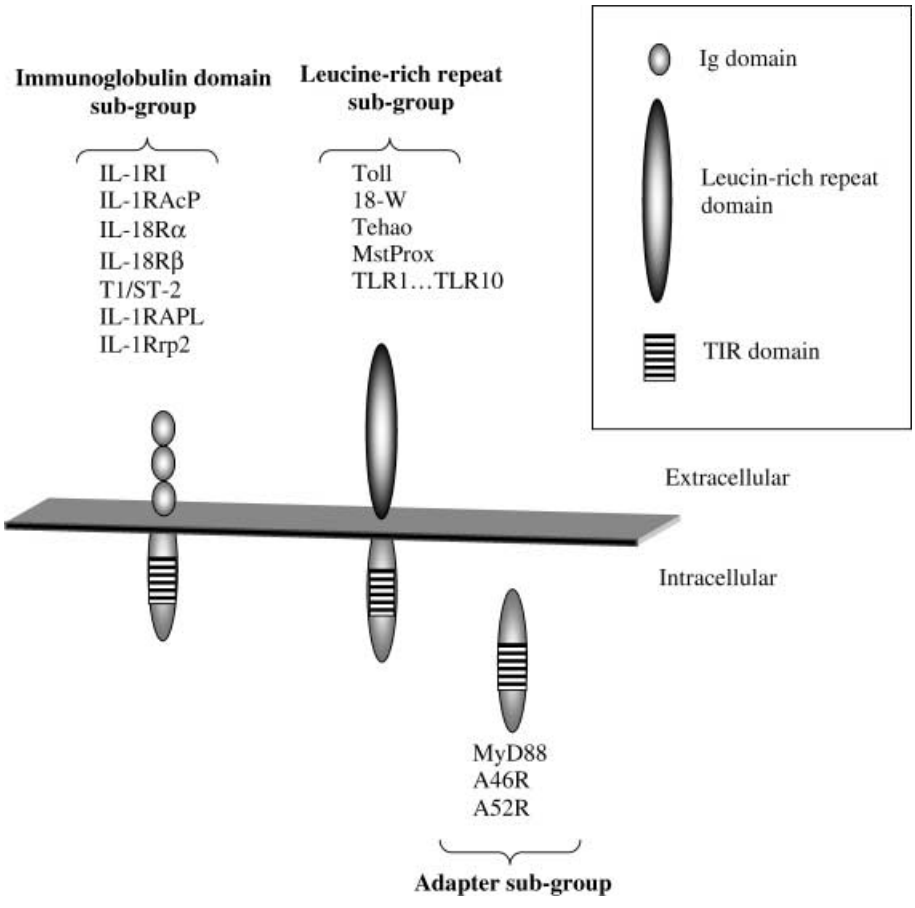

Fig. 2 The Toll/IL-1R (*TIR*) superfamily. The TIR superfamily is defined by a homologous TIR domain and can be subdivided into three main groups. Members of the first group contain immunoglobulin like domains and include IL-18Rα and IL-18Rβ. The second group is defined by the presence of leucine-rich 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 suggest 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) κB activation cascade involves IL-18 mediated IL-18Rα 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-κB binding kinase (NIK) to two I-κB kinases (IKK-1 and IKK-2). This finally leads to the release of the NF-κB subunits p65/p50 from I-κB and its translocation into the nucleus to bind to κB-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-1β are related to the same family in terms of structure, processing, receptor family, and signal transduction pathways. As with IL-1β, 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.

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

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

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-12p70 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-κB 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 κB-binding 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-1α, and monocyte chemotactic 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.

