

# Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process<sup>1-4</sup>

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## ABSTRACT

In this review, 2 cytokines are discussed with respect to the inflammatory processes that are fundamental to aging and mortality. Both interleukin (IL)-1 and IL-18 are members of the same structural family (IL-1 family, or IL-F); there are presently 9 members of this family, but with the exception of IL-1 $\alpha$ , IL-1 $\beta$ , and IL-18, the others are antagonists or remain without known function. IL-1 $\alpha$  is an intracellular cytokine with properties of both a cytokine and a transcription factor. IL-1 $\beta$  and IL-18 are closely related; both possess a similar three-dimensional structure, and their respective precursor forms are inactive until cleaved by the intracellular cysteine protease caspase-1. Patients with mutations in the *NALP3* gene, which controls the activity of caspase-1, readily secrete more IL-1 $\beta$  and IL-18 and suffer from systemic inflammatory diseases. Patients with defects in this gene have high circulating concentrations of IL-6, serum amyloid A, and C-reactive protein, each of which decrease rapidly upon blockade of the IL-1 receptor, which suggests that IL-1 $\beta$  contributes to the elevation of these markers of the inflammatory mechanisms of aging. Animal studies support the concept that IL-1 $\beta$  and IL-18 participate in the pathogenesis of atherosclerosis. For example, overexpression of the IL-18 binding protein, a naturally occurring, specific inhibitor of IL-18, prevents the spontaneous development of atherosclerosis in apolipoprotein E-deficient mice. From human and animal studies, one may conclude that IL-1 $\beta$  and IL-18 participate in fundamental inflammatory processes that increase during the aging process. *Am J Clin Nutr* 2006;83(suppl):447S-55S.

**KEY WORDS** Cytokines, inflammation, atherosclerosis, aging

## INTERLEUKIN 1 AND SYSTEMIC DISEASE

*Editor's note: This article is based on the Keynote Address at the Living Well to 100 Conference held at Tufts University. The author was specifically asked to review the historical perspective of the interleukin 1 gene family as mediators of the aging process to set the tone and context for the importance of inflammation and genetics as presented in the remaining session. As such, this article should be viewed as laying the scientific foundation for the emerging directions presented.*

For many years, the molecule that was later termed interleukin (IL)-1 was described for its ability to affect various and different biological properties. It remained uncertain whether a single polypeptide was capable of several distinctly different biological

properties. It was a highly contentious issue that a single molecule caused fever, induced hepatic acute-phase proteins, activated lymphocytes, and upregulated prostanoid synthesis. Recombinant IL-1 was needed to prove that IL-1 did in fact possess such a wide and varied spectrum of biological activities. In 1984 it was predicted that IL-1 was responsible for many of the acute-phase responses to infection and inflammation (1). The cDNAs for mouse IL-1 $\alpha$  (2) and human IL-1 $\beta$  (3) were cloned, and soon thereafter, experiments with recombinant IL-1 showed that IL-1 was indeed pyrogenic (4). In addition, recombinant IL-1 mediated many components of the acute-phase response. Thus, a single, endogenously produced molecule, in this case IL-1, was proven to cause a wide variety of biological effects associated with infection, inflammation, and autoimmune processes (4). In many ways, the biology of IL-1 played a major role in the new field of cytokines.

## PRODUCTION OF INTERLEUKIN 1 AND ITS ROLE IN NUTRITION

Many studies measuring concentrations of IL-1 in various body fluids have been published. Some have been remarkable in that the concentrations were unexpectedly low for the severity of disease. For example, circulating concentrations of IL-1 $\beta$  in patients with sepsis and bacteremia have been found to be in the low pg/mL range (5, 6). On the basis of the human response to intravenously administered IL-1 $\alpha$  or IL-1 $\beta$ , such concentrations of circulating IL-1 are consistent with the potency of IL-1 in humans (reviewed in reference 7). Intravenous infusion of IL-1 $\beta$  into humans at 10 ng/kg results in fever and elevated cortisol and IL-6 concentrations (8), and infusion of IL-1 $\alpha$  at 50 ng/kg requires pressors to maintain blood pressure (9).

IL-1 is a potent anorectic cytokine that is at least 1000-fold more effective than leptin (10, 11). In mice infected with influenza virus, blocking IL-1 with the IL-1 receptor antagonist (IL-1Ra) attenuates the decrease in food intake and increases survival (12). In a model of local inflammation in which weight loss is a prominent manifestation, IL-1Ra reverses the decrease in food

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intake (13). Mice deficient in IL-1 $\beta$  also do not lose weight when subjected to a local inflammatory process (14). In humans, the loss of mean body mass is often reported in patients with autoimmune diseases such as rheumatoid arthritis, and the peripheral blood mononuclear cells of rheumatoid arthritis patients produce more IL-1 than do the cells of healthy controls (15). In this and similar studies, the elevation of IL-1 $\beta$  production correlates with decreased food intake and is consistent with the anorectic property of IL-1. The loss in mean body mass is also associated with poor survival; in the Framingham Study population, it was shown that elevations in IL-6 correlate with mortality (16). IL-6 concentrations in serum are a reflection of IL-1 activity in vivo (17–19).

