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IL-18: A T_{H1}-inducing, proinflammatory cytokine and new member of the IL-1 family

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Formerly called IFN- γ -inducing factor, IL-18 is the new name of a novel cytokine that plays an important role in the T_{H1} response, primarily by its ability to induce IFN- γ production in T cells and natural killer cells. Mice deficient in IL-18 have suppressed IFN- γ production despite the presence of IL-12. IL-18 is related to the IL-1 family in terms of both structure and function. In terms of structure, IL-18 and IL-1 β share significant primary amino acid sequences and are similarly folded as all- β pleated sheet molecules. Also similar to IL-1 β , IL-18 is synthesized as a biologically inactive precursor molecule lacking a signal peptide. Studies have shown that similar to the IL-1 β precursor, the IL-18 precursor requires cleavage into an active, mature molecule by the intracellular cysteine protease called IL-1 β -converting enzyme (ICE), which is also known as caspase-1. Therefore inhibitors of ICE activity may limit the biologic activity of IL-18 and may be useful as T_{H1} immunosuppressive agents. The activity of mature IL-18 is closely related to that of IL-1. IL-18 induces gene expression and synthesis of TNF, IL-1, Fas ligand, and several chemokines. The activity of IL-18 is by means of a signaling chain of a putative IL-18 receptor (IL-18R) complex. This IL-18R complex is made up of a binding chain termed IL-18R α , a member of the IL-1R family previously identified as the IL-1R-related protein (IL-1Rrp), and a signaling chain, the IL-18R β , also a member of the IL-1R family. The IL-18R complex recruits IL-1R-activating kinase and TNF receptor-associated factor-6, which phosphorylates nuclear factor κ B (NF κ B)-inducing kinase with subsequent activation of NF κ B. Thus on the basis of primary structure, 3-dimensional structure, receptor family, signal transduction pathways, and biologic effects of IL-18 appear to place this cytokine in the IL-1 family. Similar to IL-1, IL-18 participates in both innate and acquired immunity. (J Allergy Clin Immunol 1999;103:11-24.)

Key words: Cytokines, inflammation, IFN- γ

Abbreviations used

AP-1:	Activating protein-1
AcPL:	Accessory protein-like
ICAM-1:	Intercellular adhesion molecule-1
ICE:	IL-1 β -converting enzyme
IDDM:	Insulin-dependent diabetes mellitus
IKK:	Inhibitory κ B kinase
IL-1Ra:	IL-1 receptor antagonist
IL-1R-AcP:	IL-1 receptor accessory protein
IL-1Rrp:	IL-1R-related protein
IL-18BP:	IL-18 binding protein
IRAK:	IL-1 receptor-activating kinase
MAPK:	Mitogen-activated protein kinase
NF κ B:	Nuclear factor κ B
NIK:	NF κ B-inducing kinase
NK cell:	Natural killer cell
NOD:	Nonobese diabetic
proIL-18:	Precursor form of IL-18
STAT:	Signal transducer and activator of transcription
TRAF-6:	TNF receptor-associated factor-6

IL-18 was first described as an IFN- γ -inducing factor, which circulated during endotoxemia in mice preconditioned with a prior infection of *Propionibacterium acnes*. Because of its property to induce IFN- γ , IL-18 is by default a member of the T_{H1}-inducing family of cytokines (IFN- γ , IL-2, IL-12, and IL-15). However, because antibodies to IL-18 also reduced the hepatotoxicity of endotoxemia, IL-18 was considered to possess other biologic properties beyond that of inducing IFN- γ . In this review the biologic activities of IL-18 are examined and discussed. The first conclusion that can be made is that IL-18 is related to IL-1 β more than to any other cytokine. The similarities between IL-1 β and IL-18 exist at several levels. First, the IL-18 precursor form (proIL-18), like proIL-1 β , does not contain a signal peptide, and proIL-18 requires cleavage to an active cytokine by the IL-1 β -converting enzyme (ICE), which is also known as caspase-1. IL-1 β and IL-18 are structurally related because both cytokines are primarily all- β pleated sheet-folded molecules. This structural relationship is of significant importance because there are very few all- β sheet molecules among the different cytokines.

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At the receptor level, the activity of IL-18 takes place through a signaling chain of a putative IL-18 receptor (IL-18R) complex. In this review the IL-18R binding chain is termed IL-18R α . IL-18R α is a member of the IL-1 receptor family, previously identified as the IL-1R-related protein (IL-1Rrp).¹ A nonbinding signaling chain (IL-18R β), also termed accessory protein-like (AcPL), is related to the IL-1R accessory protein. Following binding of IL-18 to the IL-18R α , the AcPL then binds to form a high-affinity heterodimeric complex with the ligand. The high-affinity IL-18R complex recruits the IL-18R-activating kinase (IRAK), resulting in nuclear factor κ B (NF κ B)-inducing kinase (NIK) with subsequent translocation of NF κ B to the nucleus. Initially identified as part of the IL-1R signaling event, IRAK is recruited to the IL-1R complex after exposure to IL-1 (Table I). In cells possessing IL-18R β , nuclear translocation of NF κ B is observed after incubation with IL-18, and this property helps explain the pleotropic nature of IL-18. In IL-18-deficient mice, production of IFN- γ and cytotoxic T cells are markedly diminished despite ample amounts of IL-12. A similar finding exists in mice deficient in ICE. The role of IL-12 in IFN- γ production therefore seems to require IL-18.

PRODUCTION OF IL-18

Production from monocytes/macrophages

Most of the information on the production of IL-18 is derived from mice preconditioned by *Propionibacterium acnes* and then challenged 4 to 6 days later with LPS. In preconditioned mice LPS induces a more rapid and higher level of IFN- γ compared with that of nonpreconditioned mice. When preconditioned mice are passively immunized with a neutralizing antibody to IL-18, markedly reduced circulating levels of IFN- γ are observed. The preconditioning results in a dramatic increase in the number and activity of liver Kupffer cells and splenic macrophages. Therefore the role of IL-18 in LPS-induced IFN- γ production is likely exaggerated. Similar to other activators of the reticuloendothelial system, preconditioning in this model does not provide information on the role of IL-18 under "normal" conditions. In terms of the role of IL-18 in human disease, the production of IL-18 in freshly obtained human PBMCs and in splenic macrophages from nonpreconditioned mice have been studied.² By using reverse-transcribed PCR and Western blot analysis, the production of IL-18 was compared with that of IL-1 β in the same preparations.

Previous studies demonstrated that there is no constitutive gene expression for IL-1 β in freshly obtained human PBMCs from healthy donors in over 40 cycles of PCR.^{3,4} Although this observation was confirmed, it was surprising that the same PBMCs expressed constitutive mRNA for IL-18. This was also observed with Western blot analysis for proIL-18 in lysates from the same PBMCs. Yet there was no proIL-1 β in the same cells. Constitutive IL-18 gene expression and the presence of proIL-18 protein were also observed in freshly

obtained murine splenocytes.² In these splenocytes there was no constitutive expression of the IL-1 β gene or protein. The promoter regions for IL-1 β and IL-18 gene expression have been studied and may provide insight into these observations. The promoter for IL-18 is TATA-less, and IL-18 promoter activity upstream of exon 2 acts constitutively.⁵ Therefore it is not unexpected that IL-18 mRNA is constitutively expressed, even in whole blood freshly obtained from healthy donors in whom no procedural artifact is introduced. The additional finding that the 3' untranslated region of human IL-18 lacks the AUUUA destabilization sequence⁵ is also consistent with these observations. This would allow for more sustained levels of the polyadenylated species and translation into protein. Other than to distinguish differences between IL-1 β and IL-18 in the same cells, the clinical significance of constitutive gene and protein expression for IL-18 in mononuclear cells remains unclear, but it certainly would focus on regulation at the level of processing the precursor and secretion of the mature form or forms (Table II). Osteoclasts also produce IL-18,⁶ and regulation of bone density may be a property of IL-18 as it is for IL-1 β .

Production from keratinocytes

In addition to monocytes/macrophages, the epidermal cells from mouse skin produce IL-18 constitutively.⁷ The keratinocyte is the major source of IL-18. Moreover, use of contact sensitizers increased IL-18 synthesis, whereas skin irritants did not. A murine keratinocyte cell line (PAM 212) also produced IL-18 constitutively.⁷ Cultured keratinocytes from the sensitized skin released IL-18 into the supernatant as measured by a specific murine ELISA for IL-18, but in addition, the IL-18 in the supernatants was biologically active because it stimulated IFN- γ production from T cells.

Production of IL-18 from the adrenal cortex and pituitary gland

Similar to IL-1, IL-18 is found in the rat adrenal cortex and pituitary gland.⁸ The adrenal IL-18 was increased by cold stress, whereas the pituitary IL-18 was not. Two transcripts of adrenal IL-18 were sequenced: an abundant form was clearly the rat homologue of mouse IL-18 and shared 91% amino acid homology. The other transcript was less abundant. It had a 59 bp deletion and the corresponding 19 amino acids at the 3' end of the molecule.⁸ It is possible that this truncated rat IL-18 molecule may act as a receptor antagonist. However, unlike the IL-1 receptor antagonist (IL-1Ra), this putative IL-18Ra results from an entire exon deletion and not from a new gene product.

Stimulators of IL-18 production

In general, macrophage stimulators, such as LPS, exotoxins from gram-positive bacteria, and a variety of microbial products, induce each of the macrophage cytokines. These include IL-1, TNF, IL-6, IL-10, and several others. Thus it is not surprising that these same activators also stimulate IL-18 production. GM-CSF was

used to induce human blood monocytes into macrophages that were then infected with the influenza A virus. The resulting supernatant induced T cells to release IFN- γ , which was prevented by using a neutralizing antibody to IFN- α/β .⁹ IFN- α and IL-18 acted synergistically to increase IFN- γ production, yet there was a demonstrable role for IL-12 in these cultures.