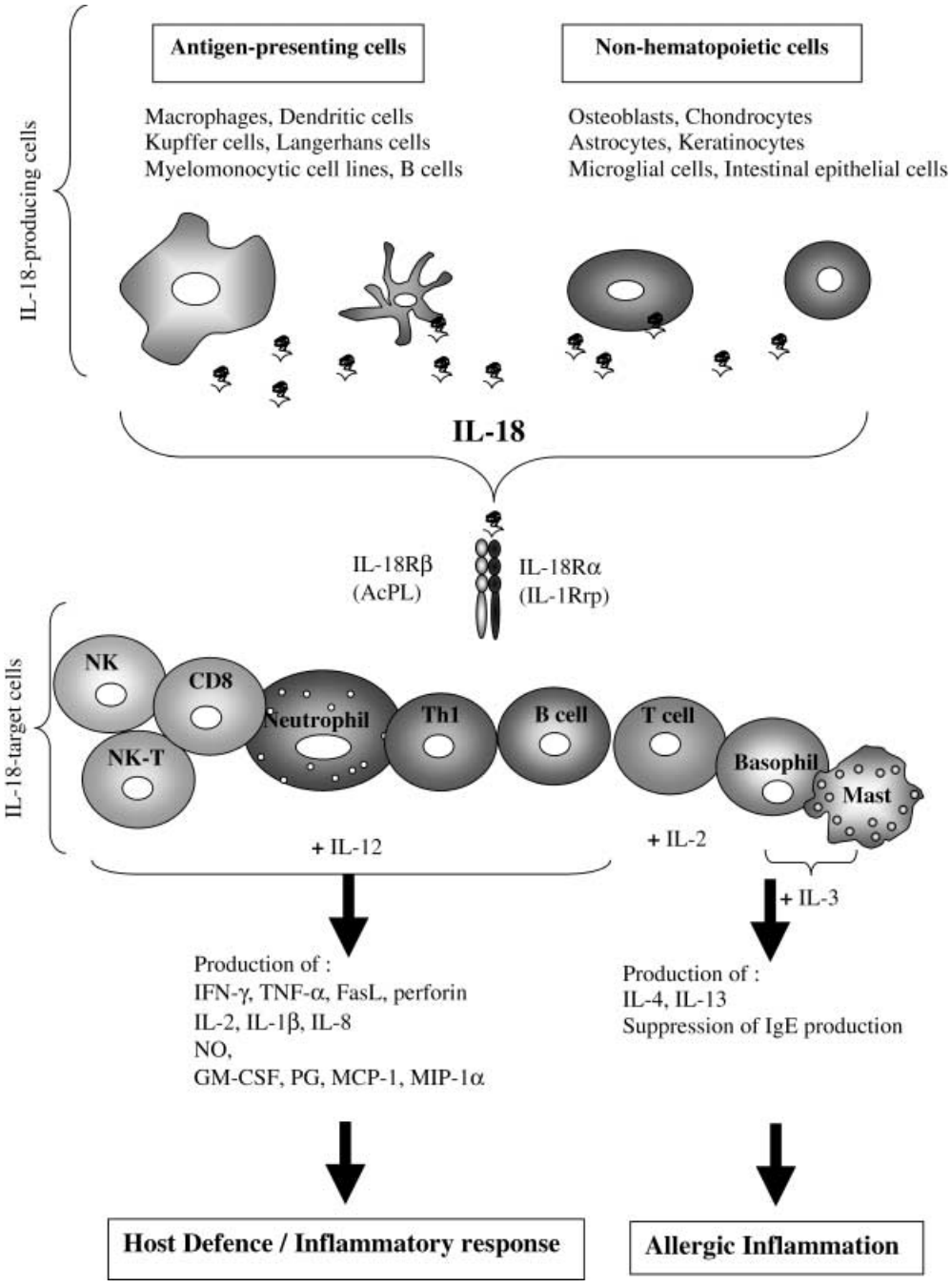

Fig. 3 Cellular sources and the targets of IL-18. Both APCs and nonhematopoietic cells have been reported to produce IL-18. IL-18 and IL-12 synergize to induce the differentiation of $CD4^+$ Th0 cells to enhance the cytotoxic response of $CD8^+$ T cells, NK, and NK-T cells, and to induce IFN-γ production by T cells, NK cells, and macrophages. The inflammatory response is mainly mediated by activated macrophages that produce inflammatory cytokines, nitic oxide (*NO*), monocyte chemotactic protein 1 (*MCP-1*), macrophage inflammatory protein 1α (*MIP-1α*), 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
IFNγ 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 antitumor 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-1β, 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].

Table 3 IL-18 inducing infectious agents

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

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.

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 wildly 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 coincubation 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, *Legionalla 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*.

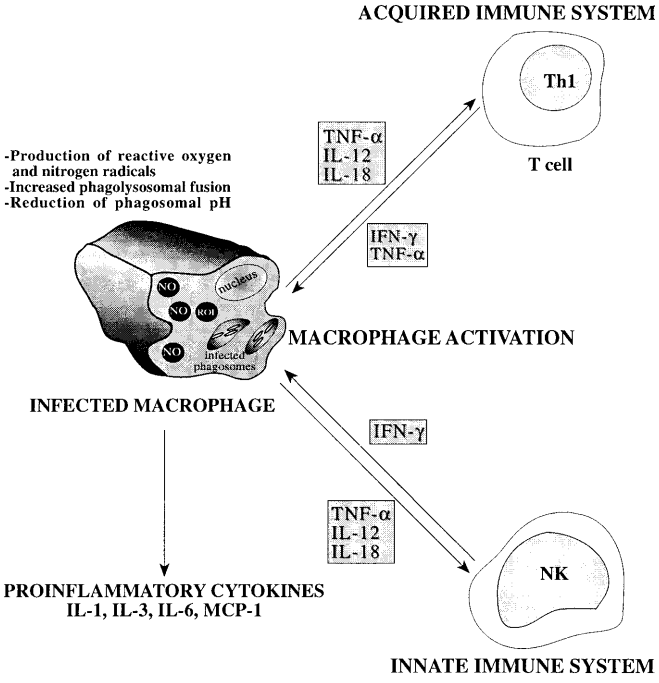

Fig. 4 Basic pathway of immune responses to mycobacterial infections. Infected macrophages become activated, leading to the release of TNF-α, IL-12, and IL-18 that are required to activate NK cells and to establish a Th1 response early in the infectious process. In response to these cytokines Th1 cells 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 chemotactic 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 granulomas 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 together 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 spenocytes 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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References

1. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, et al (1995) Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 378:88–91
2. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, Quintal L, Sekut L, Talanian R, Paskind M, Wong W, Kamen R, Tracey D, Allen H (1997) Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. Nature 386:619–623
3. Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, Fleming MA, Hayashi N, Higashino K, Okamura H, Nakanishi K, Kurimoto M, Tanimoto T, Flavell RA, Sato V, Harding MW, Livingston DJ, Su MS (1997) Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. Science 275:206–209
4. Akita K, Ohtsuki T, Nukada Y, Tanimoto T, Namba M, Okura T, Takakura-Yamamoto R, Torigoe K, Gu Y, Su MSS, Fujii M, Satoh-Itoh M, Yamamoto K, Kohno K, Ikeda M, Kurimoto M (1997) Involvement of caspase-1 and caspase-3 in the production and processing of mature human interleukin 18 in monocytic THP.1 cells. J Biol Chem 272:26595–26603
5. Fantuzzi G, Puren AJ, Harding MW, Livingston DJ, Dinarello CA (1998) Interleukin-18 regulation of interferon gamma production and cell proliferation as shown in interleukin-1beta-converting enzyme (caspase-1)-deficient mice. Blood 91:2118–2125
6. Ushio S, Namba M, Okura T, Hattori K, Nukada Y, Akita K, Tanabe F, Konishi K, Micallef M, Fujii M, Torigoe K, Tanimoto T, Fukuda S, Ikeda M, Okamura H, Kurimoto M (1996) Cloning of the cDNA for human IFN-gamma-inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. J Immunol 156:4274–4279
7. Nolan KF, Greaves DR, Waldmann H (1998) The human interleukin 18 gene IL18 maps to 11q22.2-q22.3, closely linked to the DRD2 gene locus and distinct from mapped IDDM loci. Genomics 51:161–163
8. Conti B, Jahng JW, Tinti C, Son JH, Joh TH (1997) Induction of interferon-gamma inducing factor in the adrenal cortex. J Biol Chem 272:2035–2037
9. Muneta Y, Mori Y, Shimoji Y, Yokomizo Y (2000) Porcine interleukin 18: cloning, characterization of the cDNA and expression with the baculovirus system. Cytokine 12:566–572
10. Gillespie MT, Horwood NJ (1998) Interleukin-18: perspectives on the newest interleukin. Cytokine Growth Factor Rev 9:109–116
11. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001) Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 19:423–474
12. Bazan JF, Timans JC, Kaselein RA (1996) A newly defined interleukin-1? Nature 379:591
13. Smith DE, Renshaw BR, Ketchem RR, Kubin M, Garka KE, Sims JE (2000) Four new members expand the interleukin-1 superfamily. J Biol Chem 275:1169–1175
14. Pan G, Risser P, Mao W, Baldwin DT, Zhong AW, Filvaroff E, Yansura D, Lewis L, Eigenbrot C, Henzel WJ, Vandlen R (2001) IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. Cytokine 13:1–7
15. Torigoe K, Ushio S, Okura T, Kobayashi S, Taniai M, Kunikata T, Murakami T, Sanou O, Kojima H, Fujii M, Ohta T, Ikeda M, Ikegami H, Kurimoto M (1997) Purification and characterization of the human interleukin-18 receptor. J Biol Chem 272:25737–25742
16. Parnet P, Garka KE, Bonnert TP, Dower SK, Sims JE (1996) IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP. J Biol Chem 271:3967–3970
17. Born TL, Thomassen E, Bird TA, Sims JE (1998) Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. J Biol Chem 273:29445–29450
18. Wheeler RD, Culhane AC, Hall MD, Pickering-Brown S, Rothwell NJ, Luheshi GN (2000) Detection of the interleukin 18 family in rat brain by RT-PCR. Brain Res Mol Brain Res 77:290–293
19. O'Neill LA, Dinarello CA (2000) The IL-1 receptor/toll-like receptor superfamily: crucial receptors for inflammation and host defense. Immunol Today 21:206–209
20. Coyle AJ, Lloyd C, Tian J, Nguyen T, Erikkson C, Wang L, Ottoson P, Persson P, Delaney T, Lehar S, Lin S, Poisson L, Meisel C, Kamradt T, Bjerke T, Levinson D, Gutierrez-Ramos JC (1999) Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. J Exp Med 190:895–902
21. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86:973–983
22. Williams MJ, Rodriguez A, Kimbrell DA, Eldon ED (1997) The 18-wheeler mutation reveals complex antibacterial gene regulation in Drosophila host defense. EMBO J 16:6120–130
23. Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, Aderem A (1999) The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature 401:811–815
24. Poltorak A, He X, Smirnova I, Liu MY, Huffel CV, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282:2085–2088
25. Kawai T, Adachi O, Ogawa T, Takeda K, Akira S (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity 11:115–122
26. Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z (1997) MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity 7:837–847
27. Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M (1999) Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity 10:127–136
28. Aizawa Y, Akita K, Taniai M, Torigoe K, Mori T, Nishida Y, Ushio S, Nukada Y, Tanimoto T, Ikegami H, Ikeda M, Kurimoto M (1999) Cloning and expression of interleukin-18 binding protein. FEBS Lett 445:338–342
29. Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, Dinarello CA (2000) Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci U S A 97:1190–1195
30. Xiang Y, Moss B (1999) IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. Proc Natl Acad Sci U S A 96:11537–11542
31. Kim SH, Azam T, Yoon DY, Reznikov LL, Novick D, Rubinstein M, Dinarello CA (2001) Site-specific mutations in the mature form of human IL-18 with enhanced biological activity and decreased neutralization by IL-18 binding protein. Proc Natl Acad Sci U S A 98:3304–3309
32. Dinarello CA (1998) Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. Int Rev Immunol 16:457–499
33. Liebermann DA, Hoffman B (1998) MyD genes in negative growth control. Oncogene 17:3319–3329
34. Robinson D, Shibuya K, Mui A, Zonin F, Murphy E, Sana T, Hartley SB, Menon S, Kastelein R, Bazan F, O'Garra A (1997) IGIF does not drive Th1 development but synergizes

