The IL-1 family and inflammatory diseases

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ABSTRACT

IL-1 and its related family member IL-18 are primarily proinflammatory cytokines by their ability to stimulate the expression of genes associated with inflammation and autoimmune diseases. For IL-1 (IL-1α and IL-1β), the most salient and relevant properties are the initiation of cyclooxygenase type 2 (COX-2), type 2 phospholipase A and inducible nitric oxide synthase (iNOS). This accounts for the large amount of prostaglandin-E2 (PGE2), platelet activating factor and nitric oxide (NO) produced by cells exposed to IL-1 or in animals or humans injected with IL-1. Another important member of the pro-inflammatory IL-1 family is IL-18. IL-18 is also an important player in autoimmune disease because of its ability to induce IFNγ, particularly in combination with IL-12 or IL-15. Both IL-1 and IL-18 increase the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) on mesenchymal cells and vascular-cell adhesion molecule-1 (VCAM-1) on endothelial cells. This latter property promotes the infiltration of inflammatory and immunocompetent cells into the extravascular space. IL-1 and IL-18 are also an angiogenic factors by increasing the expression of vascular endothelial growth factor; IL-1and IL-18 thus play a role in pannus formation and blood vessel supply. The strongest case for the importance of IL-1 in disease processes come from the administration of the IL-1 receptor antagonist, also a member of the IL-1 family and IL-18 binding protein (IL-18BP), a constitutively expressed and secreted protein that binds and neutralizes IL-18. Data from the human genome project have revealed other members of the IL-1 family. However, these appear to be antagonists rather than agonists. IL-1 also acts as an adjuvant during antibody production and stimulates bone marrow stem cells for differentiation in the myeloid series. IL-1 is distinct from tumor necrosis factor (TNF); IL-1 and TNFα share several biological properties but the salient difference is that TNF receptor signaling induces programmed cell death whereas IL-1 receptor signaling does not. In fact, IL-1 is a hematopoietic growth factor and IL-1 was administered to humans to reduce the nadir of white blood cells and platelets in patients during bone-marrow transplantation. This property of IL-1 is not observed in the responses to TNFα. Furthermore, in animal models of destructive rheumatoid arthritis, IL-1 is necessary but TNFα is not.

Introduction

A great deal can be learned from studies specifically blocking a single cytokine in the context of a disease process. In the case of IL-1, the administration of IL-1 receptor antagonist (IL-1Ra) to wild-type mice or the severity of disease in mice deficient in either isoform of IL-1 or deficient in IL-1 receptors has clearly established IL-1 as a key mediator of autoimmune and inflammatory diseases. Although IL-1Ra is discussed in detail elsewhere in this journal, injections IL-1Ra into humans with rheumatoid arthritis has resulted in a reduction in the inflammatory and joint destructive nature of their disease (1-3). In mice lacking IL-1 receptor type I, there is a failure to develop proliferative lesions of vascular smooth muscle cells in mechanically injured arteries (4). Mice deficient in tumor necrosis factor-α (TNFα) also exhibit decreased neointimal hyperplasia but in these mice, there is no expression of IL-1α, suggesting that the effect in TNFα deficient mice is due to a lack of IL-1α expression. This conclusion is supported by the observation that TNFα is expressed in mice deficient in IL-1 receptors, but there is reduced intimal hyperplasia. These and similar experiments are consistent with the concept that some effects of TNFα are mediated by IL-1. Early studies on the effects of TNFα revealed that TNFα induces IL-1 (5). It is also possible that

the effect of blocking TNFα in patients with rheumatoid arthritis is due to a reduction in IL-1 production and/or activity. For example, in rheumatoid arthritis patients injected with anti-TNFα monoclonal antibodies, there is a rapid reduction in circulating IL-1β levels. Mice overexpressing TNFα develop a spontaneous rheumatoid arthritis-like joint disease; however, if treated early in their disease process with anti-IL-1 receptor antibody, there is no development of arthritis (6).

The IL-1agonist family, IL-1α, IL-1β and IL-1, are unique in the cytokine families. Each is initially synthesized as precursor molecules without a signal peptide. After processing by the removal of N-terminal amino acids by specific proteases, the resulting peptides are called "mature" forms. The 31 kDa precursor form of IL-1β and the 24 kDa IL-18 precursor are biologically inactive and require cleavage by specific intracellular cysteine protease called IL-1β converting enzyme (ICE). ICE is also termed caspase-1, the first member of a large family of intracellular cysteine proteases with important roles in programmed cell death. However, there is little evidence that ICE (caspase-1) participates in programmed cell death (7). Rather, ICE seems to be primarily used by the cell to cleave the IL-1β and IL-18 precursors. As a result of the cleavage, the mature form of IL-1β is a 17.5 kDa molecule and of IL-18 is an 18 kDa peptide. Although ICE is primarily responsible for cleavage of the precursor intracellularly, other proteases such as proteinase-3 can process the IL-1β precursor extracellularly into an active cytokine (8). However, IL-1 and IL-18 are truly pleiotropic cytokines and affect the innate as well as the acquired immune systems.

In terms of host-defense, mice deficient in the IL-1 receptor type I, IL-1α, IL-1β or double deficient in IL-1α and IL-1β exhibit no phenotype different from the same strain wild-type mice. A similar observation has been made with mice deficient in IL-18 or the IL-18 receptor. Thus, IL-1- and IL-18-deficient mice live in routine, microbially unprotected animal facilities. From these observations, one can conclude that these three agonist members of the IL-1 family, which play important roles is disease, are not essential for normal embryonic development, post-natal growth, homeostasis, reproduction or resistance to routine microbial flora. These mice also do not exhibit evidence of spontaneous carcinogenesis and their lifespan appears normal. Lymphoid organ architecture is also normal. Nevertheless, in the context of an inducible disease, a deficiency in any one of these three members of the IL-1 superfamily reveals a role in disease severity. In contrast, as described below, mice deficient in IL-1Ra do not exhibit normal reproduction, have stunted growth and in selected strains develop spontaneous diseases such as rheumatoid arthritis-like polyarthropathy and a fatal arteritis (9, 10).

Historical background

The history of IL-1 begins with studies on the pathogenesis of fever. These were studies performed on the fever-producing properties of proteins called endogenous pyrogens, which were released from activated rabbit peritoneal exudate cells. Menkin and Beeson initially studied endogenous pyrogens in 1943-1948, although there is evidence that Menkin's preparations were contaminated with bacterial endotoxins. However the work of Beeson was followed by contributions of several investigators, who were primarily interested in the link between fever and infection/inflammation. In 1972, Waksman and Gery made an important contribution with the discovery that soluble factors augmented lymphocyte proliferation in response to antigenic or mitogenic stimuli. Kamschmidt also contributed to the "discovery phase" of IL-1 in describing macrophage products that induced the synthesis of acute phase proteins. The basis for the name "interleukin" was to streamline the growing number of biological properties attributed to soluble factors from macrophages and lymphocytes. IL-1 was the name given to the macrophage product whereas IL-2 was used to define the lymphocyte product. At the time of the assignment of these names, there was no amino acid sequence analysis known and the terms were used to define biological properties. In the field of rheumatoid arthritis, Krane and Dayer described IL-1 as an inducer of collagenases and Saklatvala described IL-1 for its property to destroy cartilage. The large number of diverse multiple biological activities attributed to a single molecule engendered considerable skepticism in the scientific community but with the cloning of IL-1 in 1984 (11,12), the use of recombinant IL-1 established that IL-1 was indeed a pleiotropic cytokine mediating inflammatory as well as immunological responses. With the use of targeted gene disruption, a more precise role for IL-1 in immune responses has been possible. For example, immunization with sheep red blood cells fails to elicit an antibody response in IL-1β deficient mice and hypersensitivity responses to antigens are suppressed in IL-1b deficient mice.