Although there are consistent reports of the anorectic property of IL-1 affecting the nutritional status of the host, strategies to reduce the biological effects of IL-1 have focused on drug-mediated programs and on nutritional approaches. Of these, diets high in n–3 fatty acids have been shown to produce a highly consistent reduction in both inflammation and cytokine production. In healthy humans, supplementation of a normal American diet with n–3 fatty acids results in a >50% reduction in the ex vivo production of IL-1 $\beta$  and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) from stimulated peripheral blood mononuclear cells (20). Of importance, on cessation of supplementation, the production of these cytokines returns to their baseline measurement after a washout period of 6 wk (20). A similar reduction was reported in older women (21). It is not necessary to provide supplements of n–3 fatty acids to the diet to observe a reduction in IL-1 $\beta$  and TNF- $\alpha$ . Increasing the amount of n–3 fatty acids in food results in a similar reduction in cytokines (22). If the aging process is negatively affected by inflammation, and assuming that cytokines such as IL-1 $\beta$  are responsible, in part, for the inflammation, then diets rich in n–3 fatty acids may be beneficial to longevity.

### BLOCKING INTERLEUKIN 1 ACTIVITY IN DISEASE

Regardless of the results from in vitro or in vivo use of IL-1, both of which have contributed to the knowledge base for IL-1, it was the specific blockade of IL-1 activity in the context of a complex disease model that led to a full appreciation of the role of IL-1 in disease. IL-1Ra is a member of the IL-1 family and is a specific receptor antagonist for IL-1 $\alpha$  and IL-1 $\beta$  that binds to the IL-1 receptor type I. IL-1Ra has been approved for the treatment of rheumatoid arthritis. More than 50 000 patients have been treated, and many continue to experience benefit from its use. Because of its specificity, IL-1Ra blockade of the IL-1 receptor is used to prove IL-1 causality in a disease process. Causality in disease can only be ascertained with specific neutralization or receptor blockade. Specific gene deletion or polymorphisms can also contribute to an understanding of causality. In the case of IL-1 $\beta$ , there appears to be a single amino acid mutation in the *NALP3* gene that results in a highly inflammatory disease that is treated with specific IL-1 receptor blockade (23–28)

Increased secretion of IL-1 $\beta$  from cultured peripheral blood mononuclear cells has also been reported for a growing number of inherited, chronic autoinflammatory syndromes, each of which responds to IL-1Ra. In these syndromes, increased secretion of IL-1 $\beta$  is due to a single amino acid mutation in the *NALP3* gene, which controls the activation of caspase-1. In general, circulating concentrations of IL-1 $\beta$  are not detected in these

patients but increased secretion of IL-1 $\beta$  is observed in vitro. The single point mutation in the *NALP3* gene, the protein of which is found in the IL-1 $\beta$  inflammasome (29), results in loss of the tight control of IL-1 $\beta$  processing in that relatively minor stresses such as exposure to cold result in increased secretion of IL-1 $\beta$  with consequent systemic disease (25). Other chronic inflammatory syndromes that are not caused by the *NALP3* mutations but that exhibit elevated IL-1 $\beta$  release in vitro also respond to IL-1Ra (30). Two other genetic diseases with the hallmarks of chronic systemic and local inflammation are associated with increased secretion of IL-1 $\beta$  and respond to IL-1Ra therapy: the syndrome known as PAPA (pyogenic arthritis, pyoderma gangrenosum, and acne) (31, 32) and familial Mediterranean fever. Both diseases are associated with the intracellular protein known as pyrin, which participates in maintaining procaspase-1 as an inactive enzyme. Mutations of the pyrin gene in mice, similar to those found in humans with familial Mediterranean fever, result in increased caspase-1 activity and increased secretion of IL-1 $\beta$  (33).

Although these systemic, multisystem syndromes are not common diseases, they reveal a fundamental role for IL-1 in systemic inflammation regardless of the cause. IL-1 affects several targets that account for the manifestations of systemic disease. These are recurrent fevers, neutrophilia, thrombocytosis, elevated serum amyloid A and C-reactive protein, and anemia. Skin rashes and urticaria are also observed. Hearing loss, developmental delay, and aseptic meningitis can also be observed in early childhood. The endothelium is a prime target for IL-1-mediated inflammation because IL-1 receptors on the endothelium can be triggered by systemic IL-1, which results in prostaglandin E production, bone marrow release of neutrophils, and the production of IL-6. In fact, IL-1 induction of IL-6 accounts for hepatic acute-phase protein synthesis and thrombocytosis. The dominant role of IL-1 in mediating systemic inflammation is revealed when stopping and restarting IL-1Ra therapy. On cessation of IL-1Ra therapy, clinical signs and symptoms of disease as well as biochemical and hematologic abnormalities rebound within days and resolve on resumption of IL-1 receptor blockade (24, 26, 34, 35).