IL-18 AND IFN- γ PRODUCTION IFN- γ -inducing factors

Until the discovery of IL-18, the dominant IFN- γ -inducing factor from the macrophage was the heterodimeric cytokine IL-12.¹⁰ In some studies IL-1 can also be shown to augment IFN- γ production,¹¹ but compared with IL-12 and IL-18, this is not a dominant biologic property of IL-1. Once IL-12 binds to its cell surface receptor, nuclear translocation of signal transducer and activator of transcription (STAT) 3 and STAT4 take place, triggering promoter regions of the IFN- γ gene. Mice deficient in the p40 chain of IL-12 manifest decreased IFN- γ production. However, on closer examination, the induction of IFN- γ by IL-12 was, in fact, a coinduction. IL-12 itself induced only a small increase in IFN- γ production from primary human CD4⁺ T cells¹²; however, in the presence of costimulants, such as anti-CD3/CD28, mitogens, or specific antigens, large amounts of IFN- γ are produced. The role of these costimulants in IL-12-augmented IFN- γ production now appears to be related to activation pathways for the production of IL-18. Until the generation of IL-18-deficient mice,¹³ the precise role of IL-18 in endotoxin-induced IFN- γ production by IL-12 was uncertain because macrophage (or other cell) production of IL-18 could not be ruled out. As discussed below, little or no IFN- γ is produced from mice or cells deficient in IL-18¹³ or ICE.¹⁴⁻¹⁶ Importantly, the lack of IFN- γ production in these experiments takes place in the presence of ample IL-12.¹⁶ It should be noted here that mitogens, such as concanavalin A and PHA, induce IFN- γ production in ICE-deficient mice¹⁶ and also under conditions of near-total neutralization of IL-18 activity.¹⁷

IL-18 as an IFN- γ -inducing factor

In general, IL-18 induction of IFN- γ is similar to that of IL-12 in that as a sole cytokine, IL-18 induces low levels of IFN- γ , but in the presence of costimulants, IFN- γ production is greatly enhanced. When LPS is used as a costimulant, macrophages present in the culture produce IL-18, and it is likely that it is the endogenous IL-18 that acts as the cofactor. In the absence of a costimulant, primary human CD4⁺ T cells produce a small amount of IFN- γ (4-fold increase over background level), but in the same cultures IL-12 has no effect.¹² These findings were confirmed by using a human IFN- γ promoter linked to a luciferase reporter transiently transfected into human CD4⁺ T cells. IL-18 alone stimulated strong promoter activity, whereas IL-12 did so only when added with the costimulants anti-CD3 and anti-CD28.¹² IL-18 appeared to activate the IFN- γ pro-

motor at an activating protein (AP)-1 site, whereas IL-12 resulted in binding to a STAT4 site; the combination of a costimulant and IL-12 resulted in activation of both sites. By supershift, IL-18 activated the binding of c-jun to the AP-1 site.¹² The conclusions made from these studies resolve basic issues regarding the relative roles of IL-18 and IL-12 in the production of IFN- γ in CD4⁺ primary human T cells. IFN- γ promoter activation can take place by binding of c-jun to the AP-1 site, whereas in the same cells STAT4 binding takes place by IL-12. The activation of AP-1 in the IFN- γ promoter is sufficient for IFN- γ gene expression and synthesis, but IL-12 activation of STAT4 does not trigger the IFN- γ promoter unless another signal is provided, such as anti-CD3/anti-CD28.

Synergism between IL-18 and IL-12 for IFN- γ production

Although T cells are the major source of IFN- γ , IL-18 and IL-12 act synergistically to increase IFN- γ production from murine bone marrow-derived macrophages.¹⁸ Similar to several studies in T cells, the combination of IL-18 plus IL-12 was far more effective in inducing IFN- γ from these macrophagic cells than either cytokine alone. In fact, the most consistent conclusion that can be made about IFN- γ production is that IL-12 is needed for IL-18-induced IFN- γ production and that IL-18 induces IFN- γ only when its receptor is unregulated by IL-12. Therefore synergism with these two IFN- γ -inducing factors is reported in each study to date. There are some exceptions; KG-1 cells¹⁹ and U1 macrophagic cells²⁰ respond to IL-18 without a need for IL-12, but it is likely that in these cell lines, IL-18R β is already expressed. However, in primary cells both cytokines are required for IFN- γ production.

IFN- γ production in IL-18-deficient mice

Mice with a null mutation for IL-18 were preconditioned with *P. acnes* and, after 4 days, injected with LPS. Circulating levels of IL-18 were not detectable compared with the wild-type controls, which were similarly preconditioned and injected. In another experiment, mice were preconditioned with *P. acnes* for 7 days and then injected with LPS; circulating IFN- γ levels in IL-18-deficient mice were one fifth of those in wild-type controls.¹³ The levels of IL-12 in the IL-18-deficient mice were nearly the same as in wild-type controls. Therefore IL-18 deficiency is associated with a near, but not absolute, absence of IFN- γ production. These findings are similar to those reported for mice injected with neutralizing antibodies to IL-18²¹ and in mice deficient in ICE.^{15,16,22}

IFN- γ production in ICE-deficient mice

Mice preconditioned with *P. acnes* and then injected with LPS have markedly elevated circulating levels of IFN- γ compared with nonconditioned mice. However, in preconditioned mice deficient in ICE, circulating IFN- γ levels were markedly reduced.^{14,15} In vitro, production of

TABLE I. A comparison of postreceptor properties of IL-18 and IL-1 β

Property	IL-18	IL-1 β
Binding to IL-1Rrp	+	—
Binding to IL-1R-AcP	—	+
Recruitment of IRAK	+	+
Recruitment of MyD88	+	+
Phosphorylation of TRAF-6	+	+
Activation of NIK	+	+
Translocation of NF κ B	+	+
Target for AP-1 promoter	+	+
Activation of p38 MAPK	+	+

IFN- γ with nonendotoxin stimulants, such as zymosan or *Staphylococcus epidermidis* in spleen cells from ICE-deficient mice, were also significantly decreased (>75%).¹⁶ In the latter case IL-12 levels were elevated, demonstrating the requirement for IL-18 in LPS-induced IFN- γ production even in the presence of high levels of IL-12. In contrast to microbial stimulants, mitogen-stimulated IFN- γ production was unaffected in spleen cell cultures from ICE-deficient mice.¹⁶ In addition to a deficiency in ICE, specific inhibitors of ICE added to spleen cell cultures from wild-type mice also produce less IFN- γ . Also, antibodies to murine IL-18 suppress LPS-induced IFN- γ in vitro.¹⁶

Other effects of IL-18 on T cells

IL-18 activates T cells to synthesize IL-2, GM-CSF, and TNF- α . There are also reports that IL-18 suppresses the production of IL-10 and does not induce the production of IL-1Ra. In general, the ability of IL-18 to induce different cytokines depends on the cellular targets. Mouse spleen cells and human PBMCs are mixed populations of T cells and monocytic cells. Frequently used, freshly obtained human PBMCs are mostly (70% to 80%) T cells. Of these, approximately 50% are CD4⁺ cells. The percentage of monocytes in PBMC populations varies from 10% to 30%. In addition, there are natural killer (NK) cells and some basophils, but in general, PBMCs are predominantly T cells. Therefore the response of PBMCs to IL-18 should be made by using cultures of selected cells. Each cell type is separated by using magnetic beads coated with specific antibodies. In PBMCs stimulated with IL-18 for 24 hours, the production of cytokines IL-1 β , TNF- α , and IL-8 is observed, but in fact, the cells that respond to the IL-18 are the resting T cells and NK cells.²³

Role of IL-18 in NK cell activity

IFN- γ is the dominant cytokine activating NK cells for killing various tumor cells, and therefore, not surprisingly, stimulation of NK cells with exogenous IL-18 enhances NK cell activity.^{21,24,25} Endogenous NK cell activity, however, is reduced but not absent in IL-18-deficient mice.¹³ Intraperitoneal administration of

TABLE II. A comparison of biologic properties of IL-18 and IL-1 β

Property	IL-18	IL-1 β
Precursor requires ICE cleavage	+	+
Induction of IFN- γ	+	$\pm$
Synergy with IL-12 for IFN- γ	+	+
Increased expression in NOD mice	+	+
Induction of IL-8/IL-1 β /TNF- α	+	+
Increased HIV-1 production	+	+
Activation of T _{H1} responses	+	$\pm$
B-cell activation	+	+
Activation of T _{H2} responses	—	$\pm$
Adjuvant for tumor immunity	+	+
Increased protection in infection	+	+
Elevated levels in leukemias	+	+
Increased expression in adhesion molecules	+	+

exogenous IL-18 to these IL-18-deficient mice not only restored NK function but actually enhanced the lytic activity over that of the wild-type controls. The combination of IL-18 plus IL-12 increased NK activity in both knockout and wild-type controls.¹³ The enhancement of NK activity by exogenous IL-18 in the IL-18R-deficient mice was due to an increase in gene expression of IL-18R β , which was particularly elevated in mice preconditioned with *P. acnes*.¹³ IL-12 was also administered to IL-18-deficient mice in an attempt to restore NK activity. At 5 and 10 ng per mouse, no increase in NK activity was observed in the IL-18-deficient mice, but after 2 days of 100 ng of IL-12 per mouse (8000 ng/kg), spleen cell NK activity was equal to that of the wild-type mice. These latter results suggest that at high doses of exogenous IL-12, NK activity can be restored in IL-18-deficient mice. These results are somewhat difficult to reconcile with the finding that NK activity in IL-18-deficient mice is only one third that of the wild-type, despite the same level of IL-12 production in both mouse strains.

