Interleukin-18 and the Pathogenesis of Inflammatory Diseases

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Summary: Several autoimmune diseases are thought to be mediated in part by interleukin (IL)-18. Many are those with associated increased interferon-γ (IFNγ) levels such as systemic lupus erythematosus, macrophage activation syndrome, rheumatoid arthritis, Crohn’s disease, psoriasis, and graft-versus-host disease. In addition, ischemia, including acute renal failure in human beings, appears to involve IL-18. Animal studies also support the concept that IL-18 is a key player in models of lupus erythematosus, atherosclerosis, graft-versus-host disease, and hepatitis. Unexpectedly, IL-18 plays a role in appetite control and the development of obesity. IL-18 is a member of the IL-1 family; IL-1β and IL-18 are related closely, and both require the intracellular cysteine protease caspase-1 for biological activity. The IL-18 binding protein, a naturally occurring and specific inhibitor of IL-18, neutralizes IL-18 activities and has been shown to be safe in patients. Other options for reducing IL-18 activities are inhibitors of caspase-1, human monoclonal antibodies to IL-18, soluble IL-18 receptors, and anti–IL-18 receptor monoclonal antibodies.

Select cytokines appear to be pivotal in lupus. Based on experimental and human evidence, interleukin (IL)-18 is central in the pathogenesis of tissue destruction in this illness. IL-18 levels are increased substantially in lupus patients as compared with normal individuals.1 Similarly, IL-18 is upregulated in the kidney of the MRL-Fas

IL-18 is a member of the IL-1 family and is related structurally to IL-1β.2 Recently, a new member of the IL-1 family, IL-33, was reported; structurally IL-33 is related closely to IL-18.6 But unlike IL-18, IL-33 binds to its own receptor, ST2, a long-time orphan receptor in the IL-1 family of cytokines.6 The IL-1β and the IL-18 precursors require caspase-1 for processing and release.6 Therefore, antiproteases that inhibit caspase-1 reduce both the processing and release of IL-1β and IL-18. Now, IL-33 can be added to the list of members of the IL-1 family, which require caspase-1 for processing and release.6 However, it is important to note that IL-18 is not a recapitulation of the biology or clinical significance of IL-1 or similar to the biological activity of IL-33; in fact, IL-18 is a unique cytokine showing inflammatory and immunoregulatory processes that are distinct from IL-1β or IL-33. For example, IL-1β is not required for interferon-γ (IFNγ) production whereas IL-18 is required.10 Initially thought of as primarily a T-helper 1 (Th1)-polarizing cyto-
kine, IL-18 also is relevant to T-helper 2 (Th2) diseases. As discussed in this review, animal models reveal that targeting IL-18 holds promise for the treatment of autoimmune and inflammatory diseases.

**IL-18 AS AN IMMUNOREGULATORY CYTOKINE**

The importance of IL-18 as an immunoregulatory cytokine is derived from its prominent biological property of inducing IFNγ. IL-18 first was described in 1989 in the serum after an injection of endotoxin into mice pretreated with *Propionibacterium acnes* and shown to induce IFNγ; however, at that time many investigators concluded that the serum factor was nothing but IL-12. With a great deal of diligence, the putative IFNγ-inducing factor activity was purified from thousands of mouse livers and the N-terminal amino acid sequence revealed a unique cytokine, not IL-12. With molecular cloning of the "IFNγ-inducing factor" in 1995, the name was changed to IL-18. Surprisingly, the new cytokine was related to IL-1, particularly to IL-1β. Both cytokines, lacking signal peptides, are first synthesized as inactive precursors, and neither is secreted via the Golgi. After cleavage by caspase-1, the active mature cytokines are released. Macrophages and dendritic cells are the primary sources for active IL-18, but the IL-18 precursor is found 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-1β, but it became clear that IL-18 activity also would be affected. In fact, any phenotypic characteristic of caspase-1–deficient mice undergoing inflammatory challenges must be differentiated as caused by reduced IL-1β or IL-18 activity. For example, the IL-1β–deficient mouse is susceptible to models of colitis whereas the caspase-1–deficient mouse is resistant; antibodies to IL-18 are protective whereas the IL-1–receptor antagonist is not. Because of its role in the production of IFNγ, T-cell polarization is a characteristic of IL-18 whereas IFNγ induction is not a prominent characteristic of IL-1. IL-18 shows 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 (FasL). The induction of fever, an important clinical property of IL-1, tumor necrosis factor α (TNFα), and IL-6, is not a property of IL-18. Injection of IL-18 into mice, rabbits, or human beings does not produce fever. Unlike IL-1 and TNFα, IL-18 does not induce cyclooxygenase-2 and hence there is no production of prostaglandin E2. IL-18 has been administered to human beings for the treatment of cancer to increase the activity and expansion of cytotoxic T cells. Although the results of clinical trials are presently unknown, several preclinical studies have shown the benefit of IL-18 administration in certain models of rodent cancer. Not unexpectedly and similar to several cytokines, the therapeutic focus on IL-18 has shifted from its use as an immune stimulant to inhibition of its activity.

Because IL-18 can increase IFNγ production, blocking IL-18 activity in autoimmune diseases is potentially an attractive therapeutic target. However, anti–IL-12 has been shown to reduce the severity of Crohn’s disease and psoriasis. Therefore, IL-12 can induce IFNγ in the absence of IL-18. However, there are many models of IL-18 activity independent of IFNγ. For example, we recently reported a new cytokine, IL-32, which was discovered in the total absence of IL-12 or IFNγ. Furthermore, models of inhibition of proteoglycan synthesis is IL-18 dependent, but IFNγ independent. In addition, IL-18–dependent melanoma metastasis to the liver is IFNγ independent. The results of preclinical studies and the targeting of IL-18 to treat autoimmune and inflammatory diseases are discussed in this article.