### THE BIOLOGY OF INTERLEUKIN 18

The importance of IL-18 as an immunoregulatory cytokine is derived from its prominent biological property of inducing interferon  $\gamma$  (IFN- $\gamma$ ) (36). IL-18 was first described in 1989 as an IFN- $\gamma$ -inducing factor in the circulation of endotoxin-injected mice. With the molecular cloning of IFN- $\gamma$ -inducing factor in 1995 (37), the name was changed to IL-18. Macrophages and dendritic cells are the primary sources for active IL-18, but the IL-18 precursor is constitutively expressed in epithelial cells throughout the body. Previously, it was thought that inhibition of caspase-1 as a therapeutic target was specific for reducing the activity of IL-1 $\beta$ , but it became clear that IL-18 activity would also be affected. In fact, any phenotypic characteristic of caspase-1-deficient mice undergoing inflammatory challenges must be differentiated as being due to reduced IL-1 $\beta$  or reduced IL-18 activity.

Because of its role in the production of IFN- $\gamma$ , T cell polarization is a characteristic of IL-18, whereas IFN- $\gamma$  induction is not a prominent characteristic of IL-1. IL-18 exhibits characteristics of other proinflammatory cytokines, such as increases in cell adhesion molecules, nitric oxide synthesis, and chemokine

production. A unique property of IL-18 is the induction of Fas ligand. The induction of fever, an important clinical property of IL-1, TNF- $\alpha$ , and IL-6, is not a property of IL-18. Injection of IL-18 into mice, rabbits, or humans does not produce fever (38, 39). Unlike IL-1 and TNF- $\alpha$ , IL-18 does not induce cyclooxygenase-2 and hence there is no production of prostaglandin E<sub>2</sub> (40, 41). Although the results of clinical trials are presently unknown, several preclinical studies suggest a benefit of IL-18 administration in certain models of rodent cancer (42, 43). Not unexpectedly, the focus on IL-18 has shifted from its use as an immune stimulant to inhibition of its activity. Because IL-18 is required for IFN- $\gamma$  production, blocking IL-18 activity in autoimmune diseases is a particularly attractive therapeutic target.

### THERAPEUTIC STRATEGIES FOR REDUCING INTERLEUKIN 18 ACTIVITY

The strategies for reducing IL-18 activity include neutralizing monoclonal antibodies to IL-18, caspase-1 inhibitors, and blocking antibodies to the IL-18 receptor chains. Caspase-1 inhibitors are oral agents and are presently being tested in clinical trials of rheumatoid arthritis. Caspase-1 inhibitors prevent the release of active IL-1 $\beta$  and IL-18 and therefore may have clinical benefit by reducing the activities of both cytokines. A naturally occurring IL-18 binding protein (IL-18BP) was discovered in 1999; IL-18BP is effective in neutralizing IL-18 activity (44). IL-18BP is not a soluble form of either chain of the IL-18 receptor but rather is a constitutively secreted, high-affinity and specific inhibitor of IL-18 (45, 46). IL-18BP is currently in clinical trials for the treatment of rheumatoid arthritis and severe psoriasis, but the results have not yet been published.

### CASPASE-1 AND NON-CASPASE-1 PROCESSING OF INTERLEUKIN 18

The importance of caspase-1 in inflammation has been shown in patients with mutations in the *NALP3* gene as discussed above. The products of the *NALP3* gene participate in the conversion of procaspase-1 to active caspase-1. Single amino acid point mutations in the gene product result in increased processing and release of IL-1 $\beta$  and IL-18. Although the use of IL-1Ra in these patients results in a near total reversal in both the symptoms and the biochemical abnormalities of the disease (24), it remains likely that IL-18 also contributes to disease in these patients.

The non-caspase-1 enzyme associated with processing both the IL-1 $\beta$  and the IL-18 precursors is proteinase-3 (47). Agonistic autoantibodies to proteinase-3 are pathologic in Wegener granulomatosis and may contribute to the non-caspase-1 cleavage of the IL-18 precursor and IFN- $\gamma$  production in this disease. Epithelial cells stimulated with proteinase-3 in the presence of endotoxin release active IL-18 into the supernatant fluid (48). Because lactate dehydrogenase activity is not released, the appearance of active IL-18 is not due to cell leakage or death. Injecting mice with recombinant Fas ligand results in hepatic damage, which is IL-18 dependent (49). However, the same results are observed in caspase-1-deficient mice (50). Fas ligand-mediated cell death is IL-18-dependent and caspase-1-independent (49, 50), but ischemia-reperfusion injury resulting in cell death is via an IL-18-dependent as well as caspase-1-dependent pathway (51, 52).