T_{H1} responses and IL-18

The role of IL-18 in the T_{H1} response has been studied primarily in T cells from the BALB/C mouse strain. Subsets of T cells exhibit a differential responsiveness to IFN- γ production by IL-12. This seems to be due to a reciprocal regulation of the IL-12 receptor β 2-chain, in that IL-4 suppresses the expression of this receptor, whereas IFN- γ increases its expression and counteracts the inhibitory effect of IL-4.²⁶ Because the BALB/C mouse strain is a high producer of IL-4, a T_{H2} response to various antigens tends to develop in these mice. In BALB/C mice, IL-18 is clearly a cofactor with IL-12 for the development of the T_{H1} response. This is likely because of the IFN- γ -inducing nature of IL-18. In contrast, C57BL/6 mice do not exhibit a requirement for IL-18 for the development of the T_{H1} response. Any role of IL-18 in the T_{H1} response appears to be a result of the production of IFN- γ , which counters the suppressive effect of IL-4 on the

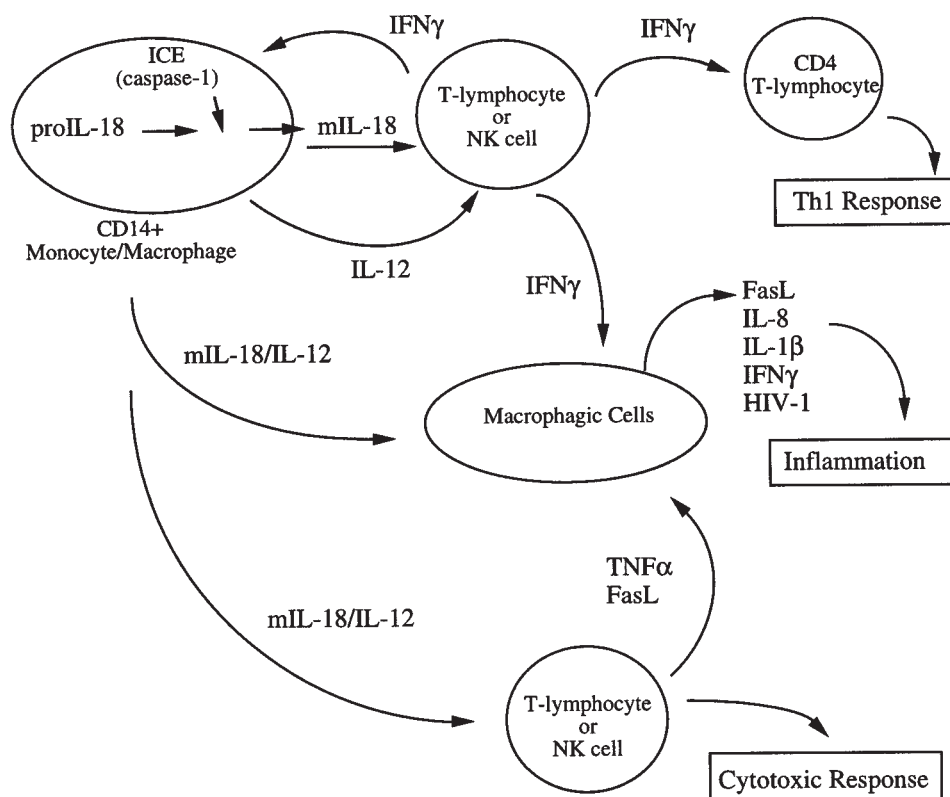


FIG 1. Pathways of IL-18 in the T_{H1} response.

expression of the IL-12R β 2-chain. The addition of anti-IL-4 antibodies reduces the effect of IL-18 presumably because the requirement for IFN- γ is reduced.²⁷

Using ovalbumin-sensitized T-cell receptor–overexpressing CD4 cells, Robinson et al²⁷ demonstrated that IL-18 potentiated the IL-12–induced development of the T_{H1} response by measuring increased production of IFN- γ after 48 hours.²⁷ This observation of IL-12 synergy with IL-18 is consistent with the observations of others who claimed that IL-18 alone was able to drive the T_{H1} response independently of IL-12,^{21,28} but it is unlikely that the effect of IL-18 in those studies was truly free of IL-12 because the presence of small numbers of macrophages could have contributed IL-12 in the culture. However, in a macrophage-free culture in which endogenous IL-12 production was excluded, a marked synergism was observed with IL-12 and IL-18 in both BALB/C and C57BL/6 mice.²⁷ The addition of IL-18 in the secondary stimulation of T cells revealed that the T_{H1} response can take place in the absence of IL-12.²⁷

It seems consistent with the current studies to conclude that there is an absolute requirement for IL-12 in the development of the T_{H1} response. It is well established that IL-12 induces the development of naive CD4⁺ cells into T_{H1} cells by their ability to secrete IFN- γ . On the other hand, IL-18 itself does not shift naive T cells into the T_{H1} phenotype. Although one can propose that in both in vivo and in vitro settings small amounts of IL-18 are

coproduced, IL-12–driven development of naive T cells into T_{H1} cells was observed in mice deficient in IL-18.¹³

IL-18 does not activate T_{H2} T cells

IL-18 does not stimulate T_{H2} cells.^{27,29} This was shown by using gel-shift assays in which IL-18 induced translocation of NF κ B in T_{H1} clones but not T_{H2} cells. T_{H2} –committed clones are generated by using ovalbumin-sensitized splenic T cells after incubation in IL-4 and anti-mouse IL-12.²⁷ In these T_{H2} clones nuclear translocation of NF κ B was not observed after incubation with IL-18, whereas in the same cells IL-1 α induced NF κ B activation and cellular proliferation.²⁷ In another study T_{H2} clones did not express mRNA for IL-18R α ,³⁰ which may be the best explanation for the lack of IFN- γ production by T_{H2} cytokines.

IL-12 increases the responsiveness to IL-18 through an increase in IL-18R expression

The hallmark of IL-18 induction of IFN- γ is the near-absolute requirement for a second signal provided by LPS and other stimuli but particularly by IL-12. Because these other stimuli are themselves inducers of IL-12, it is likely that IFN- γ production is the result of an IL-18/IL-12 effect. In general, in mouse and human cells IL-12 itself, even at nanomolar concentrations, does not induce IFN- γ . However, in the presence of low picomolar concentrations of IL-12, IL-18 becomes essential for the production of

IFN- γ . This has been shown by using LPS induction of IFN- γ in ICE-deficient mice¹⁶ and in mice deficient in IL-18.¹³ In both models IL-12 is present in normal to even high levels. However, in IL-12-deficient mice, IFN- γ production is also absent. The explanation for these observations is that IL-12 is needed to upregulate the receptor for IL-18. Initial studies indicated that there was increased responsiveness to IL-18 after exposure of T cells to IL-12 in T_{H1}-committed clones.²⁹ Data clearly demonstrate that IL-12 increases gene expression and surface expression of IL-18R α .³⁰ As discussed below, IL-12 appears to increase the responsiveness of cells to IL-18, suggesting that IL-12 may affect the surface expression of IL-18R α . By using a specific antibody, one can assume that the increased binding of IL-18 in IL-12-stimulated cells is indeed due to an increase in the receptor number. This would explain the synergy often observed between IL-12 and IL-18 in IFN- γ production from T cells. Fig 1 shows some of the cellular pathways of IL-18.

IL-12 and IL-18 have different signaling pathways in T_{H1} cells

IL-12 binding to its specific receptors triggers a cascade of phosphorylations during which nuclear translocation of 2 transcription factors, STAT3 and STAT4, takes place.³¹ IL-12-deficient and STAT4-deficient mice have an impaired T_{H1} response.^{32,33} Unlike IL-12, IL-18 does not activate STAT4.²⁷ In fact, by using a supershift assay, IL-18 on T_{H1} clones does not appear to activate any of the STAT transcription factors associated with T_{H1} development.²⁷ By using transfection with the IFN- γ promoter linked to a luciferase reporter, IL-18 directly induced IFN- γ promoter activity,¹² whereas for IL-12 activation of this promoter, costimulation with anti-CD3 was required. Either IL-12 or IL-18 plus anti-CD3 activated AP-1. Mutation of the AP-1 site reduced both IL-12- and IL-18-mediated promoter activity. However, mutation of the STAT4 site only affected IL-12 activity.¹² The conclusions of these studies suggest that both AP-1 and STAT4 sites are required for IL-12-dependent IFN- γ promoter activity, but IL-18 induces a direct activation pathway through the AP-1 site.

Effect of IL-18 on B cells

IL-18, together with IL-12, inhibits the production of IgE by activated B cells.³⁴ By using anti-CD40/IL-4-activated murine B cells, IL-18 and IL-12 produce IFN- γ , which in turn suppresses IgE synthesis. This inhibition is not unexpected because suppression of Ig synthesis is a well-established property of IFN- γ . Splenocytes from mice with chronic graft-versus-host disease produce Ig κ , IgM, and IgG1 *in vitro*, and the addition of IL-12 markedly suppressed this production, an effect mediated by IFN- γ production.³⁵ However, by using purified B cells from the splenic population, IL-12 had only marginal suppressing effects, and IL-18 had no effect. The addition of IL-18 plus IL-12, however, resulted in striking inhibition of Ig synthesis in these cultures.³⁵

The combination of IL-12 plus IL-18 acts synergistically to induce a phenotypic change in T cells and puri-

fied splenic B cells to become IFN- γ -secreting cells without engaging their respective antigen receptors.³⁰ Similar to T cells, the role of IL-12 on B cells appears to be an upregulation of IL-18R β ,³⁰ the signaling receptor chain for IL-18 activity.

IL-18R COMPLEX

Current understanding of the IL-18R complex

Torigoe et al³⁶ described the purification and characterization of a human IL-18 receptor by using an antibody directed against a cell surface protein of the human Hodgkin's cell line L428. The purified protein had a molecular weight between 60 and 100 kd, and its amino acid sequence revealed 100% identity to a previously known member of the IL-1 receptor family, which had been termed the IL-1Rrp.¹ IL-1Rrp had remained an orphan receptor until recently. When IL-1Rrp was first reported, it was clear that it was indeed a member of the IL-1 receptor family, although it did not bind IL-1 α , IL-1 β , or IL-1Ra.¹ This was an unexpected finding because IL-1Rrp was isolated from a library by using degenerate oligonucleotides from conserved amino acids in the extracellular domains of the IL-1R type I.

Until the studies by Torigoe, the ligand for IL-1Rrp was unknown, but it was likely that the ligand was structurally related to members of the IL-1 family. However, despite its relatedness, neither IL-1 α nor IL-1 β bind to IL-1Rrp.¹ When the extracellular domain of IL-1Rrp was expressed as a fusion protein linked to the Fc domain of IgG, IL-1Rrp did not bind IL-1 on a Biacore chip.¹ However, a chimeric molecule was constructed that was comprised of the extracellular domains of the IL-1 type I receptor linked to the transmembrane and cytosolic domain of IL-1Rrp. When transfected into COS cells, this chimeric receptor did exhibit IL-1 functional activity on binding of IL-1. Therefore although IL-1Rrp is a member of the IL-1R family, and its extracellular domains do not bind IL-1 itself, IL-1Rrp transmits a signal similar to that of the IL-1R.

IL-1Rrp is the binding component of the IL-18R complex

Torigoe et al³⁶ reported that IL-1Rrp is the functional component of IL-18R primarily on the basis of the observation that antibodies to IL-1Rrp prevent IL-18 activity. Yet in terms of other cytokine receptors, IL-18 binding to the L428 cell line used in the purification process was low (dissociation constant [Kd] of 18.5 nmol/L). Boraschi et al³⁷ reported a Kd of 25 nmol/L for IL-18. The Kd for IL-18 binding to IL-1Rrp expressed transiently on COS cells was 45 nmol/L.³⁶ These are unusually low affinities for a cytokine with a biologic activity in the picomolar range. Nevertheless, IL-1Rrp is unquestionably essential for IL-18 activity and an integral part of the IL-18 signaling complex. Therefore the term IL-18R α replaces the term IL-1Rrp. However, a second binding chain in a putative IL-18R complex likely exists in order to form a high-affinity complex that characterizes all cytokine receptor signaling.