**Therapeutic Strategies for Reducing IL-18 Activities**

The strategies for reducing IL-18 activity include neutralizing anti–IL-18 monoclonal antibodies, caspase-1 inhibitors, and blocking antibodies to the IL-18–receptor chains. Caspase-1 inhibitors are oral agents and are presently in clinical trials in rheumatoid arthritis; a reduction in the signs and symptoms of the disease has been observed. Caspase-1 inhibitors pre-
vent the release of active IL-1β and IL-18, and therefore may derive 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. IL-18BP is not a soluble form of either chain of the IL-18 receptor but rather a constitutively secreted, high-affinity, and specific inhibitor of IL-18. IL-18BP is currently in clinical trials for the treatment of rheumatoid arthritis and severe psoriasis. Although the results of these trials have not been published, in phase I and phase II clinical trials, IL-18BP was safe even at the highest doses in more than 6 weeks of treatment.

Caspase-1 and Non–Caspase-1 Processing of IL-18

The importance of caspase-1 in inflammation has been shown in patients with mutations in the NALP3 gene locus, which participates 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-1β. Clinical manifestations include mental retardation, hearing loss, exquisite sensitivity to cold, and deforming arthritis. Some patients have extremely high levels of serum amyloid A protein with renal deposits and terminal renal failure; within a few days of IL-1 blockade using the IL-1 receptor antagonist, these patients show a near-total reversal in both the symptoms and the biochemical abnormalities of the disease. It remains likely that IL-18 also contributes to disease in these patients.

The non–caspase-1 enzyme associated with processing both the IL-1β and the IL-18 precursors is proteinase-3. Agonistic autoantibodies to proteinase-3 are pathologic in Wegner’s granulomatosis and may contribute to the non–caspase-1 cleavage of the IL-18 precursor and IFNγ production in this disease. Epithelial cells stimulated with proteinase-3 in the presence of endotoxin release active IL-18 into the supernatant. Because lactate dehydrogenase activity is not released, the appearance of active IL-18 is not a result of cell leakage or death. Injecting mice with recombinant FasL results in hepatic damage, which is IL-18 dependent. However, FasL-mediated cell death is IL-18 dependent and caspase-1 independent, but ischemia-reperfusion injury results in cell death via an IL-18– and caspase-1–dependent pathway.

P2X7 Receptor Targeting

The P2X7 receptor is involved in the secretion of IL-1β and IL-18. Stimulation of this receptor by adenosine triphosphate is a well-described event in the release of IL-1β and IL-18. A tyrosine derivative named KN-62 shows selective P2X7-receptor–blocking properties. In a study of small molecule inhibitors of this receptor, analogues of KN-62–related compounds were characterized for their ability to affect the human P2X7 receptor on monocyte-derived human macrophages. Although several analogues inhibited the secretion of IL-1β, no data exist on the effect of these inhibitors on IL-18 secretion. Unlike IL-1β, the secretion of IL-18 is mostly studied in vivo in mice that have been treated with Corynebacterium parvum, rather than in vitro. In vitro, the release of IL-18 requires the presence of activated T cells.

Targeting the IL-18 Receptors

Antibodies to either chain of the IL-18–receptor complex are attractive options for treating IL-18–mediated diseases. The IL-18 receptor chains (IL-18Rα and IL-18Rβ) are members of the IL-1–receptor family. The binding sites for IL-18 to the IL-18–receptor α chain are similar to those for IL-1 binding to the IL-1–receptor type I. Two sites bind to the ligand binding chain (IL-18Rα) and a third site binds to the IL-18Rβ chain, 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 (Fig. 1). The Toll domains of the IL-18 receptors are similar to the same domains of the Toll-like receptors, which recognize various microbial products, viruses, and nucleic acids. As a therapeutic option, however, commercial antibodies generated to the IL-18–receptor α and β chains are 100-fold less effective in neutralizing IL-18 activity compared with the IL-18BP. Nevertheless, the development of blocking antibodies to IL-18–receptor chains remains a vi-
able therapeutic option because an antibody to the type I IL-1–receptor chain is in clinical trials in rheumatoid arthritis. Unless converted into a fusion protein in somewhat the same manner as that of other soluble cytokine receptors, it is unlikely that the soluble form of the monomeric form of the IL-18Rα is a candidate therapeutic agent because of its low affinity. Another member of the IL-1 family (IL-1F), IL-1F7, 43 may be the naturally occurring receptor antagonist of IL-18. IL-1F7 binds to the IL-18Rα chain with a high affinity but this binding does not recruit the IL-18Rβ chain. The occupancy of the IL-18Rα without formation of the heterodimer with the IL-18Rβ 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 44, 45 and the biological significance of IL-1F7 binding to the IL-18Rα remains unclear. However, in the presence of low concentrations of IL-18BP, IL-1F7 reduces the activity of IL-18. 46

IL-18 Binding Protein

The discovery of the IL-18BP took place during the search for the extracellular (soluble) receptors for IL-18 in human urine. Nearly all the soluble cytokine receptors are found in human