### INTERLEUKIN 18 AND INTERLEUKIN 18 RECEPTORS

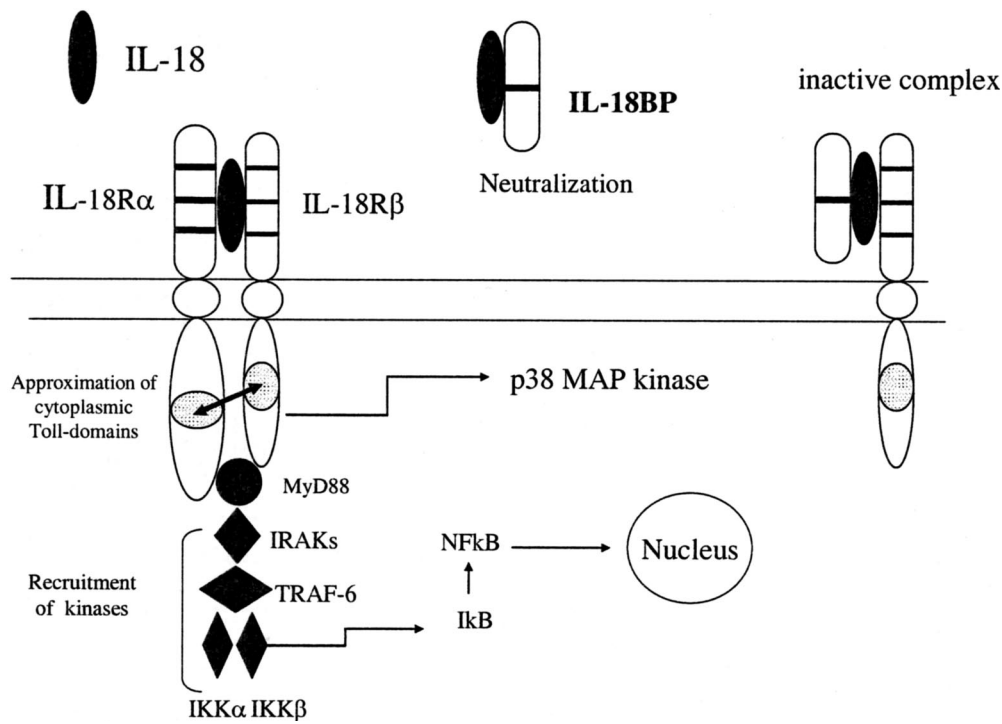
The IL-18 receptor chains (IL-18R $\alpha$  and IL-18R $\beta$ ) are also members of the IL-1 receptor family. The binding sites for IL-18 to the IL-18R $\alpha$  chain are similar to those for IL-1 binding to the IL-1 receptor type I (53, 54). Two sites bind to the ligand-binding chain (IL-18R $\alpha$ ) and a third site binds to the IL-18R $\beta$  chain, which is also called the signal-transducing chain. The intracellular chains of the IL-18 receptors contain the Toll domains, which are essential for initiating signal transduction (see Figure 1). The Toll domains of the IL-18 receptors phylogenetically link IL-18 to the Toll-like receptors, which recognize microbial products. Compared with IL-18BP, the combination of the IL-18R $\alpha$  and  $\beta$  chains is 100-fold less effective in neutralizing IL-18 activity (55). It is unlikely that the soluble form of IL-18R $\alpha$  is a candidate therapeutic agent because of its low affinity. Another member of the IL-1 family, IL-1F7 (56), may be the naturally occurring receptor antagonist of IL-18. IL-1F7 binds to the IL-18R $\alpha$  chain with high affinity, but this binding does not recruit the IL-18R $\beta$  chain. The occupancy of the IL-18R $\alpha$  without formation of the heterodimer with the IL-18R $\beta$  is the same mechanism by which the IL-1 receptor antagonist prevents the activity of IL-1. But IL-1F7 does not affect the activity of IL-18 (57, 58), and the biological significance of IL-1F7 binding to IL-18R $\alpha$  remains unclear. However, in the presence of low concentrations of IL-18BP, IL-1F7 reduces the activity of IL-18 (59).