The IL-1 ligand superfamily

The intron-exon organization of the IL-1 genes suggests duplications of a common gene some 350 million years ago. Before this common IL-1 gene, there may have been another ancestral gene from which fibroblast growth factors (FGF) such as acidic and basic FGF also evolved, since IL-1 and FGFs share significant amino acid homologies, and similar to IL-1, form an all–beta-pleated sheet tertiary structure. To date, ten individual members of the IL-1 gene superfamily have been described. Of these, four gene products have been thoroughly studied. The other six members have been shown to exit in various human tissues, but their role in health or disease is presently unknown. The four primary members of the IL-1 gene superfamily are IL-1α, IL-1β, IL-18 and IL-1 receptor antagonist (IL-1Ra). IL-1α, IL-1β and IL-18 are each agonists; IL-1Ra, on the other hand, is the specific receptor antagonist for IL-1α and IL-1β but not for IL-18. When IL-1Ra occupies the IL-1 receptor, bona fide IL-1 cannot bind to the receptor and there is no biological response to IL-1. The existence of a highly specific and naturally occurring receptor antagonist in cytokine biology appears to be

Table I. IL-1 Superfamily members.

New Name	Former Name(s)	Property
IL-1F1	IL-1α	Agonist
IL-1F2	IL-1β	Agonist
IL-1F3	IL-1Ra	Antagonist
IL-1F4	IL-18; IFNγ inducing factor	Agonist
IL-1F5	IL-1Hy1, FIL1δ, IL-1Hζ, IL-1RPζ, IL-1L1, IL-1δ	Unknown
IL-1F6	FIL-1, IL-1ε	Unknown
IL-1F7	FIL-1ζ, IL-1H4, IL-1RP1	Unknown
IL-1F8	FIL-1h, IL-1H2	Unknown
IL-1F9	IL-1H1, IL-1RP2	Unknown
IL-1F10	IL-1Hy2, FKSG75	Unknown

unique to the IL-1 family. Similar to the use of anti-TNFα monoclonal antibodies or soluble TNF receptors, the beneficial effects of these anti-cytokine strategies is limited to amelioration of disease activity without affecting the dysfunctional autoimmune nature of rheumatoid arthritis.

Members of the IL-1 superfamily have been assigned a new nomenclature using the expression IL-1F reflecting their being part of a "family" of related ligands. Table I lists the current members of the IL-1 superfamily. In this review, the terms IL-1α, IL-1β and IL-18 as well as IL-1Ra will be retained. Most members of the IL-1 superfamily are located on the long arm of chromosome 2. IL-18 and IL-18 binding protein (IL-18BP) are located on chromosome 11. The intron-exon organization of the new members is also similar to that of the primary four members of the IL-1 superfamily. The six new members are closely related to IL-1β and IL-1Ra. From the intron-exon organization, some members represent gene duplications. In the case of IL-1F5, and possibly other newly described members, the duplication of the IL-1Ra gene has taken place (13). IL-1F7 and IL-1F9 are also closely related to IL-1Ra (14).

IL-1F5 shares 47% amino acid identity with IL-1Ra and is expressed in human monocytes activated by endotoxins. From the gene sequence, the predicted amino acids sequence of IL-1F5 does not have a leader peptide for secretion, which is in sharp contrast to the IL-1Ra (IL-1F3). IL-1F5 failed to exhibit agonist activity using induction of IL-6 from fibroblasts, a well-described biological property of IL-1α and IL-1β (15). Furthermore, IL-1F5 did not block the IL-1α or IL-1β-induced IL-6 or IL-18-induced production of IFNγ (15). Therefore, IL-1F5 possesses neither IL-1- or IL-18 like neither agonist activities nor the property to act as a receptor antagonist for IL-1, despite it close amino acid identity to IL-1Ra.

Although IL-1F7 (formerly IL-1ζ, IL-1H4, IL-1H and IL-1RP1) is structurally related to IL-1Ra (36%), this member of the IL-1 superfamily binds to the IL-18 receptor α chain and therefore has attracted attention as being related to IL-18 (16). IL-1F7 has no leader peptide and the recombinant form has been expressed with a N-terminus from a predicted caspase-1 site (17). There are two forms of IL-1F7, a full-length peptide and a splice variant with an internal 40 amino acids deletion (16). The binding of IL-1F7 to the soluble IL-18Rα-chain has also been observed. However, compared to IL-18, recombinant IL-1F7 does not induce IFNγ from in whole human blood cultures, in peripheral blood mononuclear cells (PBMC) or various cell lines. Therefore, it is unlikely that IL-1F7 is a true agonist for the IL-18 receptor. Whether IL-1F7 is a receptor antagonist for IL-18 remains to be determined.

IL-1F9 is constitutively expressed primarily in the placenta and the squamous epithelium of the esophagus. The three-dimensional folding of IL-1F9 is similar to that of IL-1Ra; therefore, IL-1F9 appears to be a possible IL-1 receptor antagonist rather than an agonist. IL-1F10 shares 37% amino acid identity with the IL-1Ra and a similar three-dimensional structure (18). This cytokine is secreted from cells and is expressed in human skin, spleen, and tonsil. To date, recombinant IL-1F10 has been shown to bind to the recombinant soluble IL-1 receptor type I but it is unclear whether IL-1F10 binds to cell surface IL-1 receptors. Although these data suggest that IL-1F10 is likely to be a receptor antagonist, compared to IL-1Ra, its role in health and disease remains unclear.

In general, the function(s) of the newly described members of the IL-1 superfamily (IL-1F5-10) is presently unclear. It is unlikely that any possess pro-inflammatory properties since recombinant forms have not revealed detectable effects in primary cells similar to those for IL-1α, IL-1β or IL-18. Since most share significant amino acid identities with IL-1Ra and since the intron-exon organization appears to reveal gene duplication of the IL-1Ra gene, these IL-1 superfamily members may be receptor antagonists. Whether these IL-1Ra-like homologues can block IL-18 is also presently unclear. Because deletion of only the IL-1Ra gene has resulted in a significant disease-producing phenotype in mice (see below), one can assume that the genes coding for the IL-1Ra homologues (IL-1F5-10) do not play a significant role in health. At present, the effect of deletion of IL-1F5-10 in mice is unknown.

What is the significance of these new members of the IL-1 family to human disease and particularly to rheumatoid arthritis? It appears that there are three members of the IL-1 family, which are clearly agonists (IL-1α, IL-1β and IL-18) whereas all other members are antagonists. During cytokine evolution, it apparently was important to maintain some control over the biological activities of the IL-1 agonists and so one can conclude that several genes in the family counter regulate the activities of only three agonist IL-1. It is of note that the large family of TNFα-like cytokines and related proteins have not

Table II. Nomenclature of IL-1R family.