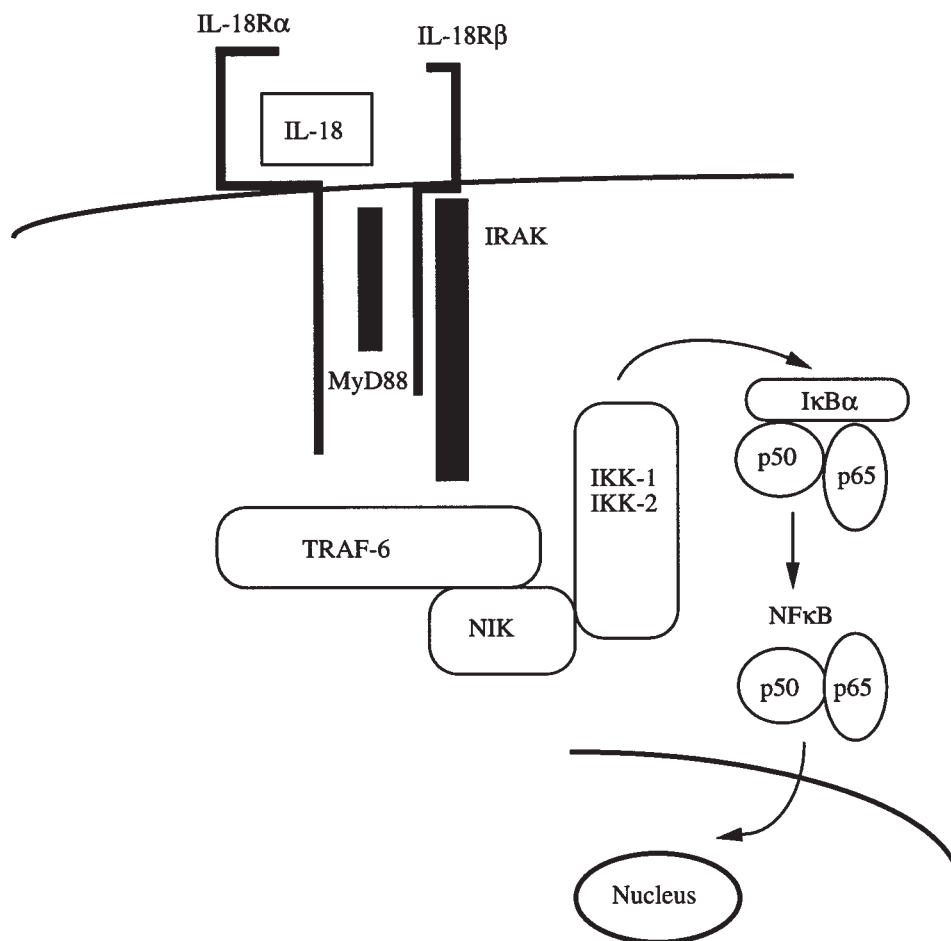


FIG 2. Model for IL-18 signal transduction. IL-18 binds to the IL-18R α chain (not identified to date) and recruits the IL-18R β chain³⁶ (also known as IL-1R β). This heterodimeric complex recruits MyD88 and IRAK. Phosphorylated IRAK activates TRAF-6, and in turn NIK is phosphorylated. This results in activation of IKK and then phosphorylation of I κ B. Phosphorylated I κ B degrades and releases the p50 and p65 components of NF κ B. Free NF κ B migrates through nuclear pores and translocates to nuclear DNA for gene transcription. Adapted from *J Leukoc Biol* 1998;63:650-7.

Yoshimoto et al³⁰ have reported that there are both high-affinity (0.4 nmol/L) and low-affinity (40 nmol/L) binding sites for IL-18 in murine primary T cells. Clearly IL-18R α could account for the low-affinity IL-18 binding sites. Thus a second chain resulting in a greater affinity would explain the higher affinity. This "other" receptor chain has been identified as the AcPL receptor³⁸ and is related to the IL-1 accessory protein identified by Greenfeder.³⁹ Similar to the IL-1 accessory protein, the IL-18 AcPL does not bind to its ligand but rather binds to the complex formed by IL-18 with the IL-18R α chain.³⁸ This heterodimeric is likely the high-affinity complex.

There is a comparable finding in the identification of the IL-1R complex. For nearly 9 years, IL-1 activity was thought to be caused by a single-chain receptor, the 80 kd IL-1R type I.⁴⁰ The second chain was discovered in 1995; it was termed the IL-1R accessory protein (IL-1R-AcP).³⁹ This latter receptor is essential to IL-1 signaling, although IL-1R-AcP does not bind to any IL-1 ligand.

Similar to many cytokine receptors, it is a heterocomplex of a ligand-binding chain (often termed the α -chain of the receptor) plus an accessory chain (often termed the β -chain) which transmits the signal. The importance of the IL-1R-AcP for IL-1 activity was shown by using antibodies to IL-1R-AcP, which is an essential part of the heterodimeric IL-1R complex.

Therefore the IL-18R α is unlikely the sole component of the IL-18R-signaling apparatus and is certainly not the high-affinity ligand-binding receptor chain. It is likely that the IL-18R complex is comprised of a ligand-binding chain of IL-1R β (IL-18R α), with the second chain being the non-IL-18-binding AcPL (IL-18R α).³⁸ As described below,¹⁷ an IL-18 binding protein (IL-18BP) has been purified from human urine and would likely be the soluble (extracellular) form of the IL-18R α chain. But a transmembrane form of the IL-18BP has not been isolated to date despite murine cDNA cloning and human genomic analyses.^{17,41,42} Thus the IL-18BP is a

third gene product and appears to be the "decoy" receptor of IL-18, similar to the IL-1R type II, which is the decoy receptor for IL-1.

Isolation and purification of an IL-18BP from human urine

By using methods established for the purification and characterization of ligand-binding cytokine receptors in human urine,⁴³⁻⁴⁶ a 38-kd IL-18BP was purified to homogeneity from urine by using ligand-affinity chromatography, and the N-terminal amino acids were determined.¹⁷ The amino acid sequence of the IL-18BP is not that of a known cytokine receptor presently listed in the data banks. Although this binding protein likely represents the extracellular domain of the ligand-binding receptor chain, to date a transmembrane and cytosolic component to the IL-18BP has not been found.

Similar to neutralizing antibodies to IL-18,¹⁶ the IL-18BP prevents LPS-induced IFN- γ production.¹⁷ Also similar to anti-IL-18 antibodies, the IL-18BP does not affect IFN- γ production after stimulation with mitogens such as concanavalin A. However, unlike antibodies to IL-18, the IL-18BP does not exhibit species specificity, and therefore human IL-18BP neutralizes both human and murine IL-18.¹⁷

IL-18 signal transduction is similar to that of IL-1

It is clear that IL-1 and IL-18 do share signaling pathways, particularly in activating the processes by which NF κ B translocates to the nucleus. In terms of IFN- γ production, IL-1 α , IL-1 β , and IL-18 each induce IFN- γ in the presence of IL-2 or IL-12.^{11,47} IL-18 is 5 to 10 times more potent than either form of IL-1,⁴⁷ which suggests, as expected, that signal transduction of IL-1 and IL-18 cannot be identical (Table I). However, the recruitment of IRAK to the IL-18R complex places IL-18 signaling high in the signaling cascade that it shares with IL-1. For IL-1 signaling, one of the earliest events is the formation of the heterocomplex of the ligand IL-1 with the type I IL-1R and the IL-1R-AcP.³⁹ This complex then recruits IRAK.⁴⁸ The topic of IL-1 signal transduction related to NF κ B activation has been reviewed in detail.^{49,50} The IL-1R-AcP is essential for the recruitment and activation of IRAK.^{51,52} In fact, deletion of specific amino acids in the IL-1R-AcP cytoplasmic domain results in loss of IRAK association,⁵¹ and these same amino acids are present in IL-1Rrp (IL-18R α). In addition, an intracellular adapter molecule termed MyD88 appears to dock to the complex, allowing IRAK to become phosphorylated.^{48,53} IRAK then dissociates from the IL-1R complex and associates with TNF receptor-associated factor-6 (TRAF-6).⁵⁴ TRAF-6 then phosphorylates NIK,⁵⁵ and NIK phosphorylates the inhibitory κ B kinases (IKK-1 and IKK-2).⁵⁶ Once phosphorylated, I κ B is rapidly degraded by a ubiquitin pathway liberating NF κ B, which translocates to the nucleus for gene transcription.

IRAK also associates with the IL-18R complex.^{27,57} This was demonstrated by using IL-12-stimulated T cells followed by immunoprecipitation with anti-IL-18R or

anti-IRAK.⁵⁷ Furthermore, IL-18-triggered cells also recruited TRAF-6.⁵⁷ Like IL-1 signaling, MyD88 has a role in IL-18 signaling. MyD88-deficient mice do not produce acute-phase proteins and have diminished cytokine responses. Recently, T_{H1}-developing cells from MyD88-deficient mice were unresponsive to IL-18-induced activation of NF κ B and c-Jun N-terminal kinase.⁵⁸ Thus MyD88 is an essential component in the signaling cascade that follows IL-1R, as well as IL-18R binding. It appears that the cascade of sequential recruitment of MyD88, IRAK, and TRAF-6 followed by the activation of NIK, degradation of I κ BK, and release of NF κ B are nearly identical for IL-1, as well as for IL-18. Indeed, in cells transfected with IL-18R α (IL-1Rrp) and then stimulated with IL-18, translocation of NF κ B is observed with the electromobility shift assay.³⁶ In U1 macrophages, which already express the gene for IL-1Rrp, there is translocation of NF κ B and stimulation of HIV-1 production.²⁰

Fig 2 is a working model of the proposed IL-18 signaling process, which results in translocation of NF κ B to the nucleus. However, not shown is the evidence that IL-18 signaling activates p38 mitogen-activated protein kinase (MAPK). In U1 cells stimulated with IL-18, IL-8 synthesis is reduced by nearly 90% in the presence of a specific p38 MAPK inhibitor.²⁰ In murine T cells, IL-18 stimulated the phosphorylation of p42 MAPK and p56lck.⁵⁹