### INTERLEUKIN 18 BINDING PROTEIN

The discovery of IL-18BP took place during the search for extracellular (soluble) receptors for IL-18 in human urine. Nearly all the soluble cytokine receptors are found in human urine (60). In searching for IL-18 soluble receptors, IL-18 was covalently bound to a matrix, and highly concentrated human urine was passed over the matrix and eluted with acid to disrupt the ligand (IL-18) for its soluble receptors. Unexpectedly, instead of the elution of soluble forms of the cell surface IL-18 receptors, IL-18BP was discovered (44). This was the result of the higher affinity of IL-18BP for the ligand compared with the soluble receptors.

IL-18BP is a constitutively secreted protein with high-affinity (400 pmol/L) binding to IL-18. There is limited amino acid sequence homology between IL-18BP and the cell surface IL-18 receptors; IL-18BP lacks a transmembrane domain and contains only one immunoglobulin-like domain (46, 61). IL-18BP shares many characteristics with the soluble form of the IL-1 receptor type II in that both function as decoys to prevent the binding of their respective ligands to the signaling receptor chains. In fact, there is limited amino acid homology between IL-18BP and the IL-1 receptor type II, suggesting a common ancestor. In humans, IL-18BP is highly expressed in spleen and the intestinal tract, both of which are immunologically active tissues (44). Alternate mRNA splicing of IL-18BP results in 4 isoforms (44, 46). Of considerable importance is that the prominent 'a' isoform is present in the serum of healthy humans at a 20-fold molar excess compared with IL-18 (45). This level of IL-18BP may contribute to a default mechanism by which a Th1 response to foreign organisms is blunted to reduce triggering an autoimmune response to a routine infection. The promoter for IL-18BP contains 2 IFN- $\gamma$  response elements (62), and constitutive gene expression for IL-18BP is dependent on IFN- $\gamma$  (63), which suggests a





**FIGURE 1.** Interleukin (IL)-18 activation of cell signaling. Mature IL-18 binds to the IL-18 receptor  $\alpha$  (IL-18R $\alpha$ ) chain and recruits the IL-18R $\beta$  chain, which results in the close approximation of the Toll domains in the cytoplasmic segment of these chains. The intracellular protein MyD88, which is common to IL-1 and Toll-like receptor signal transduction, is recruited and leads to the phosphorylation of the IL-1 receptor activating kinases (IRAKs), of which there are 4. The tumor necrosis factor receptor activating factor (TRAF)-6 also becomes phosphorylated, which is followed by the activation of the inhibitory  $\kappa$ B kinases (IKK)  $\alpha$  and  $\beta$ . This results in the phosphorylation of inhibitory  $\kappa$ B (I $\kappa$ B) and translocation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) to the nucleus. In addition, IL-18-activated cells phosphorylate mitogen activating protein (MAP) kinase p38. IL-18 binding protein (IL-18BP) is present in the extracellular milieu as a constitutively expressed protein, where it can bind and neutralize IL-18, thus preventing activation of the cell surface receptors. In addition, formation of inactive complexes of IL-18BP with IL-18 and IL-18R $\beta$  deprives the cell of the participation of IL-18R $\alpha$  chain in activating the cell.

compensatory feedback mechanism. Thus, elevated concentrations of IFN- $\gamma$  stimulate more IL-18BP in an attempt to reduce IL-18-mediated IFN- $\gamma$  production. For example, in mice deficient in interferon regulatory factor 1, a transcription factor for IFN- $\gamma$ , low to absent tissue concentrations of IL-18BP are found compared with those in wild-type mice (64). Mice deficient in interferon regulatory factor 1 are exquisitely sensitive to colitis but when treated with exogenous IL-18BP exhibit reduced disease (65).

#### VIRAL INTERLEUKIN 18 BINDING PROTEIN

*Molluscum contagiosum* is a common viral infection of the skin often seen in children and individuals with HIV-1 infection. The infection is characterized by raised but bland eruptions; there are large numbers of viral particles in the epithelial cells of the skin, but histologically there are few inflammatory or immunologically active cells in or near the lesions. Clearly, the virus fails to elicit an inflammatory or immunologic response. A close amino acid similarity exists between human IL-18BP and a gene found in various members of the Pox viruses. The greatest homology is with *Molluscum contagiosum* (44, 66, 67). The viral genes encoding for viral IL-18BP have been expressed and the recombinant proteins neutralize mammalian IL-18 activity (66, 67). The ability of viral IL-18BP to reduce the activity of mammalian IL-18 likely explains the lack of inflammatory and immune cells in the infected skin and the blandness of the lesions. One may conclude from the natural experiment of *Molluscum*

*contagiosum* infection that blocking IL-18 reduces immune and inflammatory processes, such as the function of dendritic and inflammatory cells.