Figure 1. IL-18 activation of cell signaling. Mature IL-18 binds to the IL-18Rα chain and recruits the IL-18Rβ chain resulting in the formation of a heterodimeric complex. As a result of the formation of the extracellular complex, the intracellular chains also form a complex which brings the Toll domains of each receptor chain into close proximity. Although poorly understood, the close proximity of the Toll domains recruits the intracellular protein MyD88 to the receptor chains. MyD88 is common to cells activated by IL-1, IL-18 and TLR-4 ligands (endotoxins). Following MyD88 recruitment, there is a rapid phosphorylation of the IL-1 receptor activating kinases (IRAK). There are four IRAK proteins. Depending on the cell type, other kinases have been reported to undergo phosphorylation. These are the TNF receptor activating factor (TRAF)-6 and inhibitory kappa B kinases (IKK) α and β (not shown). Phosphorylation of IKK results in the phosphorylation of IκB and translocation of NFκB to the nucleus. However, this is not observed uniformly in all cell types and there are distinct differences in NFκB activation in different cells stimulated with IL-18. 13 In addition, IL-18-activated cells phosphorylate mitogen activating protein kinase (MAPK) p38. In IL-18-activated cells, new genes are expressed and translated. Those shown in the figure represent the pro-inflammatory genes. Preventing IL-18-induced cellular activation is accomplished by the presence of IL-18BP. 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 the IL-18Rβ-chain deprives the cell of the participation of IL-18Rβ-chain in activating the cell.
For example, the TNF p75 soluble receptor, used widely for the treatment of rheumatoid arthritis, ankylosing spondylitis, and psoriasis, initially was purified and sequenced using ligand-specific affinity chromatography. In searching for IL-18 soluble receptors, IL-18 was bound covalently to a matrix and highly concentrated human urine, donated by Italian nuns, was passed over the matrix and eluted with acid to disrupt the ligand (in this case 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. This was a result of the higher affinity of the IL-18BP for the ligand compared with the soluble receptors.

The IL-18BP is a constitutively secreted protein, with high-affinity (400 pmol/L) binding to IL-18. There is very limited amino acid sequence homology between IL-18BP and the cell surface IL-18 receptors; IL-18BP lacks a transmembrane domain and contains only 1 immunoglobulin (Ig)-like domain. 18BP shares many characteristics with the soluble form of the IL-1 type II receptor 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 human beings, IL-18BP is expressed highly in spleen and the intestinal tract, both immunologically active tissues. Alternate messenger RNA (mRNA) splicing of IL-18BP results in 4 isoforms. Of considerable importance is that the prominent ‘a’ isoform is present in the serum of healthy human beings at a 20-fold molar excess compared with IL-18. 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 responses to a routine infection. The promoter for IL-18BP contains 2 IFNγ response elements and constitutive gene expression for IL-18BP is IFNγ dependent, suggesting a compensatory feedback mechanism. Thus, increased levels of IFNγ stimulate more IL-18BP in an attempt to reduce IL-18–mediated IFNγ production. For example, in mice deficient in IFN regulatory factor-1, a transcription factor for IFNγ, low to absent tissue levels of IL-18BP are found compared with wild-type mice. These IFN regulatory factor-1–deficient mice are exquisitely sensitive to colitis, but when treated with exogenous IL-18BP they show reduced disease.

**Viral IL-18BP**

The most convincing evidence that IL-18 is a major player in inflammatory conditions and that IL-18BP is functional in combating inflammation comes from a natural experiment in human beings. *Mollusca contagiosa* is a common viral infection of the skin often seen in children and individuals with human immunodeficiency virus-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 Poxviruses. The greatest homology is with in *M contagiosa*. The viral genes encoding for viral IL-18BP have been expressed and the recombinant proteins neutralize mammalian IL-18 activity. 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 this natural experiment of *M contagiosa* infection that blocking IL-18 reduces immune and inflammatory processes such as the function of dendritic and inflammatory cells.

**IL-18:IL-18BP Imbalance in Macrophage-Activating Syndrome**

Also known as *hemophagocytic syndrome*, macrophage-activating syndrome (MAS) is characterized by an uncontrolled and poorly understood activation of Th-1 lymphocytes and macrophages. In a study of 20 patients with MAS secondary to infections, autoimmune disease, lymphoma, or cancer, the concentrations of circulating IL-18, IL-18BP, IFNγ, and IL-12 were determined and matched with clinical parame-
Evidence of stimulation of macrophages and natural killer (NK) cells was highly increased in MAS but not in control patients. Most importantly, concentrations of IL-18BP were increased only moderately, resulting in a high level of biologically active free IL-18 in MAS (4.6-fold increase compared with controls; \( P < 0.001 \)). Others have reported marked expression of IL-18 in fatal MAS. Free IL-18 but not IL-12 concentrations significantly correlated with clinical status and the biological markers of MAS such as anemia, hypertriglyceridemia, and hyperferritinemia, and also with markers of Th-1 lymphocyte or macrophage activation such as increased concentrations of IFN\( \gamma \), soluble IL-2, and TNF-receptor concentrations. Therefore, treatment of life-threatening MAS with IL-18BP is a logical therapeutic intervention to correct the severe IL-18:IL-18BP imbalance resulting in Th-1 lymphocyte and macrophage activation.

Neutralizing Antibodies to IL-18

Although there are no clinical trials of neutralizing antibodies to IL-18, preclinical studies have used IL-18 antibodies to reduce IL-18 activity in animal models of disease. The results of these studies are shown in Table 1. Assuming that neutralizing antibodies to IL-18 are developed and tested in human diseases, what are the anticipated differences between a neutralizing antibody and a neutralizing soluble receptor or a binding protein such as IL-18BP? First, to evaluate such differences, the agent with the highest affinity is preferable. From a pharmacokinetic viewpoint, a long half-life is preferable. One can increase the binding affinity for a ligand by converting a soluble receptor or binding protein to a divalent fusion protein. However, the danger here is the increased risk of creating a novel epitope for antibody production. The advantage of monoclonal antibodies is that they are human and the risk of developing antibodies to a human antibody is reduced significantly. At first glance, one would conclude that high-affinity human antibodies to IL-18 are preferable to the IL-18BP. However, if a divalent fusion protein of IL-18BP has a high affinity and is not immunogenic, the next issue is a comparison of the half-life of a monoclonal antibody with that of a fusion protein. Here the issue is one of safety. A short half-life is preferential for rapid cessation of therapy in the event of a life-threatening infection whereas long half-life antibodies exert their effects of suppressing host defense for weeks. In fact, the large body of evidence for comparing infections associated with anti-TNF\( \alpha \) monoclonal antibodies (infliximab or adalimumab) with the soluble TNF p75 receptor fusion protein (etanercept) may, in part, be owing to differences in half-life and mechanisms of action. In the case of neutralizing IL-18, the suppression of IFN\( \gamma \) is of concern for host defense against intracellular organisms such as *Mycobacterium tuberculosis*.