IL-18 AS A PROINFLAMMATORY CYTOKINE IL-18 induces chemokines in monocytes and NK cells

There is no dearth of evidence for the role of chemokines in promoting inflammation. For example, the neutrophil-activating chemokine IL-8 is a primary agonist in several pathologic processes of the lung. It is presently unclear whether IL-18 has a role in the eosinophilic infiltration of asthma, but the global stimulation of both CXC and CC chemokines by IL-18²³ warrants consideration of the hypothesis that eosinophilic chemokines are induced from cells expressing receptors for IL-18. In this regard, the macrophage cell line U1, which is derived from the histiocytic lymphoma cell U937, releases IL-8 when stimulated by IL-18.²⁰ In addition, the myelomonocytic leukemia cell line KG-1 also releases IL-8 when stimulated with IL-18.¹⁹

This topic has recently been reviewed.⁶⁰ Is there a broader role for IL-18? Does the cytokine possess other biologic activities not only as a costimulant for T_{H1} cytokines but also as a direct acting proinflammatory cytokine? The ability of IL-18 to induce CC and CXC chemokines places IL-18 in a strategic role in inflammation. By using freshly obtained human PBMCs, mature recombinant IL-18 induced IL-8 gene expression and synthesis.²³ By using IL-1Ra to block IL-1 activity in these cells, IL-18-induced IL-8 production was reduced by 40%. When TNF activity was inhibited by soluble receptors for TNF, IL-18-induced IL-8 production was reduced by 80%, suggesting that the primary action of

IL-18 was through a TNF-dependent pathway. Although IL-8 in PBMC cultures was derived from the CD14⁺ cell population (and not the CD3⁺ cells), the source of TNF was the CD3⁺ and NK cells. Therefore the inflammatory cytokine cascade initiated by IL-18 in the mixed PBMC population starts with triggering of constitutive IL-18 receptors on lymphocytes and NK cells, leading to TNF production; in turn, TNF stimulates IL-1 and IL-8 from the CD14⁺ cells. The induction of TNF can be observed at the level of gene expression within 2 hours after exposure to IL-18. IL-18 also stimulates the synthesis of macrophage chemoattractant protein-1 in these cultures.²³ These results are consistent with the ability of IL-18 to activate the translocation of NF κ B in peripheral T cells and fibroblasts.^{27,36,61}

Consistent with the induction of TNF in these models is the observation that IL-18 induces HIV-1 expression. At concentrations as low as 100 pmol/L, mature IL-18 induces p24 synthesis in the chronically infected macrophage cell line U1.²⁰ The increase in p24 is an indication of production of competent virus by these cells. Similar to the observation in PBMCs, HIV-1 p24 production by IL-18 is inhibited by blocking the activity of endogenously produced TNF.²⁰ Furthermore, the induction of p24 by IL-18 is reduced by inhibition of endogenous nitric oxide synthesis.⁶² Again, these observations are consistent with the ability of IL-18 to activate NF κ B translocation. As discussed below, one chain of the IL-18 receptor complex is the IL-1R α , which, on transient transfection into COS-1 cells, results in NF κ B translocation.³⁶ In murine-cloned T cells IL-18 stimulation results in a macromolecular complex precipitated by an antibody to IRAK; however, in these same cells IL-18 does not activate STAT4, which characteristically occurs after IL-12 treatment.²⁷ Because IRAK activates a kinase cascade leading to degradation of I κ B and activation of NF κ B, these findings are consistent with IL-18 induction of TNF from T cells and IL-8 from macrophages.²³

IL-18 upregulates expression of Fas ligand

IL-18 enhances Fas ligand expression and induces apoptosis in Fas-expressing human myelomonocytic KG-1 cells.¹⁹ KG-1 cells are monocyte-like and also produce the intercellular adhesion molecule-1 (ICAM-1). IL-18 increases ICAM-1 expression in these macrophagic cells.⁶³ These cells also express constitutively Fas antigen (CD95), and after exposure to IL-18, KG-1 cells became apoptotic. Fas ligand is upregulated in T cells and NK cells after incubation with IL-18.⁶⁴ In the liver cytotoxic T cells are thought to contribute to the destruction of hepatic cells, particularly when infected by viruses. Cytotoxic T cells and NK cells isolated from the liver and then stimulated with IL-18 exhibit enhanced killing of Jurkat T cells.⁶⁵ The IL-18-induced enhanced killing by liver-derived cytotoxic T lymphocytes was through the perforin-dependent pathway and not CD95.⁶⁵ Liver-derived NK cells also exhibited enhanced killing of Jurkat cells after exposure to IL-18, but the mechanism was thought to be independent of TNF.

However, failure to detect TNF in cell culture supernatants is not an argument that any cellular change is TNF independent because membrane forms of TNF are biologically active but often hardly measurable in supernatant. In fact, the ability of IL-18 to induce HIV-1 in U1 cells²⁰ and IL-8 in PBMCs²³ is TNF dependent. In both studies the dependence on TNF was demonstrated by reducing the IL-18 effect with soluble TNF p55 receptor. Therefore it is likely that IL-18-induced killing by liver NK cells can still be caused by induction of membrane TNF.

IL-18 stimulates HIV-1 expression in a macrophage cell line

The U1 promonocytic cell line is derived from the U937 human histiocytic lymphoma cell line. This cell contains two copies of HIV-1 in its genome. After stimulation with cytokines, such as TNF or IL-1, U1 cells produce large amounts of live, infectious HIV-1 virus. In these cells IL-18 increased HIV-1 production as measured by synthesis of p24 HIV-1 antigen.²⁰ This increase in HIV-1 production was markedly reduced by inhibition of nitric oxide synthase.⁶⁶ By gel-shift analysis, activation of the NF κ B transcription factor was seen after a 1-hour exposure of cells to IL-18. IL-18 induced a 30-fold increase in p24 over unstimulated U1 cells. However, neutralization of endogenous TNF activity reduced IL-18-induced HIV-1 production by 55%. In the same cultures IL-18-induced synthesis of the chemokine IL-8 was reduced by 96%. In addition, neutralization of endogenous IL-6 activity also reduced IL-18-induced HIV-1 production (by 63%) but did not affect IL-8 production. Specific inhibition of the p38 MAPK reduced IL-18-stimulated p24 production by 73% and IL-8 by 87%. One can conclude from these studies that the stimulation of HIV-1 and IL-8 production in these cells by IL-18 takes place by means of a cascade involving direct NF κ B activation because both HIV-1 and IL-8 have NF κ B-binding consensus nucleotides in their respective promoters. In addition, indirect activation of HIV-1 expression likely occurs by means of the induction of TNF and IL-6, which themselves stimulate HIV-1 expression. The role for activation of p38 MAPK in this process is consistent with the known role for this kinase in the synthesis of cytokines⁶⁷ and HIV-1.⁶⁸

Using acutely infected T-lymphoblastic Hut78 cells and U937 macrophagic cells, IL-18 was added to the cultures for 5 weeks, and the amount of infectious virus was assessed. In the T cells the presence of IL-18 increased HIV-1 production 3- to 4-fold, whereas no effect was observed in the U937 cells.⁶⁹ The amount of IL-18 added to the cultures of acutely infected cells was 100 ng/mL, which is 10-fold greater than that used to stimulate HIV-1 production in U1 cells that are latently infected.²⁰

IL-18 and insulin-dependent diabetes mellitus (IDDM)

In islet cultures IL-1, TNF, and/or IFN- γ induce nitric oxide, which mediates selective β -cell death.^{70,71} Expression of IL-1 β in cells infiltrating the islets in the

nonobese diabetic (NOD) mouse and the BioBreeder rat add further to the concept that IL-1 β plays a role in these spontaneous models of diabetes. Recently, a role for IL-18 has been postulated in the development of autoimmune diabetes in the mouse.⁷² The rationale of these studies was based on the well-established observation that IFN- γ contributes to β -cell death. In addition, IL-18 increases Fas ligand expression^{19,73} and NF κ B activation.^{36,61} Expression of IL-18 transcripts occurred with the onset of insulinitis in the NOD mouse.⁷² The location of the IL-18 gene within the Idd2 interval on chromosome 9 implicates IL-18 as one of the NOD susceptibility genes.⁷⁴ The human IL-18 gene maps to 11q22.2-q22.3, which is closely linked to the DRD2 gene locus and distinct from mapped IDDM loci.⁷⁵

There seems to be a clear association with the development of the T_{H1} response in the NOD mouse and local IL-18 expression.⁷⁶ It was also reported that the T_{H1} response in the NOD mouse leading to the destruction of the islet is preceded by expression of IL-18 in the spleen.⁷² Cells expressing IL-4 were evenly distributed around the infiltrate, whereas IFN- γ -expressing cells were restricted to the area around the β cells.⁷⁶ Therefore the precise role for IL-18 in the pathogenesis of IDDM is presently unclear. The well-established ability of IL-18 to induce Fas ligand^{19,64,73} would provide provisional evidence for a role of this cytokine in IDDM in addition to its T_{H1} cytokine profile.

Is there a role for IL-18 in autoimmune demyelinating disease?

Fas-mediated T-cell death worsens autoimmune diseases in some models.⁷⁷ For example, neutralizing antibodies to IFN- γ worsen autoimmune experimental allergic encephalomyelitis.⁷⁸ On the other hand, transgenic mice overexpressing central nervous system IFN- γ under the control of an oligodendrocyte-specific promoter exhibited several of the characteristic lesions seen in models of autoimmune demyelination.⁷⁹ Because the prominent biologic property of IL-18 is IFN- γ , as well as Fas-ligand, expression, it is possible that IL-18 plays an agonist role in autoimmune demyelinating disease. Blocking IL-1 β with IL-1Ra or soluble IL-1RI also reduces autoimmune allergic encephalomyelitis.^{80,81}

Other proinflammatory activities of IL-18

IL-18 stimulates ICAM-1 expression on monocytic cell lines.⁶³ Anti-IFN- γ did not reduce the effect of IL-18 in inducing ICAM-1 expression on monocytes, and therefore this was a direct effect of IL-18 and not one mediated by IFN- γ . In murine peritoneal macrophages murine IL-18 stimulated production of IL-6 and IL-1 β ; there was no stimulation of TNF or IL-1 α in the same cultures.⁸² By using macrophages from mice deficient in both TNF- α and lymphotoxin- α , IL-18 stimulated IL-6 production. IL-18 inhibits osteoclast-like multinucleated cells by induction of GM-CSF and not through induction of IFN- γ .⁸³ In a mixture of osteoblasts and hematopoietic cells, IL-18 inhibited osteoclast formation in the pres-

ence of osteoclast-generating agents. With mice deficient in GM-CSF, a similar role for IL-18 was reported,⁸⁴ and thus this is a non-IFN- γ effect of IL-18.