with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity 7:571–581
35. Matsumoto S, Tsuji-Takayama K, Aizawa Y, Koide K, Takeuchi M, Ohta T, Kurimoto M (1997) Interleukin-18 activates NF-kappaB in murine T helper type 1 cells. Biochem Biophys Res Commun 234:454–457
36. Kanakaraj P, Ngo K, Wu Y, Angulo A, Ghazal P, Harris CA, Siekierka JJ, Peterson PA, Fung-Leung WP (1999) Defective interleukin (IL)-18-mediated natural killer and T helper cell type 1 responses in IL-1 receptor-associated kinase (IRAK)-deficient mice. J Exp Med 189:1129–1138
37. Burns K, Clatworthy J, Martin L, Martinon F, Plumpton C, Maschera B, Lewis A, Ray K, Tschopp J, Volpe F (2000) Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. Nat Cell Biol 2:346–351
38. Tsuji-Takayama K, Matsumoto S, Koide K, Takeuchi M, Ikeda M, Ohta T, Kurimoto M (1997) Interleukin-18 induces activation and association of p56 (lck) and MAPK in a murine TH1 clone. Biochem Biophys Res Commun 237:126–130
39. Shapiro L, Heidenreich KA, Meintzer MK, Dinarello CA (1998) Role of p38 mitogen-activated protein kinase in HIV type 1 production in vitro. Proc Natl Acad Sci USA 95:7422–7426
40. Trinchieri G (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 13:251–276
41. Snijders A, Hilkens CM, van der Pouw Kraan TC, Engel M, Aarden LA, Kapsenberg ML (1996) Regulation of bioactive IL-12 production in lipopolysaccharide-stimulated human monocytes is determined by the expression of the p35 subunit. J Immunol 156:1207–1212
42. Gillessen S, Carvajal D, Ling P, Podlaski FJ, Stremlo DL, Familletti PC, Gubler U, Presky DH, Stern AS, Gately MK (1995) Mouse interleukin-12 (IL-12) p40 homodimer: a potent IL-12 antagonist. Eur J Immunol 25:200–206
43. Mattner F, Ozmen L, Podlaski FJ, Wilkinson VL, Presky DH, Gately MK, Alber G (1997) Treatment with homodimeric interleukin-12 (IL-12) p40 protects mice from IL-12-dependent shock but not from tumor necrosis factor alpha-dependent shock. Infect Immun 65:4734–4737
44. Ahn HJ, Maruo S, Tomura M, Mu J, Hamaoka T, Nakanishi K, Clark S, Kurimoto M, Okamura H, Fujiwara H (1997) A mechanism underlying synergy between IL-12 and IFN-gamma-inducing factor in enhanced production of IFN-gamma. J Immunol 159:2125–2131
45. Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, Akira S, Nakanishi K (1998) IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. J Immunol 161:3400–3407
46. Xu D, Chan WL, Leung BP, Hunter D, Schulz K, Carter RW, McInnes IB, Robinson JH, Liew FY (1998) Selective expression and functions of interleukin 18 receptor on T helper (Th) type 1 but not Th2 cells. J Exp Med 188:1485–1492
47. Barbulescu K, Becker C, Schlaak JF, Schmitt E, Meyer zum Buschenfelde KH, Neurath MF (1998) IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN-gamma promoter in primary CD4+ T lymphocytes. J Immunol 160:3642–3647
48. Yang J, Murphy TL, Ouyang W, Murphy KM (1999) Induction of interferon-gamma production in Th1 CD4+ T cells: evidence for two distinct pathways for promoter activation. Eur J Immunol 29:548–555
49. Brossart P, Grunebach F, Stuhler G, Reichardt VL, Mohle R, Kanz L, Brugger W (1998) Generation of functional human dendritic cells from adherent peripheral blood monocytes by CD40 ligation in the absence of granulocyte-macrophage colony-stimulating factor. Blood 92:4238
50. Stoll S, Muller G, Kurimoto M, Saloga J, Tanimoto T, Yamauchi H, Okamura H, Knop J, Enk AH (1997) Production of IL-18 (IFN-gamma-inducing factor) messenger RNA and functional protein by murine keratinocytes. J Immunol 159:298–302
51. Dinarello CA (1999) Interleukin-18. Methods 19:121–132
52. Lebel-Binay S, Berger A, Zinzindohoue F, Cugnenc P, Thiounn N, Fridman WH, Pages F (2000) Interleukin-18: biological properties and clinical implications. Eur Cytokine Netw 11:15–26
53. Sugawara I (2000) Interleukin-18 (IL-18) and infectious diseases, with special emphasis on diseases induced by intracellular pathogens. Microbes Infect 2:1257–1263
54. Puren AJ, Fantuzzi G, Dinarello CA (1999) Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1beta are differentially regulated in human blood mononuclear cells and mouse spleen cells. Proc Natl Acad Sci USA 96:2256–2261
55. Takeda K, Tsutsui H, Yoshimoto T, Adachi O, Yoshida N, Kishimoto T, Okamura H, Nakanishi K, Akira S (1998) Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity 8:383–390
56. Micallef MJ, Yoshida K, Kawai S, Hanaya T, Kohno K, Arai S, Tanimoto T, Torigoe K, Fujii M, Ikeda M, Kurimoto M (1997) In vivo antitumor effects of murine interferon-gamma-inducing factor/interleukin-18 in mice bearing syngeneic Meth A sarcoma malignant ascites. Cancer Immunol Immunother 43:361–367
57. Micallef MJ, Tanimoto T, Kohno K, Ikeda M, Kurimoto M (1997) Interleukin 18 induces the sequential activation of natural killer cells and cytotoxic T lymphocytes to protect syngeneic mice from transplantation with Meth A sarcoma. Cancer Res 57:4557–4563
58. Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, Lotze MT, Tahara H (1999) Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin-induced tumor apoptosis, respectively. J Immunol 163:583–589
59. Akira S (2000) The role of IL-18 in innate immunity. Curr Opin Immunol 12:59–63
60. Rothe H, Hausmann A, Casteels K, Okamura H, Kurimoto M, Burkart V, Mathieu C, Kolb H (1999) IL-18 inhibits diabetes development in nonobese diabetic mice by counterregulation of Th1-dependent destructive insulitis. J Immunol 163:1230–1236
61. Pizarro TT, Michie MH, Bentz M, Woraratanadharm J, Smith MF Jr, Foley E, Moskaluk CA, Bickston SJ, Cominelli F (1999) IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J Immunol 162:6829–6835
62. Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, Luzza F, Fusco A, Pallone F (1999) Bioactive IL-18 expression is up-regulated in Crohn's disease. J Immunol 163: 143–147
63. Campbell E, Kunkel SL, Strieter RM, Lukacs NW (2000) Differential roles of IL-18 in allergic airway disease: induction of eotaxin by resident cell populations exacerbates eosinophil accumulation. J Immunol 164:1096–1102
64. Wild JS, Sigounas A, Sur N, Siddiqui MS, Alam R, Kurimoto M, Sur S (2000) IFN-gamma-inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. J Immunol 164:2701–2710
65. Okamura H, Tsutsui H, Kashiwamura S, Yoshimoto T, Nakanishi K (1998) Interleukin-18: a novel cytokine that augments both innate and acquired immunity. Adv Immunol 70: 281–312
66. McInnes IB, Gracie JA, Leung BP, Wei XQ, Liew FY (2000) Interleukin 18: a pleiotropic participant in chronic inflammation. Immunol Today 21:312–315
67. Tracey KJ, Cerami A (1993) Tumor necrosis factor, other cytokines and disease. Annu Rev Cell Biol 9:317–343
68. Romagnani S (1996) Understanding the role of Th1/Th2 cells in infection. Trends Microbiol 4:470–473
69. Abbas AK, Murphy KM, Sher A (1996) Functional diversity of helper T lymphocytes. Nature 383:787–793
70. Sornasse T, Larenas PV, Davis KA, de Vries JE, Yssel H (1996) Differentiation and stability of T helper 1 and 2 cells