Blocking IL-18 in Disease Models

As with any cytokine, its role 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, any reduction in severity may be caused by a reduction in the immune response such as antigens or the sensitization processes itself and does not address the effect of IL-18 on established disease. IL-18 neutralization in wild-type mice is effective in reducing collagen-induced arthritis and inflammatory arthritis. Inflammatory arthritis is of particular relevance because this is a model of cartilage loss caused by decreased proteoglycan synthesis and is independent of IFN\( \gamma \). IL-18 contributes to the lupus-like disease in mice via IFN\( \gamma \) production. These are other models that are based on a reduction in IL-18 activity in wild-type mice. Caspase-1-deficient mice provide useful models for disease but here the effect may be on IL-1\( \beta \), IL-18, or both. 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 resulted in a reduction of 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 IFN\( \gamma \) independent. Prevention of melanoma metastases is IL-18 dependent but IFN\( \gamma \) independent, and similar findings exist for ischemia-reperfusion injury in
<table>
<thead>
<tr>
<th>Disease Model</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute dextran sulphate sodium–induced colitis</td>
<td>Anti–IL-18 antibodies,11 IL-18BP&lt;sup&gt;94&lt;/sup&gt;</td>
<td>↓ clinical disease, ↓ TNFα, IFNγ, IL-1, MIP-1&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Chronic dextran sulphate sodium–induced colitis</td>
<td>Caspase-1 K&lt;sup&gt;12&lt;/sup&gt;</td>
<td>↓ IL-1β, IFNγ&lt;sub&gt;2&lt;/sub&gt;, and CD3 cells</td>
</tr>
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<td>TNBS-induced colitis</td>
<td>IL-18BP&lt;sup&gt;95&lt;/sup&gt;, Adenoviral antisense IL-18&lt;sup&gt;96&lt;/sup&gt;</td>
<td>↓ clinical disease, ↓ cytokines</td>
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<tr>
<td>CD62/CD4 T-cell–induced colitis</td>
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<td>↓ clinical disease, ↓ mucosal IFNγ</td>
</tr>
<tr>
<td>Streptococcal wall–induced arthritis</td>
<td>IL-18 antibodies&lt;sup&gt;19&lt;/sup&gt;</td>
<td>↑ cartilage proteoglycan synthesis, ↓ inflammation</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>IL-18BP, IL-18,61 Ad-viral IL-18BP&lt;sup&gt;97&lt;/sup&gt;</td>
<td>↓ clinical disease, ↓ cytokines</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>IL-18–deficient mice&lt;sup&gt;60&lt;/sup&gt;</td>
<td>↓ clinical disease, ↓ cytokines</td>
</tr>
<tr>
<td>Graft–versus-host disease</td>
<td>Anti–IL-18&lt;sup&gt;56&lt;/sup&gt;</td>
<td>↓ CD8&lt;sup&gt;+&lt;/sup&gt;–mediated mortality</td>
</tr>
<tr>
<td>Lupus-prone mice</td>
<td>IL-18 vaccination&lt;sup&gt;1&lt;/sup&gt;</td>
<td>↓ mortality, ↓ nephritis</td>
</tr>
<tr>
<td>Allergic airway hyperresponsiveness</td>
<td>IL-18 vaccination&lt;sup&gt;98&lt;/sup&gt;</td>
<td>↓ bronchoconstriction</td>
</tr>
<tr>
<td>Experimental myasthenia gravis</td>
<td>Anti–IL-18&lt;sup&gt;59&lt;/sup&gt;</td>
<td>↓ clinical disease</td>
</tr>
<tr>
<td>Autoimmune encephalomyelitis</td>
<td>Caspase-1 knock-out&lt;sup&gt;100&lt;/sup&gt;</td>
<td>↓ clinical disease, ↓ IFNγ</td>
</tr>
<tr>
<td>Con-A–induced hepatitis</td>
<td>Anti-18,101 IL-18BP,30 IL-18BP-Tg&lt;sup&gt;102&lt;/sup&gt;</td>
<td>↓ liver enzyme levels</td>
</tr>
<tr>
<td>Fas-mediated hepatic failure</td>
<td>IL-18–deficient mice,103 IL-18BP&lt;sup&gt;30&lt;/sup&gt;</td>
<td>↓ liver necrosis</td>
</tr>
<tr>
<td>Pseudomonas exotoxin-A hepatitis</td>
<td>IL-18BP&lt;sup&gt;30&lt;/sup&gt;</td>
<td>↓ liver enzyme levels, ↓ IFNγ</td>
</tr>
<tr>
<td>IL-12–induced IFNγ</td>
<td>Anti-18,104 caspase-1 knock-out&lt;sup&gt;104&lt;/sup&gt;</td>
<td>↓ IFNγ</td>
</tr>
<tr>
<td>Endotoxin-induced IFNγ</td>
<td>Anti-18,5,105 IL-18BP&lt;sup&gt;22,30&lt;/sup&gt; caspase-1 knock-out&lt;sup&gt;106&lt;/sup&gt;</td>
<td>↓ IFNγ</td>
</tr>
<tr>
<td>Lipopolysaccharide–induced hepatic necrosis</td>
<td>Anti–IL-18 monoclonal,5,105 IL-18BP&lt;sup&gt;30&lt;/sup&gt;</td>
<td>↓ necrosis, ↓ TNFα, ↓ FasL</td>
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<tr>
<td>Lipopolysaccharide–induced lung neutrophils</td>
<td>IL-18&lt;sup&gt;105&lt;/sup&gt;</td>
<td>↑ survival, ↓ myeloperoxidase</td>
</tr>
<tr>
<td>Melanoma hepatic metastasis</td>
<td>IL-18BP&lt;sup&gt;20,105&lt;/sup&gt;</td>
<td>↓ metastatic foci, ↓ VCAM-1</td>
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<tr>
<td>Ischemia-induced hepatic failure</td>
<td>Anti-18&lt;sup&gt;107&lt;/sup&gt;</td>
<td>↓ apoptosis, ↓ nuclear factor κ B</td>
</tr>
<tr>
<td>Ischemia-induced acute renal failure</td>
<td>Anti–IL-18 polyclonal,12 caspase-1 knock-out&lt;sup&gt;12&lt;/sup&gt;</td>
<td>↓ creatinine level, ↓ urea level</td>
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<tr>
<td>Ischemic myocardial dysfunction</td>
<td>IL-18BP,31 caspase-1 inhibition&lt;sup&gt;31&lt;/sup&gt;</td>
<td>↑ myocardial contractility</td>
</tr>
<tr>
<td>Lipopolysaccharide–induced myocardial suppression</td>
<td>Anti–IL-18 polyclonal&lt;sup&gt;73&lt;/sup&gt;</td>
<td>↑ heart contractility, ↓ IL-1β</td>
</tr>
<tr>
<td>Atherosclerosis in apoE knock-out mice</td>
<td>IL-18BP&lt;sup&gt;78&lt;/sup&gt;</td>
<td>↓ plaques, ↓ infiltrates, ↑ vessel collagen</td>
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</table>