#### INTERLEUKIN 18 IS A PROINFLAMMATORY CYTOKINE

Most investigations initially focused on IL-18 in Th1-mediated diseases in which IFN- $\gamma$  plays a prominent role. However, it soon became clear that blocking IL-18 results in a reduction in disease severity in models in which IFN- $\gamma$  has no significant role or in mice deficient in IFN- $\gamma$ . For example, IL-18-mediated loss of cartilage synthesis in arthritis models is independent of IFN- $\gamma$  (68). Prevention of melanoma metastases is dependent on IL-18 but independent of IFN- $\gamma$  (69), and similar findings exist for ischemia-reperfusion injury in the heart, kidney, and liver. The various animal models of Th1-, Th2-, and non-immune-mediated disease in which the effect of reducing endogenous IL-18 activity has been reported are listed in **Table 1**.

#### INTERLEUKIN 18, A TH1-DIFFERENTIATING CYTOKINE

In driving the Th1 response, IL-18 appears to act in association with IL-12 or IL-15, because IL-18 alone does not induce IFN- $\gamma$ . The effect of IL-12 is, in part, to increase the expression of IL-18 receptors on T lymphocytes, thymocytes, and natural killer cells (92–94). It appears that the role of IL-18 in the polarization of the



**TABLE 1**Effects of reduced interleukin (IL)-18 activity in various models of disease<sup>1</sup>

Disease model	Intervention	Outcome
Acute DSS-induced colitis	Anti-IL-18 (70); IL-18BP (71)	Decreased clinical disease; reduced TNF- $\alpha$ , IFN- $\gamma$ , IL-1, MIP-1 and -2
Chronic DSS-induced colitis	Caspase-1 KO (72)	Decreased clinical disease; reduced IL-1 $\beta$ , IFN- $\gamma$ , and CD3 cells
TNBS-induced colitis	IL-18BP (73)	Decreased colitis; no ulcerations; reduced cytokines
CD62/CD4 T cell-induced colitis	Adenoviral antisense IL-18 (74)	Suppression of inflammation score; decreased mucosal IFN- $\gamma$
Streptococcal wall-induced arthritis	Anti-IL-18 (68)	Restoration of cartilage proteoglycan synthesis; less inflammation
Collagen-induced arthritis	IL-18BP; IL-18 (75); Adenoviral IL-18BP (76)	Reduced clinical disease; reduced joint damage; cytokine reduction
Collagen-induced arthritis	IL-18-deficient mice (77)	Reduced clinical disease; reduced antibodies to collagen
Graft versus host disease	Anti-IL-18 (78)	Reduced CD8 <sup>+</sup> -mediated mortality
Lupus-prone mice	IL-18 vaccination (79)	Decreased lethality; reduced nephritis
Allergic airway hyperresponsiveness	IL-18 vaccination (80)	Reduced broncho-constriction
Experimental myasthenia gravis	Anti-IL-18 (81)	Reduced disease progression
Experimental autoimmune encephalomyelitis	Caspase-1 KO (82); caspase-1 inhibition (82)	Decrease disease severity scores; reduced IFN- $\gamma$
Concanavalin A-induced hepatitis	Anti-IL-18 (83); IL-18BP (49); IL-18BP-Tg (84)	Prevention of liver dysfunction
Fas-mediated hepatic failure	IL-18-deficient mice (50); IL-18BP (49)	Absence of hepatic necrosis
Pseudomonas exotoxin-A hepatic damage	IL-18BP (49)	Reduced liver damage
IL-12-induced IFN- $\gamma$	Anti-IL-18 (85); caspase-1 KO (85)	Suppression of serum and spleen cell IFN- $\gamma$ concentrations
Endotoxin-induced IFN- $\gamma$	Anti-IL-18 (37, 86); IL-18BP (44, 49); caspase-1 KO	Decreased serum and spleen concentrations of IFN- $\gamma$
Endotoxin-induced hepatic necrosis	Anti-IL-18 (87)	Prevention of hepatic necrosis; decreased TNF- $\alpha$ and FasL
Endotoxin-induced lethality	Anti-IL-18 (37, 86); IL-18BP (49)	Greater survival
Endotoxin-induced lung neutrophils	Anti-IL-18 (86)	Reduced myeloperoxidase concentrations in lung homogenates
Melanoma hepatic metastasis	IL-18BP (69, 88)	Small numbers and size of hepatic melanoma
Endothelial VCAM-1 expression in melanoma	IL-18BP (69)	Reduced gene expression and surface expression
Ischemia-induced hepatic failure	Anti-IL-18 (89)	Prevention of increases on apoptosis, chemokines, NF- $\kappa$ B
Ischemia-induced acute renal failure	Anti-IL-18 (52); caspase-1 KO (52)	Prevention of elevated creatinine and urea
Ischemia-induced myocardial dysfunction	IL-18BP (51); caspase-1 inhibition (51)	Restoration of myocardial contractility; reduced myocyte death
Endotoxin-induced myocardial suppression	Anti-IL-18 (90)	Restoration of myocardial contractility; reduced myocardial IL-1 $\beta$
Atherosclerosis in apolipoprotein E-deficient mice	IL-18BP (91)	Reduced arterial plaque lipid content and cellular infiltration