Name	New Designation	Ligand
IL-1RI	IL-1R1	IL-1α, IL-1β, IL-1Ra
IL-1RII	IL-1R2	IL-1β, IL-1α, IL-1Ra
IL-1R Ac-P	IL-1R3	IL-1α, IL-1β
ST2/Fit-1	IL-1R4	unknown
IL-18Rα/IL-1Rrp1	IL-1R5	IL-18
IL-1Rrp2	IL-1R6	?IL-1ε, IL-1δ
IL-1R18β/IL-1RAcPL	IL-1R7	IL-18
IL-1RAPL	IL-1R8	unknown
IL-R9	IL-1R9	unknown

revealed any receptor antagonists. In addition, the IL-1 family of receptors also includes a decoy receptor (IL-1 receptor type II) (19), which also blunts IL-1 responses. IL-18 also has a unique mechanism for downregulating its responses. The IL-18BP is not the soluble receptor for IL-18 but rather a separate gene product, which binds and neutralizes IL-18.

Il-1 receptor family

The IL-1 receptor family now encodes nine distinct genes of which some remain orphan receptors. As shown in Table II, these receptors have been assigned a nomenclature in the order of their discovery. The IL-18 binding protein (IL-18BP) is not listed due to its lack of being fixed to the cell via a transmembrane domain; however, the IL-18BP likely represents the former cell-bound decoy receptor for IL-18 similar to the decoy receptor for IL-1 (the IL-1 Receptor type II, see below). In fact, there is limited but significant amino acid homology between the IL-18BP and the type II IL-1 receptor, particularly in the third domain (20). IL-1R1, IL-1R2 and IL-1R3 are the bona fide receptors for IL-1. IL-1R4 (also known as ST2 and Fit) remains an orphan receptor, although proteins have been reported that bind to this receptor (21). Despite a lack of a specific ligand for this receptor, a number of studies have examined the distribution and gene regulation of this receptor in mast cells (22). IL-1R5 was formerly an orphan receptor termed IL-1R related protein-1 (23) but was subsequently discovered to be the ligand-binding chain of the IL-18 receptor (24), now termed IL-18Rα chain.

The IL-1R related protein-2 (IL-1R6) has been proposed to be the receptor for a novel member of the IL-1 family, IL-1Fε (25). The activity of this ligand for the IL-1R6 was demonstrated in a luciferase NFκB assay; another member of the IL-1 family, IL-1Fδ, appears to be its natural receptor antagonist for IL-1Fε binding to IL-1R6 (25). The IL-1R7, formerly the non-ligand binding chain of the IL-18 receptor termed IL-1R AcPL (26), is now named IL-18Rβ chain. Similar to the IL-1R-AcP, the IL-18Rβ is essential for IL-18 signal transduction (26, 27).

Two members of the IL-1 receptor family are particularly unique in that they are found on the X chromosome. These are IL-1R8 and IL-1R9, both being homologous to the IL-1 accessory protein receptor chains (IL-1R-AcP and IL-1R-AcPL). IL-1R9 (28) is highly homologous to IL-1R8 (29). Both forms have no known ligands and receptor are found in the fetal brain. In fact, non-overlapping deletions and a nonsense mutation in the IL-1R8 gene were found in patients with cognitive impairment (29) where expression in the adult hippocampal area may play a role in memory or learning. The cytoplasmic domains of IL-1R8 and IL-1R9 are longer than the other accessory chains. The IL-1R9 may function as a negative receptor. This was shown in cells overexpressing this receptor as well as the IL-1RI and IL-1R-AcP in which IL-1β signaling was blocked with a specific antibody to the IL-1R-AcP. In the presence of the antibody, IL-1β-induced luciferase was suppressed, suggesting that a possible complex of the type I receptor with IL-1β plus IL-1R9 results in a negative signal (28).

Of the three members of the IL-1 family (IL-1α, IL-1β and IL-1Ra), IL-1β has the lowest affinity for the cell bound form of IL-1RI (500 pM-1 nM). IL-1β binding to the soluble form (extracellular domains) of the IL-1RI is lower compared to the cell bound receptor. The greatest binding affinity of the three IL-1 ligands for the IL-1RI is the IL-1Ra. In fact, the off-rate is slow and binding of IL-1Ra to the cell bound IL-1RI is nearly irreversible. Compared to IL-1Ra, IL-1α binds to IL-1RI with affinities ranging from 100 to 300 pM. By comparison, IL-1β binds more avidly to the non-signal transducing type II receptor (100 pM).

The IL-1 receptor type II (IL-1RII) was described by several investigators (30, 31) and the ability of IL-1β to preferentially bind to B cells likely represents binding to the type II receptor (32, 33). The amino acid sequence of the human IL-1RII was reported in 1991 (34). The concept that this receptor functioned as a negative or "decoy" receptor was demonstrated by Colotta and Mantovani in 1993 (19, 35). The extracellular segment of the IL-1RII has three typical Ig-like domains; there is a transmembrane segment and a short cytoplasmic domain (34). The short cytoplasmic domain is unable to initiate signal transduction since there is no Toll-homology domain. Therefore, when IL-1 binds to the cell membrane, IL-1RII does not signal. Vaccinia and cowpox virus genes encode for a protein with a high amino acid homology to the type II receptor and this protein binds IL-1β (36, 37). These sames viruses also code for IL-18 binding protein-like molecules (20). The viral form of the IL-1RII likely serves to reduce the inflammatory and immune response of the host to the virus. A soluble (extracellular) form of this receptor is released from the cell surface by the action of a protease, binds IL-1β and neutralizes the biological effects of IL-1β (38). Although the short cytoplasmic domain in the rat is longer than in the human (39), this receptor does not signal. In the human and mouse, the cytoplasmic

domain of IL-1RII consists of 29 amino acids; in the rat, there are an additional 6 charged amino acids (39).

IL-1β binds with a greater affinity to the type II receptor than does IL-1α and IL-1Ra binding to this receptor is the lowest of the three ligands (38, 40, 41). Although IL-1α binds to cell surface and soluble type I receptors with approximately the same affinity (200-300 pM), IL-1α binding to surface and soluble type II receptors is nearly 100-fold less (30 and 10 nM, respectively). By comparison, IL-1β binds avidly to the non-signal transducing type II receptor (100 pM) and IL-1β binding to the soluble form of the this receptor is also high at 500 pM. Moreover, IL-1β binding to the soluble IL-1RII is nearly irreversible due to a long dissociation rate (2 hours) (38, 40, 42). The precursor form of IL-1β also preferentially binds to the soluble form of IL-1RII (30, 31). The function of the type II receptor as a "decoy" receptor is based on the binding of IL-1β to the cell surface form of this receptor, thus preventing the ability of the ligand to form a complex with the type I receptor and the accessory protein (19, 35). Another and perhaps more efficient function of the decoy receptor is to form a trimeric complex of the IL-1β ligand with the type II receptor and the accessory protein (43, 44). This mechanism serves to deprive the functional receptor type I of the accessory chain.

Figure 1 illustrates the binding of IL-1β to the cell. In this cell, IL-1β binds and recruits the IL-1R AcP for the initiation of a signal. Also shown is IL-1β binding to the type II receptor which does not initiate a signal. IL-1β binding to the soluble type II receptor is neutralized. IL-1β binding to the soluble type II receptor also recruits the IL-1R AcP and forms a non-functional complex. This complex deprives the type I receptor of the IL-1R AcP.