Abbreviations: TNBS, trinitrobenzene sulfonic acid; Con-A, concanavalin A; VCAM-1, vascular cell adhesion molecule-1.
the heart, kidney, and liver. Table 1 lists various animal models of Th1-, Th2-, and non–immune-mediated diseases in which the effect of reducing endogenous IL-18 activities has been reported.

**IL-18 in Th1-Like Diseases**

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 IFNγ. The effect of IL-12 is, in part, to increase the expression of IL-18 receptors on T lymphocytes, thymocytes, and NK cells. It appears that the role of IL-18 in the polarization of the Th1 response is dependent on IFNγ and IL-12 receptor β2 chain expression. The production of IFNγ by the combination of IL-18 plus IL-12 is an example of true synergism in cytokine biology, similar to the synergism of IL-1 and TNFα in models of inflammation. Because IFNγ is the signature cytokine of CD4+ and CD8+ T cells and NK cells, a great deal of the biology of IL-18 is considered owing to IFNγ production. Dendritic cells deficient in the IFNγ transcription factor T-bet show impaired IFNγ production after stimulation with IL-18 plus IL-12. IL-18 is present constitutively in monocytes and monocyte-derived dendritic type 1 cells. Thus, IFNγ induced by the combination of IL-12 plus IL-18 appears to be via the T-bet transcription factor.

**Graft-Versus-Host Disease**

IFNγ plays a major pathologic role in this disease because of its Th1-inducing properties and the generation of cytotoxic T cells. By using a cohort of 157 patients who received unrelated donor bone marrow transplantation and developed graft-versus-host disease, a polymorphism in the IL-18 promoter (G137C, C607A, G656T) was identified and associated with a statistically significant decreased risk of death. One hundred days after the transplant, the mortality in patients with this polymorphism was 23%, compared with 48% in those patients without the polymorphism, and after 1 year the mortality was 36% versus 65%, respectively. The probability of survival was 2-fold in patients with this haplotype. In the case of graft-versus-host disease in mice, paradoxical effects of IL-18 have been reported depending on whether the disease is CD4+ or CD8+ T-cell mediated. In human beings, T cells are responsible for the disease after allogeneic bone marrow transplantation. Administration of IL-18 to recipient mice increased survival in CD4+-mediated disease, but resulted in worsening in the CD8+-mediated disease. Neutralizing anti–IL-18 monoclonal antibodies significantly reduced CD8+-mediated mortality. Administration of IL-18 reduces the severity of the disease by inducing the production of Th2 cytokines. The importance of IL-18 in graft-versus-host disease also was shown in mice deficient in the IL-18 receptor α chain (Table 2). Other models of disease in mice deficient in this receptor chain are listed in Table 2.

**IL-18 and Th2 Diseases**

The combination of IL-18 plus IL-12 suppresses IgE synthesis via IFNγ 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, eosi-

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**Table 2. Studies on IL-18 Receptor–Deficient Mice**

<table>
<thead>
<tr>
<th>Model</th>
<th>Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 response</td>
<td>↓ IFNγ production; ↓ cytotoxicity</td>
<td>108</td>
</tr>
<tr>
<td>IL-18/IL-2–induced lung injury</td>
<td>↓ lethality</td>
<td>109</td>
</tr>
<tr>
<td>Graft-versus-host disease</td>
<td>↓ disease severity</td>
<td>110</td>
</tr>
<tr>
<td>Lupus-prone mice</td>
<td>↓ disease lethality; ↓ nephritis</td>
<td>111</td>
</tr>
<tr>
<td>NK-mediated cytotoxicity in vivo*</td>
<td>↓ cytotoxicity</td>
<td>112</td>
</tr>
<tr>
<td>Dextran sulphate sodium colitis</td>
<td>↑ disease and lethality</td>
<td>113</td>
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**NOTE.** Unless stated otherwise, IL-18 receptor–deficient mice lack the IL-18Rα chain.