<sup>1</sup> DSS, dextran sulfate sodium; FasL, Fas ligand; IFN- $\gamma$ , interferon  $\gamma$ ; IL-18BP, interleukin 18 binding protein; KO, knockout; MIP-1 and -2, macrophage inflammatory protein 1 and 2; NF- $\kappa$ B, nuclear factor  $\kappa$ B; Tg, transgenic; TNBS, trinitrobenzene sulfonic acid; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; VCAM-1, vascular cell adhesion molecule 1.

Th1 response is dependent on IFN- $\gamma$  and IL-12 receptor  $\beta$ 2 chain expression. The production of IFN- $\gamma$  by the combination of IL-18 and IL-12 is an example of true synergism in cytokine biology, similar to the synergism of IL-1 and TNF- $\alpha$  in models of inflammation. Because IFN- $\gamma$  is the "signature" cytokine of CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as of natural killer cells, a great deal of the biology of IL-18 is considered to be the result of IFN- $\gamma$  production. Dendritic cells deficient in the IFN- $\gamma$  transcription factor T-bet exhibit impaired IFN- $\gamma$  production after stimulation with IL-18 plus IL-12 (95). IL-18 is constitutively present in monocytes and monocyte-derived dendritic type 1 cells. Thus, IFN- $\gamma$  induced by the combination of IL-12 plus IL-18 appears to be via the T-bet transcription factor.

### GRAFT VERSUS HOST DISEASE

IFN- $\gamma$  plays a major pathologic role in this disease because of its Th1-inducing properties and the generation of cytotoxic T cells. With the use of a cohort of 157 patients who received unrelated

donor bone marrow transplantation and developed graft versus host disease, mutations in the IL-18 promoter (G137C, C607A, and G656T) were identified and associated with a statistically significant decreased risk of death (96). One hundred days after the transplantation, mortality in patients with this haplotype was 23% compared with 48% in those patients without the haplotype; after 1 y, mortality was 36% compared with 65%, respectively. The probability of survival was two-fold in patients with this haplotype (96). In the case of graft versus host disease in mice, paradoxical effects of IL-18 have been reported depending on whether the disease is mediated by CD4<sup>+</sup> or CD8<sup>+</sup> T cells. In humans, T cells are responsible for the disease after allogeneic bone marrow transplantation. Administration of IL-18 to recipient mice increases survival in CD4<sup>+</sup>-mediated disease but results in worsening of the CD8<sup>+</sup>-mediated disease (78). Neutralizing antibodies to IL-18 significantly reduce CD8<sup>+</sup>-mediated mortality (78). Administration of IL-18 reduces the severity of the disease by inducing the production of Th2 cytokines (97).

## INTERLEUKIN 18 AND TH2 RESPONSES

The combination of IL-18 plus IL-12 suppresses immunoglobulin (Ig) E synthesis via IFN- $\gamma$  production and suggests a role for IL-18 in Th2 polarization. For example, in models of allergic asthma, injecting both IL-12 plus IL-18 suppresses IgE synthesis, eosinophilia, and airway hyperresponsiveness (98). In contrast, administration of IL-18 alone enhances basophil production of IL-4 and histamine (99) and increases serum IgE concentrations in wild-type and IL-4-deficient mice (100). Overexpression of mature IL-18 in the skin results in worsening of allergic and nonallergic cutaneous inflammation via Th2 cytokines (101). Mice overexpressing IL-18 or overexpressing caspase-1 develop an atopic-like dermatitis with mastocytosis and the presence of Th2 cytokines; also present in these mice is elevated serum IgE (102). Although IL-18 remains a Th1 cytokine, increasing numbers of reports show a role for IL-18 in promoting Th2-mediated diseases (103). On neutralization of IL-18 in co-cultures of dendritic type 1 cells with allogeneic naive T lymphocytes, the Th1-Th2 phenotype was not affected, whereas antibodies to IL-12 down-regulated the Th1 response (104). In fact, IL-18 receptors were expressed on dendritic cells of the type 2 lineage, which suggests a Th2 response (104).

## BLOCKING INTERLEUKIN 18 IN MODELS OF AUTOIMMUNE DISEASE

As with any cytokine, the role of IL-18 in a particular disease process is best assessed by using specific neutralization of the cytokine in a complex disease model. Although mice deficient in IL-18 have been generated and tested for the development of autoimmune diseases (77), any reduction in severity may be due to a reduction in the immune response. IL-18 neutralization in wild-type mice is effective in reducing both collagen-induced arthritis (75) and inflammatory arthritis (68). Inflammatory arthritis is of particular relevance because this is a model of cartilage loss as the result of decreased proteoglycan synthesis and is independent of IFN- $\gamma$ . IL-18 contributes to the classic Th1 disease models, autoimmune encephalomyelitis (82, 105) and lupus-like disease (79), both via IFN- $\gamma$  production.