IL-1β Converting Enzyme

ICE (caspase-1) is constitutively expressed in various cells as a primary transcript of 45-kDa (inactive precursor) requiring two internal cleavages before becoming the enzymatically active heterodimer comprised of a 10 and 20-kD chain. The active site cysteine is located on the 20-kD chain. ICE itself contributes to autoprocessing of the ICE precursor by undergoing oligomerization with itself or homologs of ICE. In the presence of specific inhibitors of ICE, the generation and secretion of mature IL-1β is reduced and precursor IL-1β accumulates mostly inside but the precursor is also found outside the cell. This latter finding supports the concept that precursor IL-1β can be released from a cell independent of processing by ICE. Due to alternate RNA splicing, there are five isoforms of human ICE (ICEα, β, γ, δ, and ε); ICEα, cleaves the ICE precursor and the IL-1β precursor. It is presumed that ICEβ, and γ also process precursor ICE. ICEε is a truncated form of ICE which may inhibit ICE activity by binding to the p20 chain of ICE to form an inactive ICE complex.

In addition to ICE, the IL-1β precursor is cleaved by elastase, chymotrypsin, a mast cell chymase, proteinase-3, granzyme A, and a variety of proteases commonly found in inflammatory fluids. Some matrix metalloproteases (MMPs) commonly found in joint fluids from patients with rheumatoid arthritis also cleave the precursor of IL-1β into biologically active IL-1β. These include gelatinase-B, MMP-2, MMP-3 (stromelysin-1), and MMP-9. These alternative, extracellular proteases may account for the observation that mice deficient in ICE can exhibit a full inflammatory response to subcutaneous turpentine, an IL-1β–dependent mode. The secretion of mature IL-1β is facilitated by a fall in the intracellular levels of potassium, which takes place when a cell is exposed to high levels of ATP (45). Treatment of stimulated macrophages with millimolar concentrations

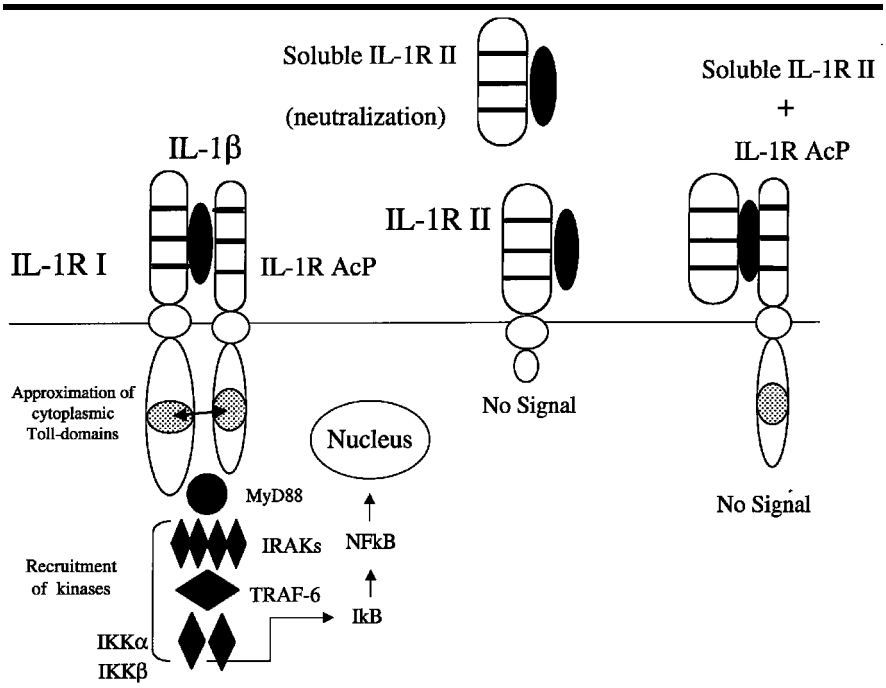

Fig. 1. IL-1 signal transduction. The IL-1RI binds IL-1β and this complex recruits the IL-1R AcPto form a ternary complex. As a result, the Toll-domains present in the cytoplasmic segments of both receptor chains approximate and this results in the binding of MyD88 to the complex. There are four kinases termed IL-1 receptor activating kinases (IRAK) which are phosphorylated by the IL-1R complex. Another kinase that is phosphorylated in the TNF receptor activating factor-6 (TRAF-6). Following phosphorylation of TRAF-6, two additional kinases are phosphorylated: inhibitory-κ B (IκB) kinase-α and IκB kinase-β (IκKK). These latter kinases, in turn, phosphorylate IκB itself resulting in proteolysis of IκB and the release of NFκB. NFκB the translocates to the nucleus and acts to trigger gene expression. When IL-1β binds to the cell-surface IL-1R type II (IL-1RII), there is no signal transduction since this receptor (decoy receptor lacks a cytoplasmic domain). Soluble IL-1RII also can bind IL-1β and this results in neutralization of IL-1β. In addition, the soluble IL-1RII-IL-1β complex may bind to the IL-1R AcPand this complex does not signal but rather deprives the IL-1RI of the IL-1R AcP to form an active complex.

of ATP also result in the processing and release of IL-1β. The effect of ATP or nigericin is due to a net decrease in the intracellular levels of potassium. Increasing the extracellular level of potassium also results in the inhibition of caspases by preventing the formation of a large intracellular complex associated with activation of caspases (46).

IL-1α as an autocrine growth factor

The concept that IL-1a can be an autocrine growth factor takes into account three distinct observations: first, precursor IL-1α is synthesized and remains inside the cell where it can bind to the nucleus; second, intracellular precursor IL-1α complexes to an intracellular pool of IL-1RI before exerting an effect as a ligand/receptor complex and thirdly, either precursor IL-1α or mature IL-1α bound to surface IL-1RI is internalized with subsequent translocation to the nucleus (similar to steroid receptors). Each mechanism has supporting experimental data. Some investigators have considered that intracellular precursor IL-1α regulates normal cellular differentiation, particularly in epithelial and ectodermal cells. In the case of keratinocytes, constitutive production of large amounts of precursor IL-1α is found in healthy human skin. In support of the concept that precursor IL-1α functions as an intracellular messenger in certain cells, an antisense oligonucleotide to IL-1α reduces senescence in endothelial cells (47, 48). In the murine Th2 cell line, IL-1a was proposed as an essential autocrine and paracrine growth factor using an antisense IL-1a oligonucleotide or anti-IL-1α antibodies. Thymic epithelium produces IL-1α and a requirement for IL-1α has been demonstrated in the expression of CD25 (IL-2 receptor a chain) and maturation of thymocytes. However, these data must be viewed with the report that in mice deficient for IL-1α, there are no demonstrable defects in growth and development, including skin, fur, epithelium and gastrointestinal function (49). The large amounts of precursor IL-1α in normal skin keratinocytes is thought to affect terminal differentiation. If there is a role for intracellular precursor IL-1α in normal cell function, this should be carefully regulated. The presence of large amounts of an intracellular form of the IL-1Ra (icIL-1Ra) (50) produced in the same cells expressing precursor IL-1α is thought to compete with the intracellular pool of precursor IL-1α for nuclear binding sites. The IL-1α deficient mouse does not support this concept (49).

Membrane IL-1α.

Precursor IL-1α can be found on the surface of several cells, particularly on monocytes and B lymphocytes, where it is referred to as membrane IL-1α (51). Membrane IL-1α is biologically active; its biological activities are neutralized by anti–IL-1α but not by anti-IL-1β. Membrane IL-1α appears to be anchored to the cell membrane via a lectin interaction involving mannose residues. A mannose-like receptor appears to bind membrane IL-1α (52). The role of membrane IL-1α in disease remains unclear. *In vitro*, the amount of IL-1Ra needed to block membrane IL-1a was ten to 50-fold greater than the amount required to block mature IL-1β (53).