* Mice are deficient in IL-18–receptor β chain.
nophila, and airway hyperresponsiveness. In contrast, the administration of IL-18 alone enhanced basophil production of IL-4 and histamine and increased serum IgE levels in wild-type and IL-4–deficient mice. Overexpression of mature IL-18 in the skin results in worsening of allergic and nonallergic cutaneous inflammation via Th2 cytokines. 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 was increased serum IgE levels. Although IL-18 remains a Th1 cytokine, there are increasing reports showing a role for IL-18 in promoting Th2-mediated diseases. In cocultures of dendritic type 1 cells with allogeneic naive T lymphocytes, the Th1/Th2 phenotype was not affected whereas anti–IL-12 downregulated the Th1 response. In fact, IL-18 receptors were expressed on dendritic cells of the type-2 lineage, suggesting a Th2 response.

**IL-18 and the Heart**

Unexpectedly, IL-18 is an important cytokine in myocardial ischemia-reperfusion injury, a model of acute infarctions, in which it functions to decrease the contractile force of the heart. It appears that the role of IL-18 in myocardial dysfunction is independent of IFNγ but likely is related to the induction of Fas ligand. Human heart tissue contains preformed IL-18 in macrophages and endothelial cells. On reducing IL-18 activity with either IL-18BP or a caspase-1 inhibitor, the functional impairment of the ischemia-reperfusion injury was reduced. A neutralizing anti–IL-18 polyclonal antibody resulted in near prevention of endotoxin-induced myocardial suppression in mice and myocardial IL-1β levels also were reduced. By using 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 compared with the wild-type mice. Caspase-1–deficient mice also had lower levels of IL-18, metalloproteinase-3 activity, and myocyte apoptosis after the injury. In human beings, myocardial tissue steady-state levels of IL-18, IL-18Rα chain, and IL-18BP mRNA and their respective protein levels were measured in patients with end-stage heart failure. Circulating plasma and myocardial tissue levels of IL-18 were increased in the patients compared with the age-matched healthy subjects. However, mRNA levels of IL-18 BP were decreased in the failing myocardium. In fact, plasma IL-18 levels were significantly higher in patients who died compared with levels in survivors.

There is increasing evidence that IL-18 contributes to atherosclerosis. Unlike the IFNγ-independent role of IL-18 in ischemic heart disease, the atherosclerotic process involves infiltration of the arterial wall by macrophages and T cells and IFNγ has been identified in the plaque and considered essential for the disease. Human atherosclerotic plaques from the coronary arteries show increased IL-18 and IL-18 receptors compared with nondiseased segments of the same artery. 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 localization of IL-18 and IL-18 receptors in smooth muscle cells is an unexpected but important finding for the pathogenesis of atherosclerosis.

Atherosclerotic arterial lesions with infiltrating, lipid-laden macrophages and T cells develop spontaneously in male apolipoprotein E (apoE)-deficient mice fed a normal diet. When injected for 30 days with IL-18, these mice showed a doubling of the lesion size without a change in serum cholesterol level. There was also a 4-fold increase in infiltrating T cells. However, when apoE-deficient mice were backcrossed into IFNγ-deficient mice, the IL-18–induced increase in lesion size was not observed. Although exogenous administration of IL-18 worsened the disease, such an experimental design can be related to the dose of IL-18. Therefore, reduction of natural levels of IL-18 in the apoE-deficient mice is a more rigorous assessment for a role for IL-18 in atherosclerosis. By using apoE-deficient mice and overexpression of IL-18BP by transfection with an IL-18BP-containing plasmid, reduced numbers of infiltrating macrophages and T cells, and decreases in cell death and lipid content of the
plaques were found. In addition, increases in smooth muscle cells and collagen content suggested a stable plaque phenotype with prevention of progression in this well-established model of human coronary artery disease.

**IL-18 and Renal Ischemia**

Similar to myocardial ischemia-reoxygenation, there is an unexpected role in renal ischemia for IL-18, which is independent of T cells and IFNγ. Clinically, loss of renal function in patients with septic shock contributes to mortality significantly. The IL-18 level was measured in patients with acute renal failure and in patients with poor renal function. There was a remarkable high level of urinary IL-18 compared with other renal diseases ($P < .001$). IL-18 also was increased in the urine of patients with delayed function of cadaveric transplants. The conclusions of the study were that urinary IL-18 level is a marker for proximal tubular injury in acute renal failure.

In a large clinical study in intensive care units, the level of IL-18 in the urine of patients correlated with the development of renal failure more than creatinine as a predictor of impending renal failure. More impressively, based on IL-18 urine levels, it was possible to predict mortality in the intensive care unit by 48 hours, a time period nearly 2 days before other indicators of impending death. These findings in human beings are consistent with animal studies. By using a reversible model of acute renal failure, mice deficient in caspase-1 were protected, which was caused by impaired processing of the IL-18 precursor by caspase-1. Furthermore, wild-type mice also were protected by a preinfusion of neutralizing anti–IL-18 polyclonal antibodies. Protection was not afforded by administration of the IL-1–receptor antagonist and therefore the model reflects the role of IL-18 rather than IL-1β processing. Although the mechanism for the role of IL-18 in causing acute renal failure remains unclear, it is not related to a decrease in neutrophilic infiltration.