HOST DEFENSE FUNCTIONS OF IL-18 IL-18 in models of infection

In BALB/C mice, which are highly susceptible to infection with Mycobacteria, there is low expression of IL-18 and IFN- γ compared with the resistant strain DBA/2, in which high levels of IFN- γ and IL-18 are observed.⁸⁵ Administration of IL-12 reduces bacterial counts, and therefore it has been proposed that the combination of IL-18 and IL-12 affords protection in these models. For nearly all cytokines, including the proinflammatory cytokines, there are well-established animal models in which cytokine treatment affords protection from infection, increased host defense, or reduced disease severity. The mechanisms are multiple, and in the case of IL-12/IL-18, it is likely that IFN- γ production accounts for this protection, although no differences were detected in the IFN- γ production from resistant or susceptible mouse strains.⁸⁶

In models of protection against bacterial infection, pretreatment or early treatment, rather than postinfection treatment, is the hallmark. By using a model of live Cryptococcal infection, pretreatment and early treatment with IL-18 reduced infection severity and briefly prolonged life but did not increase survival at 3 to 6 weeks.⁸⁷ The amount of IL-18 administered to these mice was 100-fold higher than the amount of IL-1 β needed to protect mice against *Candida* lethality.⁸⁸

In a model of *Yersinia enterocolitica* infection, mice resistant to this infection (C57BL/6) expressed 4-fold more IL-18 mRNA compared with mice sensitive (BALB/C) to *Yersinia*.⁸⁹ Anti-IL-18 antibodies resulted in a 100- to 1000-fold increase in bacterial counts in the spleen but without a significant change in IFN- γ production. This is an unusual finding because the anti-IL-18 antibody reduces IFN- γ in an LPS model.²¹ By using *Salmonella typhimurium* in mice, anti-IL-18 antibodies induce an exacerbation of the infection, with reduced IFN- γ production at the protein and mRNA levels.⁹⁰ Administration of exogenous IL-18 protects mice in this model, but in IFN- γ receptor-deficient mice no protection is afforded. These data suggest that the protective effect of IL-18 is mediated by IFN- γ . Similar findings have been observed with anti-IL-12 antibodies.⁹¹

IL-18 increases innate defense against tumors

IL-18 administered into mice as an adjuvant for increased tumor immunogenicity provided enhanced NK cell activation and production of IL-2, as well as sequentially decreased IL-10.²⁸ Osaki et al⁹² have also reported increased tumor immunogenicity by pretreating with IL-18 in a melanoma model. However, the ability of IL-18 to afford protection was unexpectedly observed in IFN- γ receptor-deficient mice. Because the protection afforded by IL-12 in this model is dependent on IFN- γ receptor

expression, it appears that IL-18 may possess host-defense functions independent of IFN- γ .

Mouse mammary carcinoma cells expressing IL-12 or IL-18 are less tumorigenic and formed tumors more slowly compared with cells not expressing these cytokines.⁹³ Moreover, injecting both cytokine-expressing tumor cells together with a nontransfected tumor resulted in a 70% reduction in tumor growth. The antitumor effect was likely caused by IFN- γ production. Moreover, tumor cells expressing either of these cytokines induced a greater systemic inhibition of angiogenesis. In human pancreatic carcinoma cells transfected with IL-12 or IL-18, IL-12 induced an antitumor effect in nude mice,⁹⁴ but IL-18 did not. Because the study was carried out in nude mice, one can conclude that the effect of IL-12 is not caused by IFN- γ production.

IL-18 IN CLINICAL MEDICINE

Levels of IL-18 in human disease

To date, there is a single study measuring circulating IL-18 in patients with various leukemias.⁹⁵ By using a specific ELISA for human mature IL-18, healthy subjects exhibited levels of plasma IL-18 between 50 and 150 pg/mL. In contrast, patients with acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, and chronic myelogenous leukemia had elevated levels (200 to 1200 pg/mL). Patients with acute lymphoblastic leukemia and chronic myelogenous leukemia had the highest levels, although patients with acute myelogenous leukemia had the greatest number of samples above the level measured in healthy subjects (>150 pg/mL). It should be noted that these are the same leukemias in which a pathogenic role for IL-1 β has been demonstrated.⁹⁶

Whether to give IL-18 or suppress IL-18

The immunologic basis for increase in asthma cases in the United States, Japan, and Europe has been thoroughly discussed in many publications. Although there are several hypotheses, most consider the importance of the T_{H1} versus T_{H2} balance (or imbalance depending on one's viewpoint) as a contributing factor.⁹⁷ The delayed hypersensitivity to tuberculin test to determine the presence of a " T_{H1} state" in patients has been used by Japanese investigators.⁹⁸ In their hypothesis a decline in tuberculosis in Japan has resulted in a loss of the dominance of the T_{H1} response, allowing T_{H2} responses to dominate during antigen (allergen) exposure. In that case one should examine inducible IFN- γ production in a large population of asthmatic subjects to determine whether any correlations exist. In fact, a simple whole blood test has been designed for this purpose, which can be applied to several hundred patients in the field.⁹⁹ However, because IFN- γ is dependent on the synthesis and processing of IL-18, levels of IL-18 activity in asthmatic patients also should be determined. A clinically relevant finding would be that a correlation between low IFN- γ and IL-18 production exists in asthmatic, compared with nonasthmatic, subjects. It may not be that the absence of infection per se is the reason for

the low T_{H1} state but rather that the T_{H1} cytokine response (IFN- γ and IL-18 production), which determines the outcome of the immunologic response to allergens, is low in the asthmatic subjects. It may be possible to incorporate IL-18 as an adjuvant in a vaccine trial for treating asthmatic subjects, assuring a T_{H1} response.

Strategies for reducing IL-18 in disease

Because IL-18 is a promoter of T_{H1} immune responses, as well as a direct proinflammatory cytokine, reducing IL-18 activity in some diseases is a sensible strategy. For example, suppression of an active T_{H1} response in acute or chronic transplant rejection is presently accomplished by using antibodies to activated T cells, corticosteroids, cyclosporine, and other immunosuppressive agents. There are also ongoing clinical trials with IL-10 and rapamycin. However, although highly effective, each of these is nonspecific and global in that they suppress the synthesis of several lymphocyte growth factors and processes of macrophage and lymphocyte activation. In addition, some are clearly toxic and particularly nephrotoxic. In the case of renal transplants the nephrotoxicity of cyclosporine can obscure rejection. Moreover, the global immunosuppression may prevent the development of graft tolerance, a desirable event in allograft transplantation. On the other hand, reducing the activity of IL-18 offers a specific suppression of the T_{H1} response. How is this accomplished? Neutralizing antibodies to IL-18 are an obvious approach. Rabbit anti-mouse IL-18 will reduce the circulating levels of IFN- γ after LPS and protect against hepatocellular damage induced by LPS.²⁴ The IL-18BP described above specifically blocks IL-18 activity in vitro and in vivo.¹⁷ The IL-18BP has a high affinity for the ligand and is an ideal candidate for specific reduction of IL-18 activity in the T_{H1} response.

Of the several disease processes in which a blocking IL-18 activity may be considered a therapeutic strategy, those associated with a T_{H1} response gain support from preclinical studies. As discussed above, these include autoimmune demyelinating diseases, rheumatoid arthritis, Crohn's disease, lupus nephritis, and psoriasis. Patients with rheumatoid arthritis have been successfully treated with either IL-1Ra,¹⁰⁰ antibodies to TNF- α ,¹⁰¹ or soluble TNF- α receptor p75 Fc fusion protein.¹⁰² However, although highly effective in reducing inflammation, inflammation returns once the anticytokine therapy is stopped. Therefore a more fundamental reduction in the T_{H1} response may offer these patients a greater reduction in their disease activity. Rheumatoid arthritis is associated with increased expression of Class II molecules and large numbers of CD4⁺ T cells. Various therapeutic strategies have targeted a reduction in T cells in patients with rheumatoid arthritis with improvement in their disease activity. Therefore a specific blockade of the T_{H1} response in rheumatoid arthritis may be a sensible therapeutic approach. A similar approach can be made for multiple sclerosis. However, a therapeutic improvement from reducing a T_{H1} response in this disease is far less certain.¹⁰³

The addition of IL-18 during the secondary stimulation of T cells reveals that the T_{H1} response can take place in the absence of IL-12.²⁷ Furthermore, the increased binding of IL-18 in IL-12-stimulated cells is likely caused by an increase in the receptor number for IL-18. Therefore one would expect a reduced T_{H1} response solely by blocking IL-18 activity. By the same reasoning, a reduced T_{H1} response would also be apparent by blocking IL-12. Similar to the case of blocking either IL-1 or TNF in patients with rheumatoid arthritis,^{100,101} the marked synergy between IL-12 and IL-18 during the T_{H1} response suggests that blocking either of these cytokines would shorten the T_{H1} response.

The extracellular domain of human IL-1Rrp is also a potential agent for specific reduction in IL-18 activity. This extracellular domain has been expressed as an Fc fusion protein (also called soluble IL-1RrpFc) for enhanced half-life activity in the circulation. However, when incubated with IL-18 at molar ratios of 1:2 or greater, the soluble IL-1RrpFc actually enhances the biologic activity of IL-18 rather than reduces its activity in vitro.¹⁰⁴ On the other hand, the presence of IL-18BP results in near-complete reduction in IL-18 activity at molar excesses of 2 or greater.¹⁷

Is blocking IL-18 safe?

This important question is asked in any trial during which a naturally occurring cytokine, such as IL-1 or TNF, is blocked. Other agents, such as cyclosporine or corticosteroids, nonspecifically reduce cytokine production and yet are widely accepted for use in patients. Although host defense functions are suppressed by agents such as cyclosporine or corticosteroids, the use of IL-1Ra or neutralizing agents for TNF- α has raised concern about their safety. At present, it appears that blocking either IL-1 or TNF- α is associated with less evidence for altered host defense function compared with that observed by using cyclosporine or corticosteroids. The production of IFN- γ will certainly be suppressed in patients receiving any agent targeted to reduce IL-18 activity. Therefore infections with *Mycobacteria*¹⁰⁵ or intracellular organisms, such as *Salmonella*, may be increased.