Autoantibodies to IL-1α.

Neutralizing autoantibodies directed against IL-1α may function as natural buffers for IL-1α. Autoantibodies to IL-1α have been detected in healthy subjects as well as in patients with various autoimmune diseases. Autoantibodies to IL-1α are neutralizing IgG antibodies that bind natural precursor form of IL-1α as well as 17 kDa recombinant IL-1α [Bendtzen, 1990 #779]. The incidence of these antibodies is increased in patients with autoimmune diseases. For example, in 318 patients with chronic arthritis, anti-IL-1α, but not anti-IL-1β or anti-TNFα, IgG antibodies were detected in 18.9% of arthritis patients but in 9% of healthy subjects. Anti-IL-1α was present more commonly and at a higher level in patients with non-destructive arthritis. An inverse correlation has been observed between the levels of anti–IL-1α antibodies and the clinical disease activity.

Effects in IL-1 knockouts

The IL-1β deficient mouse

The IL-1β deficient is without abnormal findings after six years of continuous breeding. However, upon challenge, IL-1β deficient exhibit specific differences from their wild-type controls. Mice deficient in IL-1β do not develop a destructive joint process following injection of streptococcal wall components (54). The most dramatic is the response to local inflammation followed by a subcutaneous injection of turpentine (50-100 μL). Within the first 24 hours, IL-1β deficient mice injected with turpentine do not manifest an acute phase response, do not develop anorexia, have no circulating IL-6 and no fever (55, 56). These findings are consistent with those reported in the same model using anti-IL-1R type I antibodies in wild-type mice (55). IL-1β deficient mice also have reduced inflammation following zymosan-induced peritonitis (57). Additional studies have also found that IL-1β deficient mice have elevated febrile responses to IL-1β and IL-1α (58).

In contrast, IL-1β deficient mice have nearly the same responses to LPS as do wild-type mice (59) with one notable exception. IL-1β deficient mice injected with LPS have little or no expression of leptin mRNA or protein (60). In IL-1β pregnant mice, there is a normal response to LPS-induced pre-mature delivery; however, in these mice, there is decreased uterine cytokines following LPS (61). The reduction in LPS-induced cytokines is not found in non-pregnant IL-1β deficient mice suggesting that the combination of the hormonal changes in pregnancy and the state of IL-1β deficiency act together to reduce the responsiveness to LPS. The mechanism for the reduced cytokine production in pregnant IL-1β deficient mice appears to be due to a reduction in the constitutive level of the p65 component of NFκB.

No differences were noted in plasma elevations of glucocorticoid steroids between IL-1β deficient and wild-type mice following injection of LPS, indicating that IL-1β is not required for activation of the HPA axis during endotoxemia. The data demonstrate that in the

Table III. Effects in IL-1β deficient mice

Disease model	Effect	Reference
Streptococcal Wall Arthritis	Lack of destuctive processes	(54)
Endotoxin Fever	No effect	(59)
LPS-induced leptin	↓ Circulating leptin	(60)
Zymosan Peritonitis	↓ Inflammation ↓ Mortality ↓ IL-6 and chemokines	(57)
Turpentine inflammation	↓ Inflammation ↓ Fever ↓ IL-6; ↓ SAA ↓ cortisone ↓ COX-2	(55) (49)
IL-1α-induced fever	↑ Fever ↑ Cytokines	(58)
Hepatic melanoma	↓ Metastasis	(104)
Brain ischemia	↓ Neuronal Death	(105)
Immune myasthenia gravis	Resistant to disease development	(106)
Fas-expressing tumors	↓ Neutrophil infiltration	(107)
LPS-induced shock lung	No effect on neutrophil infiltration	(108)
Turpentine coagulopathy	↓ plasminogen activator inhibitor (i)	(109)
LPS-induced coagulopathy	Plasminogen activator i unchanged	(109)
Contact hypersensitivity	↓ delayed hypersensitivity	(73)
Contact hypersensitivity	↓ Langerhans cell activation	(110)
Steady state p65 (NFκB)	↓ levels and translocation	(61)

mouse, IL-1β is critical for the induction of fever during local inflammation. Another characterized body temperature, activity and feeding live influenza virus in IL-1β deficient mice. Body temperature and activity were lower in IL-1β deficient mice (62). The anorexic effects of influenza infection was similar in both groups of mice. The mice deficient in IL-1β exhibited a higher mortality to influenza infection than the wild-type mice.

Studies in IL-1α-deficient mice

Mice deficient in IL-1α are born healthy and develop normally. Following subcutaneous injection of turpentine, which induces a local inflammatory response, wild-type and IL-1α–deficient mice develop fever, whereas IL-1Β–deficient mice do not (49). The induction of glucocorticoids after turpentine injection was suppressed in IL-1β–but not in IL-1α–deficient mice. Expression of IL-1β mRNA in the brain decreased 1.5-fold in IL-1α–deficient mice, whereas expression of IL-1α mRNA decreased more than 30-fold in IL-1β–deficient mice. These data suggest that IL-1β exerts greater control over production of IL-1α than does IL-1α over the production of IL-1β. In ICE-deficient mice, IL-1α production is also reduced (63), suggesting that production of IL-1α is under the control of IL-1β.

Differences between IL-1α and IL-1β-deficient mice

Studies on the effects of selective deficiency in IL-1β in mice are summarized in Table III. These differences are to be compared to the same models in mice deficient in IL-1α. For example, mice deficient in IL-1α develop a normal immune response to immunization with sheep red blood cells whereas mice deficient in IL-1β do not produce anti-sheep red blood cell antibodies, a T-dependent response (64). However, antibody production by T-independent antigens was normal in mice deficient in both IL-1α and IL-1 as was the proliferative response to anti-CD3. In mice deficient in IL-1Ra, there was enhanced response (64). Also mice deficient in IL-1α have a brisk inflammatory response to turpentine-induced inflammation whereas IL-1β deficient mice have nearly no response.

Studies in IL-1RI–deficient mice

As stated above, mice deficient in IL-1RI develop normally and exhibit no particular phenotype despite being housed in standard animal facilities (65). IL-1RI-deficient mice show no abnormal phenotype in health and exhibit normal homeostasis, similar to that observed in IL-1β or IL-1α deficient mice (49, 55) but distinctly different from mice deficient in IL-1Ra (66). They do, however, exhibit reduced responses to challenge with inflammatory agents. When given a turpentine abscess, for example, IL-1RI–deficient mice exhibited an attenuated inflammatory response compared with wild-type mice (67). IL-1RI–deficient mice also had a reduced delayed-type hypersensitivity responses. Similar to wild-type mice treated with anti–IL-1 antibodies or IL-1Ra, IL-1RI–deficient mice were susceptible to infection with Listeria monocytogenes. Lymphocytes from IL-1RI–deficient mice with major cutaneous leishmanial infection produced more IL-4 and IL-10, but less IFNγ, than did those from wild-type mice.