derived from naive human neonatal CD4+ T cells, analyzed at the single-cell level. J Exp Med 184:473–483
71. Kohno K, Kataoka J, Ohtsuki T, Suemoto Y, Okamoto I, Usui M, Ikeda M, Kurimoto M (1997) IFN-gamma-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. J Immunol 158:1541–1550
72. Bradley LM, Dalton DK, Croft M (1996) A direct role for IFN-gamma in regulation of Th1 cell development. J Immunol 157:1350–1358
73. Miettinen M, Matikainen S, Vuopio-Varkila J, Pirhonen J, Varkila K, Kurimoto M, Julkunen I (1998) *Lactobacilli* and *streptococci* induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. Infect Immun 66:6058–6062
74. Puren AJ, Fantuzzi G, Gu Y, Su MS, Dinarello CA (1998) Interleukin-18 (IFNgamma-inducing factor) induces IL-8 and IL-1beta via TNFalpha production from non-CD14+ human blood mononuclear cells. J Clin Invest 101:711–721
75. Fassbender K, Mielke O, Bertsch T, Muehlhauser F, Hennerici M, Kurimoto M, Rossol S (1999) Interferon-gamma-inducing factor (IL-18) and interferon-gamma in inflammatory CNS diseases. Neurology 53:1104–1106
76. Prinz M, Hanisch UK (1999) Murine microglial cells produce and respond to interleukin-18. J Neurochem 72:2215–2218
77. Okamura H, Nagata K, Komatsu T, Tanimoto T, Nukata Y, Tanabe F, Akita K, Torigoe K, Okura T, Fukuda S, et al (1995) A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. Infect Immun 63:3966–3972
78. Tsutsui H, Matsui K, Kawada N, Hyodo Y, Hayashi N, Okamura H, Higashino K, Nakanishi K (1997) IL-18 accounts for both TNF-alpha- and Fas ligand-mediated hepatotoxic pathways in endotoxin-induced liver injury in mice. J Immunol 159:3961–3967
79. Tsuji H, Mukaida N, Harada A, Kaneko S, Matsushita E, Nakanuma Y, Tsutsui H, Okamura H, Nakanishi K, Tagawa Y, Iwakura Y, Kobayashi K, Matsushima K (1999) Alleviation of lipopolysaccharide-induced acute liver injury in *Propionibacterium acnes*-primed IFN-gamma-deficient mice by a concomitant reduction of TNF-alpha, IL-12, and IL-18 production. J Immunol 162:1049–1055
80. Sakao Y, Takeda K, Tsutsui H, Kaisho T, Nomura F, Okamura H, Nakanishi K, Akira S (1999) IL-18-deficient mice are resistant to endotoxin-induced liver injury but highly susceptible to endotoxin shock. Int Immunol 11:471–480
81. Wei XQ, Leung BP, Niedbala W, Piedrafita D, Feng GJ, Sweet M, Dobbie L, Smith AJ, Liew FY (1999) Altered immune responses and susceptibility to *Leishmania major* and *Staphylococcus aureus* infection in IL-18-deficient mice. J Immunol 163:2821–2828
82. Miethke T, Wahl C, Heeg K, Echtenacher B, Krammer PH, Wagner H (1992) T cell-mediated lethal shock triggered in mice by the superantigen staphylococcal enterotoxin B: critical role of tumor necrosis factor. J Exp Med 175:91–98
83. Zhao YX, Tarkowski A (1995) Impact of interferon-gamma receptor deficiency on experimental *Staphylococcus aureus* septicemia and arthritis. J Immunol 155:5736–5742
84. Hultgren O, Kopf M, Tarkowski A (1998) *Staphylococcus aureus*-induced septic arthritis and septic death is decreased in IL-4-deficient mice: role of IL-4 as promoter for bacterial growth. J Immunol 160:5082–5087
85. Bouza E, Dominguez A, Meseguer M, Buzon L, Boixeda D, Revillo MJ, de Rafael L, Martinez-Beltran J (1980) *Yersinia enterocolitica* Septicemia. Am J Clin Pathol 74:404–409
86. Bohn E, Autenrieth IB (1996) IL-12 is essential for resistance against *Yersinia enterocolitica* by triggering IFN-gamma production in NK cells and CD4+ T cells. J Immunol 156:1458–1468
87. Bohn E, Sing A, Zumbihl R, Bielfeldt C, Okamura H, Kurimoto M, Heesemann J, Autenrieth IB (1998) IL-18 (IFN-gamma-inducing factor) regulates early cytokine production in, and promotes resolution of, bacterial infection in mice. J Immunol 160:299–307