Cardiopulmonary bypass often results in acute renal failure. In 20 patients who developed acute renal failure after bypass surgery, serial urine samples were evaluated for IL-18 levels and compared with 35 matched control patients also undergoing cardiopulmonary bypass but without acute renal injury. Acute renal injury was defined as an increase in serum creatinine level of 50% or greater. The findings were remarkable in that increased creatinine levels occurred 48 to 72 hours after bypass surgery whereas urine IL-18 level was statistically significantly increased 4 to 6 hours after the end of surgery. Peak levels of urinary IL-18 were 25-fold greater 12 hours after surgery and remained increased for 48 hours. Multivariate analysis of increased urinary IL-18 and urinary neutrophil gelatinase-associated lipocalin, also increased 25-fold early in acute renal failure, revealed that these 2 markers were associated independently with the number of days of acute renal injury. These studies suggest that the increased urinary IL-18 levels predict acute renal injury after bypass surgery and may be used as a reliable biomarker rather than serum creatinine level.

**IL-18 Deficiency Triggers Overeating, Obesity, and Insulin Resistance**

Although mice deficient in IL-18 are resistant to various exogenous challenges, an unexpected observation was that as mice aged, they gained significantly more weight than wild-type control mice. By 6 months of age, IL-18–deficient mice were IL-18.5% heavier than age- and sex-matched wild-type mice and by 12 months were 38.1% heavier. The difference in weight was caused by more body fat. The basic metabolic rate and core temperature were not different between the 2 strains but increased food intake accounted for the weight gain. Not unexpectedly, leptin levels were higher in the IL-18–deficient mice and leptin levels correlated with body weight but there was no evidence that fat mice deficient in IL-18 were resistant to leptin. IL-6 levels were similar in the 2 groups. The islets of the IL-18–deficient mice showed normal architecture but were larger than those of wild-type mice. Histologic examination of major organs did not reveal significant difference but the aorta of the IL-18–deficient mice contained lipid deposits characteristic of atherosclerosis.
Mice deficient in IL-18 at 6 months of age showed increased fasting glucose compared with wild-type controls, although at 3 months of age there were no differences between the 2 groups. Glucose tolerance testing was abnormal in the IL-18-deficient mice and was consistent with insulin resistance. Mice deficient in the α chain of the IL-18 receptor also showed similar increases in weight at 6 months and increased plasma fasting glucose and were insulin resistant. In addition, mice overexpressing the natural inhibitor of IL-18, IL-18BP, overate, gained weight, and were hyperglycemic. The administration of recombinant murine IL-18 to the IL-18-deficient mice reversed insulin resistance. The increase in glucose level was prevented by the administration of recombinant IL-18 to either the wild-type or the IL-18-deficient mice, but not the IL-18 receptor-deficient mice.

The mechanism for the increased eating in mice deficient in IL-18, deficient in the IL-18 receptor, or in transgenic mice overexpressing the IL-18BP, appears to be a defect in the control of food intake by the hypothalamic satiety center. Insulin resistance in the liver and muscle were caused by the obese condition. Of importance is the observation that unlike IL-1β, IL-18 does not cause fever and does not induce cyclooxygenase-2. Phosphorylation of signal transducer and activator of transcription (STAT) 3 was defective in mice deficient in IL-18. Nevertheless, recombinant IL-18 administered intracerebrally inhibited food intake and, in addition, recombinant IL-18 reversed hyperglycemia in mice deficient for IL-18 through activation of STAT 3 phosphorylation. Hepatic genes for glucose neogenesis were increased in mice deficient in IL-18, possibly owing to the phosphorylation of STAT 3. In mice deficient for IL-18, there was less constitutive phosphorylation of STAT 3 in the liver. Because IL-18 is expressed constitutively in healthy mice and human beings, the decrease in STAT 3 phosphorylation may be owing to the lack of IL-18 in these mice. These findings indicate a new role of IL-18 in the homeostasis of energy intake and insulin sensitivity.

Conclusions on the Therapeutic Targeting of IL-18

Exploiting discoveries such as IL-18 to improve the treatment of disease depends on the validity of preclinical research, the resources of the developer, and the influence of market forces. There are no doubts in our opinion that IL-18 plays a role in several diseases and despite its use in murine models of tumors, we do not support the administration of IL-18 as a therapeutic. The challenge for a cytokine such as IL-18 is therefore which diseases to target and which agents are best to reduce IL-18 activities. One disease that may benefit blocking IL-18 is MAS. Current treatment of life-threatening MAS is intravenous cyclosporine A, a nephrotoxic inhibitor of IFNγ and other T-cell cytokines and high doses of corticosteroids. The role of IL-18 in IFNγ production and the role of IFNγ in macrophage activation are well established. The finding that patients with active MAS have high circulating levels of free IL-18 owing to lower than expected levels of IL-18BP provides a rational basis for reducing IL-18 in MAS. To prove the concept requires treating patients with progressive or established MAS with increasing doses of IL-18BP and monitoring ferritin levels and clinical responses. Because MAS can be a fatal disease, testing the concept requires no placebo arm and few patients. Outcomes of clinical improvements, a reduction in corticosteroids, and weaning of cyclosporine A would be sufficient for orphan drug status approval. Oral caspase-1 inhibitors also are deserving of testing in patients with MAS.