Although mice deficient in IL-1RI do not exhibit significant disruption of reproduction aside from a somewhat reduced litter size (68), in some laboratories, however, the body weights of the IL-1RI-deficient mice were 30% less than wild-type, whereas the TNFRp55-deficient mice weighed 30% more than wild-type mice of equivalent age (69). Although IL-1α is constitutively expressed in the skin, the barrier function of skin remains intact in mice deficient in IL-1RI (70). Similarly, mice deficient in IL-1R-AcP appear normal but have no responses to IL-1 *in vivo* (71). However, cells deficient in IL-1R-AcP have normal binding of IL-1α and IL-1Ra (binding to the IL-1RI being intact) but a 70% reduction in binding of IL-1β (71). In these cells, there is no biological response to IL-1_ despite binding of IL-1α. The results suggest that IL-1R-AcP and not IL-1RI is required for IL-1b binding and biological response to IL-1.

Mice injected with LPS have been studied. IL-1RI deficient mice exhibit the same decrease in hepatic lipase, as do wild type mice. However, injection

of LPS directly into the eye of mice deficient in IL-1RI reveal a decrease in the number of infiltrating leukocytes whereas there was no decrease in mice deficient in both TNF receptors (72). IL-1RI-deficient mice failed to respond to IL-1 in a variety of assays, including IL-1-induced IL-6 and E-selectin expression and IL-1-induced fever. Similar to IL-1β-deficient mice, IL-1RI-deficient mice had a reduced acute phase response to turpentine. Also similar to IL-1β-deficient mice (73), IL-1RI-deficient mice had a reduced delayed-type hypersensitivity response and were highly susceptible to infection by *Listeria* monocytogenes.

Mice deficient in IL-1RI did not develop trabecular bone loss following ovariectomy compared to wild-type controls (74). Although mice deficient in both the TNF-RI and TNF-RII receptors develop experimental autoimmune encephalomyelitis (EAE) after immunization with central nervous system antigens, mice deficient in IL-1RI failed to develop inflammatory lesions in the central nervous system or evidence of clinical EAE. Mice deficient in IL-1RAcP, the essential component of the IL-1RI signaling complex, have also been generated. Although cells from IL-1RAcP–deficient mice bound IL-1, there was no activation of genes dependent on NF-kB or activator protein-1 (AP-1) (71). Interestingly, the binding affinity of IL-1β for cells deficient in IL-1RAcPwas reduced by 70% whereas the binding affinity of IL-1α was only moderately reduced. In general, mice deficient in the IL-1RI exhibit reduced disease severity as do wild-type mice injected with pharmacologic doses of IL-1Ra.

IL-18 and autoimmune and inflammatory diseases

Although IL-18 is a member of the IL-1 family of ligands, IL-18 appears to have unique characteristics some of which are important for its role in rheumatoid arthritis. The most salient biological property of IL-18 that separates this cytokine from IL-1 is its ability to induce IFNγ in the presence of IL-12. IL-18 was originally identified as an IFNγ-inducing factor. Because IL-18 appears to be essential for IFN, production, the role of IL-18 in disease must consider its role in regulating IFNγ production. IFNγ is itself unlike other proinflammatory cytokines because IFNγ has been administered in thousands of humans with a variety of diseases, including rheumatoid arthritis. IFNγ was also administered to patients with bur injuries to improve intracellular killing of bacteria, particularly by mononuclear phagocytes. There are also reports of IFNγ treatment in cancer. Overall, there has not been a worsening of disease with the exception of reports on exacerbation of CNS lesions in multiple sclerosis. Therefore, unlike the systemic inflammatory response of humans injected with IL-1 or TNFα, IFNγ appears to be tolerated by humans and in some disease states, can be considered therapeutic. For example, in patients with chronic granulomatous disease or atypical mycobacterium infections (including leprosy), IFNγ is used in conjunction with specific antibiotic therapies.

IL-18 neutralization, IL-18 deficient mice or mice deficient in IL-18 receptor-a chain reveal varying degrees of reduced severity in models of systemic and local disease. In fact, there is considerable overlap in blocking IL-1, IL-18, IFNγ and TNFα in models of endotoxemia. For the treatment of humans with rheumatoid arthritis using either antibodies against IL-18, antibodies that block the IL-18 receptor or the IL-18 binding protein (IL-18BP), the relevant models are collagen-induced arthritis (CIA), streptococcal wall (SCW) arthritis and to a lesser extent adjuvant arthritis. However, because IL-18 regulates IFNγ, primary immunization with collagen in mice deficient in IL-18 or deficient in the IL-18 receptor is problematic as low titers of anti-collagen antibodies may affect the disease model. Nevertheless, IL-18 neutralization in wild-type DBA-1 mice is effective in reducing CIA (75).

IL-18 and its functions

The discovery of IL-18 and its role in models of systemic inflammation

IL-18 was first described as an IFNγ-inducing factor found in the circulating during endotoxemia (76). In those experiments, mice had been preconditioned with a prior infection of *Propionibacterium acnes.* Because of its property to induce IFNγ, IL-18 is by default a member of the T-cell helper type I (Th1)-inducing family of cytokines (IFNγ, IL-2, IL-12, IL-15). However, because antibodies to IL-18 also reduced the hepatotoxicity of endotoxemia (77), IL-18 was considered to possess other biological properties beyond that of inducing IFNγ. Like all cytokine responses to infections, there are two sides to the coin. IL-18 functions to protect the host in that its ability to induce IFNγ and other immunostimulatory cytokines assists the immune system in a specific T and B-cell mediated response. However, the other pathological consequences of infection are, in part, also mediated by IL-18 in somewhat the same fashion that are mediated by IL-1 and TNFγ. These include the increases in cell adhesion molecules and chemokines, inflammatory mediators such as nitric oxide (NO) and neutrophil activation.

The support for a role for IL-18 in the pathological processes of systemic inflammation is derived from animal studies in which specific blockade of IL-18 reduces the impact on organ damage or improves the survival of the host. The first experiments showed that mice deficient in caspase-1, failure to process the IL-18 and IL-1β precursors survived lethal endotoxemia (78, 79) whereas mice deficient in IL-1β died (59). In fact, specific antibodies against mouse IL-18 also protected against the hepatic toxicity of endotoxemia (77, 80). On the other hand, in naive mice not preconditioned with a prior infection of *Propionibacterium acnes*, IL-18 neutralization also reduces lethal endotoxemia. Moreover, this protection is observed in mice deficient in IFNγ (81). Thus, one may conclude that preconditioning with a prior infection of *Propionibacterium acnes* is needed for an IFNγ sensitive animal model.

Since IL-18 induces synthesis of the proinflammatory cytokines TNFα, IL-1β and the chemokines IL-8 and macrophage inflammatory protein-1a, neutralization of IL-18 would have a

beneficial effect in lethal endotoxemia in naive mice. Anti-IL-18 antibodies protected mice against a lethal injection of E. coli or S. typhimurium (81). Anti-IL-18 also reduced myeloperoxidase levels in the liver and lungs (81). An increased survival was accompanied by decreased levels of IFNγ and macrophage inflammatory protein-2 in anti-IL-18-treated animals challenged with *E. coli* LPS, whereas IFNγ and TNFa concentrations were decreased in treated mice challenged with *S. typhimurium*.