88. Dance DA (1991) Melioidosis: the tip of the iceberg? Clin Microbiol Rev 4:52–60
89. Jones AL, Beveridge TJ, Woods DE (1996) Intracellular survival of *Burkholderia pseudomallei*. Infect Immun 64:782–790
90. Santanirand P, Harley VS, Dance DA, Drasar BS, Bancroft GJ (1999) Obligatory role of gamma interferon for host survival in a murine model of infection with *Burkholderia pseudomallei*. Infect Immun 67:3593–3600
91. Lertmemongkolchai G, Cai G, Hunter CA, Bancroft GJ (2001) Bystander activation of CD8+ T cells contributes to the rapid production of IFN-gamma in response to bacterial pathogens. J Immunol 166:1097–1105
92. Lauw FN, Simpson AJ, Prins JM, Smith MD, Kurimoto M, van Deventer SJ, Speelman P, Chaowagul W, White NJ, van der Poll T (1999) Elevated plasma concentrations of interferon (IFN)-gamma and the IFN-gamma-inducing cytokines interleukin (IL)-18, IL-12, and IL-15 in severe melioidosis. J Infect Dis 180:1878–1885
93. Perry LL, Feilzer K, Caldwell HD (1997) Immunity to *Chlamydia* trachomatis is mediated by T helper 1 cells through IFN-gamma-dependent and -independent pathways. J Immunol 158:3344–3352
94. Lu H, Shen C, Brunham RC (2000) *Chlamydia trachomatis* infection of epithelial cells induces the activation of caspase-1 and release of mature IL-18. J Immunol 165:1463–1469
95. Byrd TF, Horwitz MA (1989) Interferon gamma-activated human monocytes downregulate transferrin receptors and inhibit the intracellular multiplication of *Legionella pneumophila* by limiting the availability of iron. J Clin Invest 83: 1457–1465
96. Brieland JK, Jackson C, Hurst S, Loebenberg D, Muchamuel T, Debets R, Kastelein R, Churakova T, Abrams J, Hare R, O'Garra A (2000) Immunomodulatory role of endogenous interleukin-18 in gamma interferon-mediated resolution of replicative *Legionella pneumophila* lung infection. Infect Immun 68:6567–6573
97. Mastroeni P, Clare S, Khan S, Harrison JA, Hormaeche CE, Okamura H, Kurimoto M, Dougan G (1999) Interleukin 18 contributes to host resistance and gamma interferon production in mice infected with virulent *Salmonella typhimurium*. Infect Immun 67:478–483
98. Dybing JK, Walters N, Pascual DW (1999) Role of endogenous interleukin-18 in resolving wild-type and attenuated *Salmonella typhimurium* infections. Infect Immun 67:6242–6248
99. Elhofy A, Bost KL (1999) Limited interleukin-18 response in *Salmonella*-infected murine macrophages and in *Salmonella*-infected mice. Infect Immun 67:5021–5026
100. Foss DL, Zilliox MJ, Murtaugh MP (2001) Bacterially induced activation of interleukin-18 in porcine intestinal mucosa. Vet Immunol Immunopathol 78:263–277
101. Chen Y, Smith MR, Thirumalai K, Zychlinsky A (1996) A bacterial invasin induces macrophage apoptosis by binding directly to ICE. EMBO J 15:3853–3860
102. Sansonetti PJ, Phalipon A, Arondel J, Thirumalai K, Banerjee S, Akira S, Takeda K, Zychlinsky A (2000) Caspase-1 activation of IL-1beta and IL-18 are essential for *Shigella flexneri*-induced inflammation. Immunity 12:581–590
103. Neighbors M, Xu X, Barrat FJ, Ruuls SR, Churakova T, Debets R, Bazan JF, Kastelein RA, Abrams JS, O'Garra A (2001) A critical role for interleukin 18 in primary and memory effector responses to *Listeria monocytogenes* that extends beyond its effects on Interferon gamma production. J Exp Med 194:343–354
104. Boockvar KS, Granger DL, Poston RM, Maybodi M, Washington MK, Hibbs JB Jr, Kurlander RL (1994) Nitric oxide produced during murine listeriosis is protective. Infect Immun 62:1089–1100