It is also our opinion that another acute and life-threatening disease can be subjected to a proof of concept—acute renal failure. Caspase-1 inhibitors would require intravenous administration rather than the oral route. However, preclinical testing in mice deficient in caspase-1 provides a rationale for the testing of caspase-1 inhibitors in patients at risk for acute renal failure. Because IL-18BP readily can be administered subcutaneously or intravenously, IL-18BP would be used in a trial and compared with a placebo arm. There may be reluctance to test a potentially useful anticytokine in acute renal failure because this disease is essentially the consequence of septic shock. The dissap-
pointing results of TNF and IL-1 blockers in sepsis trials may reduce enthusiasm for treating these patients. However, one important benefit for testing IL-18BP in preventing or reducing acute renal failure is the predictive value of urinary IL-18 levels. Because the urinary IL-18 level is increased in patients at risk a full 48 hours before the renal dysfunction becomes apparent, the design of a trial of IL-18BP infusion would be restricted to a subgroup of those patients. The clinical application of urinary IL-18 determinations as an entry criterion is substantial because of its quantification compared with clinical scoring methods. A reduction in progression to renal failure compared with placebo-treated patients would provide a basis for approval because preventing acute renal failure is an unmet medical need.

The third clinical testing of agents that reduce IL-18 activity is acute lupus nephritis and vasculitis. Here the role of IL-18 in the production of IFNγ may be of paramount importance. Animal models of lupus indicate a pathologic role for IL-18 in the kidney. However, the effect of IL-18 on the vasculature also may be part of the lupus vasculitis. Some early trials have blocked TNFα in acute lupus nephritis using the monoclonal anti-TNFα antibodies. In this case, these antibodies reduce both TNFα and IFNγ. Taken together, these preclinical observations provide a rationale for an intervention study in lupus nephritis. Testing the concept with oral caspase-1 inhibitors or IL-18BP administered subcutaneously asks whether the vessel wall inflammation can be reduced over that presently achieved by heparin, corticosteroids, and aspirin.

**IL-18 and Host Defense**

Similar to many cytokines of the innate immune response, there are 2 sides to the coin. IL-18 functions to protect the host by increased expression of adhesion molecules, neutrophil activation, and nitric oxide production, each targeting the killing of invading microbes. IL-18-mediated IFNγ production promotes specific cytotoxic T-cell–mediated responses, essential for elimination of viral infections. But cytokines such as IL-1, TNF, and IL-18 did not evolve to cause disease, but rather to oppose microbial invasion. It is the unregulated overproduction and persistent production of these cytokines that results in autoimmune and inflammatory diseases. In its role as an IFNγ-inducing factor, several models of obligate intracellular pathogens and fungal infections are worsened by a reduction or absence of IL-18 and administration of exogenous IL-18 can enhance the host defense against these microbes. However, the administration of IL-18 to patients to enhance their ability to fight infection is an unlikely clinical exercise because IFNγ already is used as a therapy to reduce infection caused by mycobacterial infections and in patients with chronic granulomatous disease.

**Consequence of IL-18–Blocking Therapy**

The use of TNFα-neutralizing strategies to treat Crohn’s disease and rheumatoid arthritis has resulted in reactivation of *M tuberculosis* infections, particularly disseminated and extrapulmonary forms. But these findings came as no surprise because blocking TNFα increased the spread and lethality of *M tuberculosis* infections in mice. In the case of chronic IL-18 blockade, a reduction in IL-18–dependent IFNγ production may result in a similar increase in reactivation of *M tuberculosis* disease. In fact, children born with defects in IFNγ production or activity die of disseminated mycobacterial disease. IL-18–deficient mice, although an extreme example of IL-18 blockade, show increased susceptibility to *M tuberculosis* infection. A high level and chronic reduction in IL-18 activity in human beings likely will affect host defense mechanisms, as is the case with TNF blockers. However, in the case of TNFα blockade, the risks of reactivation of *M tuberculosis* have been reduced dramatically by tuberculin testing.

It is likely that within 5 years the efficacy of reducing IL-18 activity in a few diseases will be established. Although IL-18BP presently is available for clinical trials, this potent naturally occurring binding protein, even with its remarkable safety record to date, has to be compared with other cytokine antagonists that presently dominate treating diseases such as rheumatoid arthritis and psoriasis. In fact, there are shrinking numbers of naïve patients with these dis-
cases willing to enter clinical trials in which the efficacy of the agents are not known or one may be randomized to receive placebo. Trials with large numbers of patients are required to compensate for the availability of naïve patients or trials must be performed in developing countries. Therefore, market-based decisions may take precedence over decisions to test for efficacy. The same decision-making processes also may affect the development of neutralizing antibodies to IL-18 or its receptors. The costs of large trials can affect the decision to test for efficacy in diseases such as rheumatoid arthritis, psoriasis, or Crohn’s disease, in which highly competitive markets already exist for anti-TNFα monoclonal antibodies (infliximab and adalimumab), anti-IL-12, and other agents. In rheumatoid arthritis a similar competitive environment dominates the area. In psoriasis, there are no less than 6 agents that are based on interruption of the immunologic mechanisms in this disease.

The breakthrough in reducing IL-18 activity in disease may come from the testing of oral caspase-1 inhibitors. These are in clinical trial in psoriasis and also in patients with NALP3 mutations in the cold-induced autoimmune syndrome locus. IL-18 is found constitutively in the skin and because of the importance of IFNγ in psoriasis, reducing IL-18 activity by reducing the processing of the IL-18 precursor may be a therapeutic advantage. Blocking IL-1 with the IL-1–receptor antagonist does not have a significant effect in patents with psoriatic arthritis.

Similar to the biologic IFNα, approval for an oral caspase-1 inhibitor will result in availability of the drug to physicians and novel testing. IFNα was approved initially for hairy cell leukemia, a truly rare disease; nevertheless, once approved, several uses for IFNα were explored by clinicians and the benefit of IFNα in the treatment of hepatitis B and C was established. In the next 5 years, one would hope that in rare diseases such as MAS approval for caspase-1 inhibitors or IL-18BP will take place. The availability of these agents in the hands of physicians likely will expand the concept that reducing IL-18 activities is a therapeutic option.106

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