IL-18 receptors

The activity of IL-18 begins with the formation of a heterodimeric complex comprised of two chains of the IL-18 receptor (IL-18R) complex plus IL-18. The ligand binding chain is termed IL-18Rα. It was reported using amino acid sequencing of a purified protein using ligand affinity purification (24). IL-18Ra is a member of the IL-1 receptor family, previously identified as the IL-1R related protein (IL-1Rrp) (23). Following the binding of IL-18 to the IL-18Ra chain, a second chain is recruited to the complex (Figure 2). This second chain, termed IL-18Rβ chain, is a different gene product but structurally related to IL-18Rα; however, the IL-18Rβ chain does not bind to IL-18 unless IL-18 is already bound to the a-chain. Because the IL-18Rβ is structurally related to the IL-1 signal-transducing chain, IL-1R accessory protein, the IL-18Rβ chain was initially termed the IL-18R accessory protein-like (AcPL) chain (26). The binding of IL-18 to the IL-18Rα is a low affinity binding (20-40 nM) (24) but the formation of the tricomplex with the IL-18Rβ chain forms a high affinity complex (600 nM). These two distinct binding affinities can be observed experimentally on T-cells (82).

IL-18 binding protein

There are limited amino acid homologies between the IL-18BP (20) and the type II IL-1R and both function as decoy receptors for their respective ligands. However, a transmembrane domain of the IL-18BP has apparently been deleted and this decoy receptor functions solely as a secreted protein. Another soluble receptor that has apparently lost its transmembrane domain is osteoprotegerin which binds and neutralizes RANK ligand. The IL-18BP has a single Ig-domain and limited homology to the IL-18Rα chain (83). Molecular modeling of IL-18 binding to IL-18BP has identified specific amino acids, which, when mutated decrease the ability of IL-18BP to bind and neutralize IL-18 (84). The affinity of IL-18 for IL-18BP is high (Kd of 400 pM) and plasma levels of 3-4 ng/ mL in healthy subjects (85) suggests that IL-18BP functions as a natural buffer against IL-18 and the Th1 response.

Role of IL-18 in models of autoimmune disease

Models of arthritis

Studies were carried out using SCW-induced arthritis (86). Using C57BL/6 or BALB/c mice, a neutralizing rabbit anti-murine IL-18 antibody was injected shortly before induction of arthritis by intra-articular injection of SCW fragments into the right knee joint. Significant (>60%) suppression of joint swelling was noted on days 1 and 2 of SCW arthritis after blockade of endogenous IL- 18 and joint TNFα and IL-1 levels were also decreased. Severe inhibition of chondrocyte proteoglycan synthesis is a prominent component of SCW-induced arthritis but a near complete reversal of the inhibition of chondrocyte proteoglycan synthesis was observed in the anti-IL-18-treated animals. Although these studies clearly established the pathological role for endogenous IL-18 in this model, the effect of IL-18 is apparently independent of IFNγ since mice deficient in IFNγ showed similar results using anti-IL-18 antibodies (86).

IL-18 also plays a role in CIA. IL-18 was injected into DBA-1 mice immunized with collagen in incomplete Freund's adjuvant. There was an increase in the erosive and inflammatory component of the arthritis (87). Using mice deficient in IL-18, CIAwas less severe

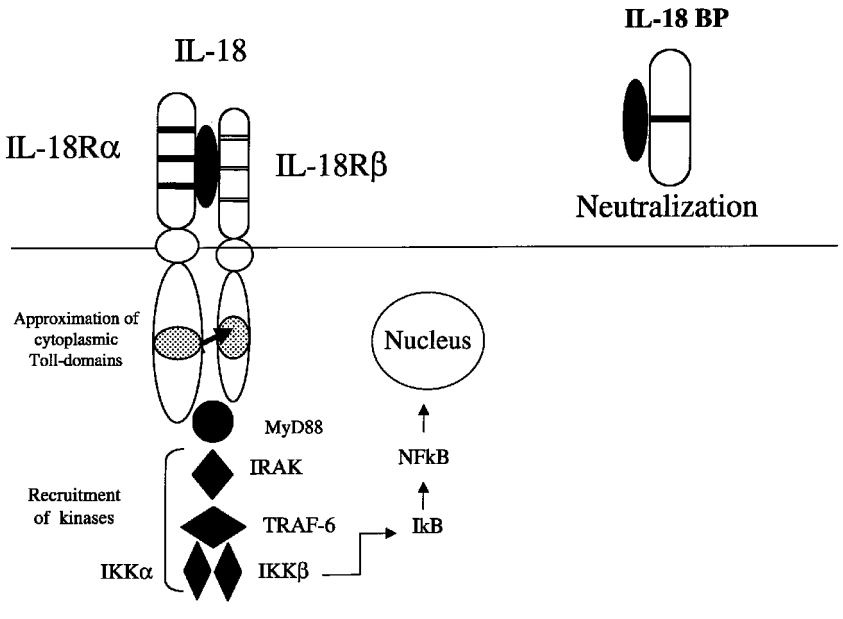

Fig. 2. IL-18 signal transduction. The IL-18Rα chain binds IL-18 and this complex recruits the IL-18Rβ chain to form a ternary complex. As a result, the Toll-domains present in the cytoplasmic segments of both receptor chains approximate and this results in the binding of MyD88 to the complex. There are four kinases termed IL-1 receptor activating kinases (IRAK) which are phosphorylated by the IL-1R complex. It is unclear which of these are activated by IL-18. Similar to IL-1, the TNF receptor activating factor-6 (TRAF-6) is phophorylated. Following phosphorylation of TRAF-6, two additional kinases are phosphorylated: inhibitory-κ B (IκB) kinase-α and IκB kinase-β (IκKK). These latter kinases, in tern, phosphorylate IκB itself resulting in proteolysis of IκB and the release of NFκB. NFκB the translocates to the nucleus and acts to trigger gene expression. When IL-18 binds to the IL-18BP, this results in neutralization of IL-18.

compared to wild-type controls (88). Histologically, there was evidence of decreased joint inflammation and the destructive component of the model. Levels of bovine collagen-induced IFNγ, TNFα, IL-6 and IL-12 from spleen cell cultures were decreased in IL-18-deficient mice. However, there was a significant reduction in serum anti-collagen antibody levels in the IL-18-deficient mice, raising the perennial issue that gene deletions on immunologically active cytokines can obscure the role of a cytokine in CIA. Nevertheless, from these studies, there is likely a pathological role for IL-18 in CIA.

Other studies in CIA used wild-type DBA-1 mice treated with either neutralizing antibodies to IL-18 or the IL-18BP after clinical onset of disease. The therapeutic efficacy of neutralizing endogenous IL-18 was assessed using different pathological parameters of disease progression. The clinical severity in mice undergoing CIA was significantly reduced after treatment with either IL-18 neutralizing antibodies or IL-18BP (75). Attenuation of the disease was associated with reduced cartilage erosion evident on histology. The decreased cartilage degradation was further documented by a significant reduction in the levels of circulating cartilage oligomeric matrix protein (an indicator of cartilage turnover). Both strategies efficiently slowed disease progression, but only anti-IL-18 antibody treatment significantly decreased an established synovitis. Serum levels of IL-6 were significantly reduced with both neutralizing strategies. In vitro, neutralizing IL-18 resulted in a significant inhibition of TNF-α, IL-6, and IFNγ secretion by macrophages (75).

Models of inflammatory bowel disease

Several studies have shown that IL-18 is expressed in the affected intestinal tissues of patients with Crohn's disease (89-92). In general, the cytokine is found in both intestinal epithelial cells as well as in the mononuclear cells of the lamina propria cells. The finding of constitutively expressed IL-18 in intestinal mucosa is not unusual since epithelial cells express IL-18 in health. However, there seems to be a pathological role in the expression of IL-18 in the mononuclear cell population in this disease. As with all cytokine-associated diseases, the role of IL-18 in inflammatory bowel disease is best revealed using specific blockade as described below.