REFERENCES

1. Parnet P, Garka KE, Bonnett TP, Dower SK, Sims JE. IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP. *J Biol Chem* 1996;271:3967-70.
2. Puren AJ, Fantuzzi G, Dinarello CA. Gene expression, synthesis and secretion of IL-1 β and IL-18 are differentially regulated in human blood mononuclear cells and mouse spleen cells. *Eur Cytokine Netw* 1998. In press.
3. Mileno MD, Margolis NH, Clark BD, Dinarello CA, Burke JF, Gelfand JA. Coagulation of whole blood stimulates interleukin-1 β gene expression: absence of gene transcripts in anticoagulated blood. *J Infect Dis* 1995;172:308-11.
4. Shapiro L, Dinarello CA. Cytokine expression during osmotic stress. *Exp Cell Res* 1997;231:354-62.
5. Tone M, Thompson SAJ, Tone Y, Fairchild PJ, Waldmann H. Regulation of IL-18 (IFN- γ -inducing factor) gene expression. *J Immunol* 1997;159:6156-63.
6. Martin TJ, Romas E, Gillespie MT. Interleukins in the control of osteoclast differentiation. *Crit Rev Eukaryot Gene Expr* 1998;8:107-23.
7. Stoll S, Mueller G, Kurimoto M, Saloga J, Tanimoto T, Yamauchi H, et al. Production of IL-18 (IFN- γ -inducing factor) messenger RNA and functional protein by murine keratinocytes. *J Immunol* 1997;159:298-302.
8. Conti B, Jeong JW, Tinti C, Son JH, Joh TH. Induction of IFN- γ -inducing factor in the adrenal cortex. *J Biol Chem* 1997;272:2035-7.
9. Sareneva T, Matikainen S, Kurimoto M, Julkunen I. Influenza A virus-induced IFN- α/β and IL-18 synergistically enhance IFN- γ gene expression in human T cells. *J Immunol* 1998;160:6032-8.
10. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immuno-regulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Ann Rev Immunol* 1995;13:251-74.
11. Hunter CA, Chizzonite R, Remington JS. IL-1 β is required for IL-12 to induce the production of IFN- γ by NK cells. *J Immunol* 1995;155:4347-54.
12. Barbulescu K, Becker C, Schlaak JF, Schmitt E, Meyer zum Büschenfelde K-H, Neurath MF. IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN- γ promoter in primary CD4+ T lymphocytes. *J Immunol* 1998;160:3642-7.
13. Takeda K, Tsutsui H, Yoshimoto T, Adachi O, Yoshida N, Kishimoto K, et al. Defective NK cell activity and Th1 response in IL-18-deficient mice. *Immunity* 1998;8:383-90.
14. Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, Fleming MA, et al. Activation of interferon- γ inducing factor mediated by interleukin-1 β converting enzyme. *Science* 1997;275:206-9.
15. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, et al. Caspase-1 processes IFN- γ -inducing factor and regulates LPS-induced IFN- γ production. *Nature* 1997;386:619-23.
16. Fantuzzi G, Puren AJ, Harding MW, Livingston DJ, Dinarello CA. IL-18 regulation of IFN- γ production and cell proliferation as revealed in interleukin-1 β converting enzyme-deficient mice. *Blood* 1998;91:2118-25.
17. Novick D, Kim S-H, Fantuzzi G, Reznikov L, Dinarello CA, Rubinstein M. A circulating IL-18 binding protein: isolation and characterization [abstract]. *Eur Cytokine Netw* 1998. In press.
18. Munder M, Mallo M, Eichmann K, Modolell M. Murine macrophages secrete interferon gamma upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. *J Exp Med* 1998;187:2103-8.
19. Ohtsuki T, Micallef MJ, Kohno K, Tanimoto T, Ikeda M, Kurimoto M. Interleukin-18 enhances Fas ligand expression and induces apoptosis in Fas-expressing human myelomonocytic KG-1 cells. *Anticancer Res* 1997;17:3253-8.
20. Shapiro L, Puren AJ, Barton HA, Novick D, Peskind RL, Su MS-S, et al. Interleukin-18 stimulates production of human immunodeficiency virus in the U1 monocytic cell line. *Proc Natl Acad Sci U S A* 1998;95:12550-5.
21. Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, et al. Cloning of a new cytokine that induces interferon- γ . *Nature* 1995;378:88-91.
22. Gu Y, Wu J, Faucheu C, Lallane J-L, Diu A, Livingston DL, et al. Interleukin-1 β converting enzyme requires oligomerization for activity of processed forms in vivo. *EMBO J* 1995;14:1923-31.
23. Puren AJ, Fantuzzi G, Gu Y, Su MS-S, Dinarello CA. Interleukin-18 (IFN- γ -inducing factor) induces IL-1 β and IL-8 via TNF α production from non-CD14+ human blood mononuclear cells. *J Clin Invest* 1998;101:711-24.
24. Okamura H, Nagata K, Komatsu T, Tanimoto T, Nukata Y, Tanabe F, et al. A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. *Infect Immun* 1995;63:3966-72.
25. Ushio S, Namba M, Okura T, Hattori K, Nukada Y, Akita K, et al. Cloning of the cDNA for human IFN- γ -inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. *J Immunol* 1996;156:4274-9.
26. Szabo S, Dighe AS, Gubler U, Murphy KM. Regulation of the interleukin-12R β 2 subunit expression in developing T helper (Th1) and Th2 cells. *J Exp Med* 1997;185:817-24.
27. Robinson D, Shibuya K, Mui A, Zonin F, Murphy E, Sana T, et al. IGF does not drive Th1 development but synergizes with IL-12 for interferon- γ production and activates IRAK and NF κ B. *Immunity* 1997;7:571-81.
28. Micallef MJ, Tanimoto T, Kohno K, Ikeda M, Kurimoto M. Inter-

- leukin-18 induces the sequential activation of natural killer cells and cytotoxic T lymphocytes to protect syngeneic mice from transplantation with Meth A sarcoma. *Cancer Res* 1997;57:4557-63.
29. Kohno K, Kataoka J, Ohtsuki T, Suemoto Y, Okamoto I, Usui M, et al. IFN γ -inducing factor (IGIF) is a co-stimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J Immunol* 1997;158:1541-50.
30. Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, et al. IL-12 upregulates IL-18 receptor expression on T cells, Th1 cells and B cells: synergism with IL-18 for IFN γ production. *J Immunol* 1998;161:3400-7.
31. Jacobson NG, Szabo SJ, Weber-Nordt RM, Zhong Z, Schreiber RD, Darnell JE Jr, et al. Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat4. *J Exp Med* 1995;181:1755-62.
32. Kaplan MH, Sun YL, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4- deficient mice. *Nature* 1996;382:174-7.
33. Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature* 1996;382:171-4.
34. Yoshimoto T, Okamura H, Tagawa Y-I, Iwakura Y, Nakanishi K. Interleukin-18 together with interleukin-12 inhibits IgE production by induction of interferon- γ production from activated B cells. *Proc Natl Acad Sci U S A* 1997;94:3948-53.
35. Lauwerys BR, Renaud JC, Houssiau FA. Inhibition of in vitro immunoglobulin production by IL-12 in murine chronic graft-vs.-host disease: synergism with IL-18. *Eur J Immunol* 1998;28:2017-24.
36. Torigoe K, Ushio S, Okura T, Kobayashi S, Taniai M, Kunikate T, et al. Purification and characterization of the human interleukin-18 receptor. *J Biol Chem* 1997;272:25737-42.
37. Boraschi D, Villa L, Volpini G, Boss'u P, Censini S, Ghlara P, et al. Differential activity of interleukin 1 α and interleukin 1 β in the stimulation of the immune response in vivo. *Eur J Immunol* 1990;20:317-21.
38. Born TL, Thomassen E, Bird TA, Sims JE. Cloning of novel receptor subunit, AcPL, required for interleukin-18 signaling. *J Biol Chem* 1998;273:29445-50.
39. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G. Molecular cloning and characterization of a second subunit of the interleukin-1 receptor complex. *J Biol Chem* 1995;270:13757-65.
40. Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, et al. Interleukin-1 signaling occurs exclusively via the type I receptor. *Proc Natl Acad Sci U S A* 1993;90:6155-9.
41. Kim S-H, Novick D, Fantuzzi G, Reznikov L, Dinareello CA, Rubinstein M. The interleukin-18 binding protein is a decoy receptor lacking a transmembrane domain. *Eur Cytokine Netw* 1998. In press.
42. Rubinstein M, Novick D, Kim S-H, Fantuzzi G, Reznikov L, Dinareello CA. A family of poxvirus-encoded homologues of the interleukin-18 binding protein. *Eur Cytokine Netw* 1998. In press.
43. Novick D, Engelmann H, Wallach D, Rubinstein M. Soluble cytokine receptors are present in normal human urine. *J Exp Med* 1989;170:1409-14.
44. Engelmann H, Novick D, Wallach D. Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. *J Biol Chem* 1990;265:1531-6.
45. Novick D, Cohen B, Rubinstein M. The human interferon α/β receptor: characterization and molecular cloning. *Cell* 1994;77:391-400.
46. Novick D, Engelmann H, Wallach D, Leitner O, Revel M, Rubinstein M. Purification of soluble cytokine receptors from normal human urine by ligand-affinity and immunoaffinity chromatography. *J Chromatogr* 1990;510:331-7.
47. Hunter CA, Timans J, Pisacane P, Menon S, Cai G, Walker W, et al. Comparison of the effects of interleukin-1 α , interleukin-1 β and interferon- γ inducing factor on the production of interferon- γ by natural killer. *Eur J Immunol* 1997;27:2787-92.
48. Croston GE, Cao Z, Goeddel DV. NF κ B activation by interleukin-1 requires an IL-1 receptor-associated protein kinase activity. *J Biol Chem* 1995;270:16514-7.
49. Martin MU, Falk W. The interleukin-1 receptor complex and interleukin-1 signal transduction. *Eur Cytokine Netw* 1997;8:5-17.
50. O'Neill LAJ, Greene C. Sigan transduction pathways activated by the IL-1 receptor family: ancient signaling machinery in mammals, insects, and plants. *J Leukoc Biol* 1998;63:650-7.
51. Wesche H, Korherr C, Kracht M, Falk W, Resch K, Martin MU. The interleukin-1 receptor accessory protein is essential for IL-1-induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases). *J Biol Chem* 1997;272:7727-31.
52. Huang J, Gao X, Li S, Cao Z. Recruitment of IRAK to the interleukin 1 receptor complex requires interleukin 1 receptor accessory protein. *Proc Natl Acad Sci U S A* 1997;94:12829-32.
53. Cao Z. Signal transduction of interleukin-1. *Cytokine* 1998. In press.
54. Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV. IRAK TRAF-6. *Nature* 1996;383:443-6.
55. Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF- κ B induction by TNF, CD95 and IL-1. *Nature* 1997;385:540-4.
56. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M. A cytokine-responsive I κ B kinase that activates the transcription factor NF- κ B. *Nature* 1997;388:548-54.
57. Kojima H, Takeuchi M, Ohta T, Nishida Y, Arai N, Ikeda M, et al. Interleukin-18 activates the IRAK-TRAF6 pathway in mouse EL-4 cells. *Biochem Biophys Res Commun* 1998;244:183-6.
58. Adachi O, Kawai T, Takeda K, Matsumoto M, Tsutsui H, Sakagami M, et al. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 1998;9:143-50.
59. Tsuji-Takayama K, Matsumoto S, Koide K, Takeuchi M, Ikeda M, Ohta T, et al. Interleukin-18 induces activation and association of p56lck and MAPK in a murine TH1 clone. *Biochem Biophys Res Commun* 1997;237:126-30.
60. Luster AD. Chemokines—chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436-45.
61. Matsumoto S, Tsuji-Takayama K, Aizawa Y, Koide K, Takeuchi M, Ohta T, et al. Interleukin-18 activates NF κ B in murine T helper type I cells. *Biochem Biophys Res Commun* 1997;234:454-7.
62. Muhl H, Shapiro L, Dinareello CA. The nitric oxide synthase inhibitor L-NMMA is a potent inhibitor of IL-18 induced expression of HIV-1 in the chronically infected macrophage U1 cell [abstract]. *Cytokine* 1997;9:947.
63. Kohka H, Yoshino T, Iwagaki H, Sakuma I, Tanimoto T, Matsuo Y, et al. Interleukin-18/interferon- γ -inducing factor, a novel cytokine, up-regulates ICAM-1 (CD54) expression in KG-1 cells. *J Leukoc Biol* 1998;64:519-27.
64. Dao T, Ohashi K, Kayano T, Kurimoto M, Okamura H. Interferon- γ -inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of murine T helper cells. *Cell Immunol* 1997;173:230-5.
65. Dao T, Mehal WZ, Crispe IN. IL-18 augments perforin-dependent cytotoxicity of liver NK-T cells. *J Immunol* 1998;161:2217-22.
66. Muhl H, Shapiro L, Dinareello CA. The nitric oxide synthase inhibitor L-NMMA is potent inhibitor of IL-18-induced expression of HIV-1 in the chronically infected macrophage U1 cell [abstract]. *Cytokine* 1997;9:947.
67. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 1994;372:739-47.
68. Shapiro L, Heidenreich KA, Meintzer MK, Dinareello CA. Role of p38 mitogen-activated protein kinase in HIV type 1 production in vitro. *Proc Natl Acad Sci U S A* 1998;95:7422-6.
69. Kalina U, Klebba C, Kauschat D, Pape M, Hoelzer D, Ottmann OG, et al. HIV-1 replication is enhanced by pro- and eukaryotically expressed recombinant interleukin-18. *Eur Cytokine Netw* 1998. In press.
70. Mandrup-Poulsen T, Bendtzen K, Dinareello CA, Nerup J. Potentiation of IL-1 mediated B-cell killing by TNF. Human tumor necrosis factor potentiates human interleukin 1-mediated rat pancreatic β -cell cytotoxicity. *J Immunol* 1987;139:4077-82.
71. Punkel C, Baquerizo H, Rabinovitch A. Destruction of rat islet cell monolayers by cytokines. Synergistic interactions of IFN γ , TNF, LT and interleukin-1. *Diabetes* 1988;37:133-6.
72. Rothe H, Jenkins NA, Copeland NG, Kolb H. Active stage of autoimmune diabetes is associated with the expression of a novel cytokine, IGIF, which is located near *Idd2*. *J Clin Invest* 1997;99:469-74.
73. Tsutsui H, Nakanishi K, Matsui K, Higashino K, Okamura H, Miyazawa Y, et al. IFN γ -inducing factor up-regulates Fas ligand-mediated