105. Daley CL, Small PM, Schecter GF, Schoolnik GK, McAdam RA, Jacobs WR Jr, Hopewell PC (1992) An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. An analysis using restriction-fragment-length polymorphisms. N Engl J Med 326:231–235
106. Di Perri G, Cruciani M, Danzi MC, Luzzati R, De Checchi G, Malena M, Pizzighella S, Mazzi R, Solbiati M, Concia E, et al (1989) Nosocomial epidemic of active tuberculosis among HIV-infected patients. Lancet II:1502–1504
107. Cisneros JR, Murray KM (1996) Corticosteroids in tuberculosis. Ann Pharmacother 30:1298–1303
108. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM (1993) Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med 178:2243–2247
109. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR (1993) An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. J Exp Med 178:2249–2254
110. Jouanguy E, Altare F, Lamhamedi S, Revy P, Emile JF, Newport M, Levin M, Blanche S, Seboun E, Fischer A, Casanova JL (1996) Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guérin infection. N Engl J Med 335:1956–1961
111. Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, Williamson R, Levin M (1996) A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. N Engl J Med 335:1941–1949
112. Cooper AM, Callahan JE, Keen M, Belisle JT, Orme IM (1997) Expression of memory immunity in the lung following re-exposure to *Mycobacterium tuberculosis*. Tuber Lung Dis 78:67–73
113. Ottenhoff TH, Kumararatne D, Casanova JL (1998) Novel human immunodeficiencies reveal the essential role of type-I cytokines in immunity to intracellular bacteria. Immunol Today 19:491–494
114. Sugawara I, Yamada H, Kaneko H, Mizuno S, Takeda K, Akira S (1999) Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice. Infect Immun 67:2585–2589
115. Vankayalapati R, Wizel B, Weis SE, Samten B, Girard WM, Barnes PF (2000) Production of interleukin-18 in human tuberculosis. J Infect Dis 182:234–239
116. Kobayashi K, Nakata N, Kai M, Kasama T, Hanyuda Y, Hatano Y (1997) Decreased expression of cytokines that induce type 1 helper T cell/interferon-gamma responses in genetically susceptible mice infected with *Mycobacterium avium*. Clin Immunol Immunopathol 85:112–116
117. Kobayashi K, Kai M, Gidoh M, Nakata N, Endoh M, Singh RP, Kasama T, Saito H (1998) The possible role of interleukin (IL)-12 and interferon-gamma-inducing factor/IL-18 in protection against experimental *Mycobacterium leprae* infection in mice. Clin Immunol Immunopathol 88:226–231
118. Garcia VE, Uyemura K, Sieling PA, Ochoa MT, Morita CT, Okamura H, Kurimoto M, Rea TH, Modlin RL (1999) IL-18 promotes type 1 cytokine production from NK cells and T cells in human intracellular infection. J Immunol 162:6114–6121
119. Qureshi MH, Zhang T, Koguchi Y, Nakashima K, Okamura H, Kurimoto M, Kawakami K (1999) Combined effects of IL-12 and IL-18 on the clinical course and local cytokine production in murine pulmonary infection with *Cryptococcus neoformans*. Eur J Immunol 29:643–649
120. Bermudez LE, Kaplan G (1995) Recombinant cytokines for controlling mycobacterial infections. Trends Microbiol 3: 22–27
121. Condos R, Rom WN, Schluger NW (1997) Treatment of multidrug-resistant pulmonary tuberculosis with interferon-gamma via aerosol. Lancet 349:1513–1515
122. Gollob JA, Mier JW, Veenstra K, McDermott DF, Clancy D, Clancy M, Atkins MB (2000) Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-gamma induction is associated with clinical response. Clin Cancer Res 6:1678–1692
123. Johnson BJ, Bekker LG, Rickman R, Brown S, Lesser M, Ress S, Willcox P, Steyn L, Kaplan G (1997) rhuIL-2 adjunctive therapy in multidrug resistant tuberculosis: a comparison of two treatment regimens and placebo. Tuber Lung Dis 78:195–203
124. Kremer L, Dupre L, Wolowczuk I, Locht C (1999) In vivo immunomodulation following intradermal injection with DNA encoding IL-18. J Immunol 163:3226–3231
125. Billaut-Mulot O, Idziorek T, Ban E, Kremer L, Dupre L, Loyens M, Riveau G, Locht C, Capron A, Bahr GM (2000) Interleukin-18 modulates immune responses induced by HIV-1 Nef DNA prime/protein boost vaccine. Vaccine 19:95–102
126. Dupre L, Kremer L, Wolowczuk I, Riveau G, Capron A, Locht C (2001) Immunostimulatory effect of IL-18-encoding plasmid in DNA vaccination against murine *Schistosoma mansoni* infection. Vaccine 19:1373–1380
127. Yamanaka K, Hara I, Nagai H, Miyake H, Gohji K, Micallef MJ, Kurimoto M, Arakawa S, Kamidono S (1999) Synergistic antitumor effects of interleukin-12 gene transfer and systemic administration of interleukin-18 in a mouse bladder cancer model. Cancer Immunol Immunother 48:297–302
128. Okamura H, Kashiwamura S, Tsutsui H, Yoshimoto T, Nakanishi K (1998) Regulation of interferon-gamma production by IL-12 and IL-18. Curr Opin Immunol 10:259–264
129. Micallef MJ, Ohtsuki T, Kohno K, Tanabe F, Ushio S, Namba M, Tanimoto T, Torigoe K, Fujii M, Ikeda M, Fukuda S, Kurimoto M (1996) Interferon-gamma-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-gamma production. Eur J Immunol 26:1647–1651
130. Yoshimoto T, Okamura H, Tagawa YI, Iwakura Y, Nakanishi K (1997) Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon-gamma production from activated B cells. Proc Natl Acad Sci USA 94:3948–4953
131. Dao T, Mehal WZ, Crispe IN (1998) IL-18 augments perforin-dependent cytotoxicity of liver NK-T cells. J Immunol 161:2217–2222
132. Okamoto I, Kohno K, Tanimoto T, Ikegami H, Kurimoto M (1999) Development of $CD8^+$ effector T cells is differentially regulated by IL-18 and IL-12. J Immunol 162:3202–3211
133. Ohtsuki T, Micallef MJ, Kohno K, Tanimoto T, Ikeda M, Kurimoto M (1997) Interleukin 18 enhances Fas ligand expression and induces apoptosis in Fas-expressing human myelomonocytic KG-1 cells. Anticancer Res 17:3253–3258
134. Olee T, Hashimoto S, Quach J, Lotz M (1999) IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses. J Immunol 162:1096–1100
135. Lu H, Yang X, Takeda K, Zhang D, Fan Y, Luo M, Shen C, Wang S, Akira S, Brunham RC (2000) *Chlamydia trachomatis* mouse pneumonitis lung infection in IL-18 and IL-12 knockout mice: IL-12 is dominant over IL-18 for protective immunity. Mol Med 6:604–612
136. Kawakami K, Koguchi Y, Qureshi MH, Miyazato A, Yara S, Kinjo Y, Iwakura Y, Takeda K, Akira S, Kurimoto M, Saito A (2000) IL-18 contributes to host resistance against infection with *Cryptococcus neoformans* in mice with defective IL-12 synthesis through induction of IFN-gamma production by NK cells. J Immunol 165:941–947
137. Shapiro L, Puren AJ, Barton HA, Novick D, Peskind RL, Shenkar R, Gu Y, Su MS, Dinarello CA (1998) Interleukin 18 stimulates HIV type 1 in monocytic cells. Proc Natl Acad Sci USA 95:12550–12555
138. Ohkusu K, Yoshimoto T, Takeda K, Ogura T, Kashiwamura S, Iwakura Y, Akira S, Okamura H, Nakanishi K (2000) Potentiality of interleukin-18 as a useful reagent for treatment and prevention of *Leishmania major* infection. Infect Immun 68:2449–2456