## INTERLEUKIN 18, INTERFERON $\gamma$ , AND THE HEART


Unrelated to IFN- $\gamma$  production, IL-18 is an important cytokine in myocardial ischemia reperfusion injury, a model of acute infarctions, where it functions to decrease the contractile force of the heart. Human heart tissue contains preformed IL-18 in macrophages and endothelial cells (51). On reducing IL-18 activity with either IL-18BP or a caspase-1 inhibitor, the functional impairment of the ischemia reperfusion injury is reduced (51). A neutralizing antibody to IL-18 results in near prevention of endotoxin-induced myocardial suppression in mice, and myocardial IL-1 $\beta$  concentrations are also reduced (90). With the use of caspase-1-deficient mice subjected to ligation of the left anterior descending coronary artery as a model for myocardial infarction, significantly lower mortality was observed in the deficient mice than in the wild-type mice (106). Caspase-1-deficient mice also had lower IL-18 concentrations, metalloproteinase-3 activity, and myocyte apoptosis after the injury. In another study, myocardial tissue steady-state concentrations of IL-18, IL-18R $\alpha$ , and IL-18BP mRNA and their respective proteins were measured in patients with end-stage heart

failure. Both circulating plasma and myocardial tissue concentrations of IL-18 were higher in the heart failure patients than in the age-matched healthy control subjects (107). In fact, plasma IL-18 concentrations were significantly higher in the patients who died than in survivors (107).

The evidence is increasing that IL-18 contributes to atherosclerosis. Unlike the IFN- $\gamma$ -independent role of IL-18 in ischemic heart disease, the atherosclerotic process involves infiltration of the arterial wall by macrophages and T cells, and IFN- $\gamma$  has been identified in the plaque and is considered essential for the disease (108). Human atherosclerotic plaques from the coronary arteries exhibit higher concentrations IL-18 and IL-18 receptors than found in nondiseased segments of the same artery (109). The post-caspase-1 cleavage IL-18 was found to colocalize with macrophages, whereas IL-18 receptors were expressed on endothelial and smooth muscle cells. The induction of IFN- $\gamma$  in smooth muscle cells by the combination of IL-18 and IL-12 is an unexpected but important finding for the pathogenesis of atherosclerosis (108, 109).

Atherosclerotic arterial lesions with infiltrating macrophages and T cells develop spontaneously in male apolipoprotein (apo) E-deficient mice fed a normal diet. When injected for 30 d with IL-18, these mice exhibit a doubling of the lesion size without a change in the concentration of serum cholesterol (108). There is also a four-fold increase in infiltrating T cells. However, when apo E-deficient mice are backcrossed into IFN- $\gamma$ -deficient mice, the IL-18-induced increase in lesion size is not observed (108). Although exogenous administration of IL-18 worsens the disease, such an experimental design can be related to the dose of IL-18. Therefore, reduction of natural concentrations of IL-18 in apo E-deficient mice is a more rigorous assessment for a role of IL-18 in atherosclerosis. With the use of apo E-deficient mice and overexpression of IL-18BP by transfection with an IL-18BP-containing plasmid, reduced numbers of infiltrating macrophages and T cells, decreases in cell death, and decreases in the lipid content of the plaques are found (91). In addition, increases in smooth muscle cells and collagen content suggest a stable plaque phenotype with prevention of progression in this well-established model of human coronary artery disease.

## CONCLUSIONS

In this review, the roles of IL-1 and IL-18 are summarized with respect to the aging process, primarily from the viewpoint that both cytokines initiate inflammation. Although a large body of evidence suggests a role of IL-1 in inflammation, less is known about IL-18. In addition to its role in autoimmune diseases, IL-18 contributes to the process of atherosclerosis and therefore by inference, to aging. Although there are no studies on IL-18 as an anorectic agent, considerable data support a role for IL-1 in loss of appetite. Clearly, reducing IL-1 and IL-18 activities can be viewed as possible therapeutic strategies to slow the aging process. Although specific, naturally occurring inhibitors of IL-1 and IL-18 can be used to reduce inflammation, dietary supplementation with n-3 fatty acids or increased consumption of foods rich in these fatty acids reduces IL-1 and TNF- $\alpha$  production. 

The author is one of the inventors of IL-1 $\beta$  and IL-18BP, neither of which generates royalties or has been a source of funding or income. The author does not hold stocks or receive remuneration from companies selling either of these reagents.



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