The role of IL-18 was examined in intestinal inflammation using a neutralizing anti-murine IL-18 antiserum in dextran sulfate sodium (DSS)-induced colitis in either BALB/c or C57BL/6 mice (93). Using increasing doses or oral DSS, levels of colonic IL-18 increased parallel with clinical worsening. With the use of confocal laser microscopy, the increased IL-18 was localized to the intestinal epithelial layer. Anti-IL-18 antibody treatment resulted in a dose-dependent reduction of the severity of colitis in both BALB/c and C57BL/6 mice. Colon shortening following DSS-induced colitis, a marker of severity in this model, was partially prevented in the anti-IL-18 treatment groups. In the colon tissue homogenates, IFNγ concentrations were lower in the anti-IL-18-treated DSS-fed mice compared with untreated DSS-fed mice. This suppressive effect of anti-IL-18 administered *in vivo* was also observed on spontaneous TNFα, IL-18, and IFNγ production from *ex vivo* colon organ cultures. Similar to spleen cells, the stimulation of lamina propria mononuclear cells by IL-18 plus IL-12 resulted in a synergistic increase in IFNγ synthesis. Using this model, IL-18 appears to be a pivotal mediator in experimental colitis.

The role of IL-18 was also studied in the trinitrobenzene sulfonic acid (TNBS)-induced colitis model in which the activity of endogenous IL-18 was neutralized using human IL-18BP isoform "a" (94). Daily injection of IL-18BP resulted in less severe clinical score, less body weight loss, and a stabilization of colon weight when compared with saline-treated mice. In IL-18BP-treated mice, the intensity of the colitis as assessed histologially was reduced. Similar to anti-IL-18 antibody treatment in DSS-induced colitis, there was a decrease in colonic levels of TNFα, IL-6, and IL-1β in mice treated with IL-18BP. However, there was no reduction in IFNγ levels in these same tissues, a finding that contrasts with the effect of anti-IL-18 antibody treatment in DSS-induced colitis.

The systemic administration of daily injections of IL-12 plus IL-18 to BALB/c mice results in a severe wasting syndrome with intestinal inflammation and fatty liver (95). Intestinal mucosal inflammation is prominent in this model with bloody diarrhea and weight loss. There are high levels of serum IFNγ in these mice associated with elevated serum nitric oxide (NO) levels. In mice deficient in inducible NO, the disease failed to develop. Moreover, the disease was also induced in mice deficient in Fas. The disease did not develop in mice deficient in IFNγ.

In this study, we show that IL-18 is strongly expressed by intestinal epithelial cells in a murine model of Crohn's disease has been used by the transfer of a population of $CD62^+$ and $CD4^+$ T cells into SCID mice. The activity of endogenous IL-18 was reduced using an adenovirus expressing IL-18 antisense mRNA (96). Local administration of the anti-sense adenovirus to mice with established colitis resulted in expression of the vector in the intestinal epithelial cells. In these mice there was a reduction in the severity of the colitis as assessed histologically. In addition, IFNγ production from mucosal but not spleen cells was observed with the use of the anti-sense adenovirus.

In acute DSS-induced colitis, mice deficient in caspase-1 exhibited a greater than 50% decrease of the clinical scores of weight loss, diarrhea, rectal bleeding, and colon length, whereas daily treatment with IL-1 receptor antagonist revealed a modest reduction in colitis severity (97). To further characterize the function of caspase-1 and its role in intestinal inflammation, chronic colitis was induced over a 30-day time period. During this chronic time course, caspase-1 deficient mice exhibited a near complete protection, as reflected by significantly reduced clinical scores and almost absent histological signs of colitis. Consistently, colon shortening occurred only in DSS-ex-

posed wild-type mice but not in caspase-1 deficient mice. Protection was accompanied by reduced spontaneous release of the proinflammatory cytokines IL-18, IL-1β, and IFNγ from total colon cultures. In addition, flow cytometric analysis of isolated mesenteric lymph node cells revealed evidence of reduced cell activation in caspase-1 deficient mice as evaluated by surface expression of CD3, CD69 and CD4/-CD25.

IL-18 in models of brain inflammation

Since IL-1 is a sleep-inducing factor, IL-18 was examined for its ability to induce sleep in rats and rabbits (98). IL-18 injected intracerebroventricularly into rabbits increased non-rapid eye movement sleep. The sleep effects of IL-18 introduced directly into the brain coincided with increases in brain temperature (98). Similar results were obtained after intracerebroventricular injection of IL-18 into rats. Intraperitoneal IL-18 failed to induce fever in mice (99) and rats (98). Anti-human IL-18 antibody significantly attenuated muramyl dipeptide-induced sleep. These data are consistent with a role for IL-18 in mechanisms of sleep responses to infection.

In caspase-1 deficient mice, experimental autoimmune encephalomyelitis was studied (100). This is the animal model for multiple sclerosis. Steady state levels of caspase-1 are elevated in this model and correlate with disease severity as well as the upregulation of cytokines such as TNFα, IL-1β, IL-6 and IFNγ. In caspase-1-deficient mice, there was a reduction in the severity of the disease, although this was dependent on the amount of the encephalitogenic myelin oligodendrocyte glycoprotein antigen used to induce the disease. The administration of the tetrapeptide inhibitor of caspase-1 to mice with the developed disease did not alter the severity index, although pretreatment was effective. It was concluded that inhibition of caspase-1, perhaps via reduction in the processing of the IL-1β as well as the IL-18 precursors is a potential treatment possibility for relapsing remitting multiple sclerosis. The importance of IFNγ in brain inflammation is supported by studies in mice showing a spontaneous neurodegenerative disease using overexpression of IFNγ in the brain with a glial promoter (101).

Models of hepatic injury

The administration of Con A or of *Pseudomonas aeruginosa* exotoxin A results in an acute hepatic injury. In both models, leptin-deficient (ob/ob) mice were protected from liver damage and showed lower induction of TNFα and IL-18 compared with their lean littermates (102). Neutralization of TNFα reduced induction of IL-18 by either Con A (70% reduction) or PEA (40% reduction). Pretreatment of lean mice with either soluble TNF receptors or with an anti-IL-18 antiserum significantly reduced Con A- and PEA-induced liver damage. The simultaneous neutralization of TNFα and IL-18 fully protected the mice against liver toxicity. However, neutralization of either IL-18 or TNF-α did not inhibit Con A-induced production of IFNγ (102). Thymus atrophy and alterations in the number of circulating lymphocytes and monocytes were observed in ob/ob mice. Exogenous leptin replacement restored the responsiveness of ob/ob mice to Con A and normalized their lymphocyte and monocyte populations. These results demonstrate that leptin deficiency leads to reduced production of TNFα and IL-18 associated with reduced T cell-mediated hepatotoxicity. In addition, both TNF-α and IL-18 appear to be essential mediators of T cell-mediated liver injury.

The daily injection of IL-12 plus IL-18 results in prominent intestinal mucosal inflammation and fatty liver changes (95). The effects on the liver, however, are both IFNγ as well as NO dependent. Administration of recombinant soluble Fas ligand to mice preconditioned with P. acnes induced elevated serum liver enzyme levels. This Fas-ligand-induced liver injury did not develop in IL-18-deficient mice. The disease also did not develop in caspase-1 deficient mice (103).

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