139. Meyer Zum Buschenfelde C, Cramer S, Trumpfheller C, Fleischer B, Frosch S (1997) *Trypanosoma cruzi* induces strong IL-12 and IL-18 gene expression in vivo: correlation with interferon-gamma (IFN-gamma) production. Clin Exp Immunol 110:378–385
140. Cai G, Kastelein R, Hunter CA (2000) Interleukin-18 (IL-18) enhances innate IL-12-mediated resistance to *Toxoplasma gondii*. Infect Immun 68:6932–6938
141. Yap GS, Ortmann R, Shevach E, Sher A (2001) A Heritable Defect in IL-12 Signaling in B10.Q/J Mice. II. Effect on Acute Resistance to *Toxoplasma gondii* and Rescue by IL-18 Treatment. J Immunol 166:5720–5725
142. Kawakami K, Qureshi MH, Zhang T, Okamura H, Kurimoto M, Saito A (1997) IL-18 protects mice against pulmonary and disseminated infection with *Cryptococcus neoformans* by inducing IFN-gamma production. J Immunol 159:5528–5534
143. Lauw FN, Simpson AJ, Prins JM, van Deventer SJ, Chaowagul W, White NJ, van der Poll T (2000) The CXC chemokines gamma interferon (IFN-gamma)-inducible protein 10 and monokine induced by IFN-gamma are released during severe melioidosis. Infect Immun 68:3888–3893
144. Yamada G, Shijubo N, Shigehara K, Okamura H, Kurimoto M, Abe S (2000) Increased levels of circulating interleukin-18 in patients with advanced tuberculosis. Am J Respir Crit Care Med 161:1786–1789