- cytotoxic activity of murine natural killer cell clones. *J Immunol* 1996;157:3967-73.
74. Sarvetnick N. IFN γ , IGIF and IDDM. *J Clin Invest* 1997;99:371-2.
 75. Nolan KF, Greaves DR, Waldmann H. The human interleukin 18 gene IL18 maps to 11q22.2-q22.3, closely linked to the DRD2 gene locus and distinct from mapped IDDM loci. *Genomics* 1998;51:161-3.
 76. Rothe H, Hibiono T, Itoh Y, Kolb H, Martin S. Systemic production of interferon- γ inducing factor (IGIF) versus local IFN- γ expression involved in the development of Th1 insulinitis in NOD mice. *Autoimmunity* 1997;10:251-6.
 77. Froyen G, Billiau F. Potential therapeutic use of antibodies directed towards HuIFN γ . *Biotherapy* 1997;10:49-57.
 78. Ferber IA, Brocke S, Carrel T-E, Ridgway W, Dinisco C, Steinman L, et al. Mice with a disrupted IFN- γ gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J Immunol* 1996;156:5-7.
 79. Horwitz MS, Evans CF, McGavern DB, Rodriguez M, Oldstone MB. Primary demyelination in transgenic mice expressing interferon- γ . *Nat Med* 1997;3:1037-41.
 80. Badovinac V, Mostarica-Stojkovic M, Dinarello CA, Stosic-Grujicic S. Interleukin-1 receptor antagonist suppresses experimental autoimmune encephalomyelitis (EAE) in rats by influencing the activation and proliferation of encephalitogenic cells. *J Neuroimmunol* 1998;85:87-95.
 81. Jacobs CA, Baker PE, Roux ER, Picha KS, Toivola B, Waugh S, et al. Experimental autoimmune encephalomyelitis is exacerbated by IL-1 α and suppressed by soluble IL-1 receptor. *J Immunol* 1991;146:2983-9.
 82. Netea MG, Kulberg BJ, Amiot F, van der Meer JWM. Preferential production of interleukin-6 and IL-1 β by murine peritoneal macrophages exposed to IL-18: no role for TNF and lymphotoxin- α . *Eur Cytokine Netw* 1998. In press.
 83. Udagawa N, Horwood NJ, Elliott J, Mackay A, Owens J, Okamura H, et al. Interleukin-18 is produced by osteoblasts and acts via granulocyte macrophage colony-stimulating factor and not via interferon- γ to inhibit osteoclast formation. *J Exp Med* 1997;185:1005-12.
 84. Horwood NJ, Udagawa N, Elliott J, Grail D, Okamura H, Kurimoto M, et al. Interleukin 18 inhibits osteoclast formation via T cell production of granulocyte macrophage colony-stimulating factor. *J Clin Invest* 1998;101:595-603.
 85. Kobayashi Y, Appella E, Yamada M, Copeland TD, Oppenheim JJ, Matsushima K. Phosphorylation of intracellular precursors of human IL-1. *J Immunol* 1988;140:2279-87.
 86. Kobayashi K, Kai M, Gidoh M, Nakata N, Endoh M, Singh RP, et al. The possible role of interleukin (IL)-12 and interferon- γ —inducing factor/IL-18 in protection against experimental *Mycobacterium leprae* infection in mice. *Clin Immunol Immunopathol* 1998;88:226-31.
 87. Kawakami K, Qureshi MH, Zhang T, Okamura H, Kurimoto M, Saito A. IL-18 protects mice against pulmonary and disseminated infection with *Cryptococcal neoformans* by inducing IFN- γ production. *J Immunol* 1997;159:5528-34.
 88. Van't Wout JW, van der Meer JWM, Barza M, Dinarello CA. Protection of neutropenic mice from lethal *Candida albicans* infection by recombinant interleukin 1. *Eur J Immunol* 1988;18:1143-6.
 89. Bohn E, Sing A, Zumbihl R, Bielfeldt C, Okamura H, Kurimoto M, et al. IL-18 (IFN γ —inducing factor) regulates early cytokine production in, and promotes resolution of, bacterial infection in mice. *J Immunol* 1998;160:299-307.
 90. Mastroeni P, Clare S, Kurimoto M, Okamura H, Harrison J, Kahn S, et al. Role of IL-18 in Salmonella resistance. *Infect Immun* 1999. In press.
 91. Mastroeni P, Harrison JA, Robinson JH, Clare S, Khan S, Maskell DJ, et al. Interleukin-12 is required for control of the growth of attenuated aromatic-compound-dependent salmonellae in BALB/c mice: role of γ interferon and macrophage activation. *Infect Immun* 1998;66:4767-76.
 92. Osaki T, Peron JM, Cai Q, Okamura H, Robbins PD, Kurimoto M, et al. IFN γ —inducing factor/IL-18 administration mediates IFN γ —and IL-12-independent antitumor effects. *J Immunol* 1998;160:1742-9.
 93. Coughlin CM, Salhany KE, Wysocka M, Aruga E, Kurzawa H, Chang AE, et al. Interleukin-12 and interleukin-18 synergistically induce murine tumor regression which involves inhibition of angiogenesis. *J Clin Invest* 1998;101:1441-52.
 94. Yoshida Y, Tasaki K, Kimurai M, Takenaga K, Yamamoto H, Yamaguchi T, et al. Antitumor effect of human pancreatic cancer cells transduced with cytokine genes which activate Th1 helper T cells. *Anticancer Res* 1998;18:333-5.
 95. Taniguchi M, Nagaoka K, Kunikata T, Kayano T, Tamauchi H, Nakamura S, et al. Characterization of anti-human interleukin-18 (IL-18)/interferon inducing factor (IGIF) monoclonal antibodies and their application in the measurement of human IL-18 ELISA. *J Immunol Method* 1997;206:107-13.
 96. Dinarello CA. Biological basis for interleukin-1 in disease. *Blood* 1996;87:2095-147.
 97. Cookson WOCM, Moffatt MF. Asthma: An epidemic in the absence of infection? *Science* 1997;275:41-2.
 98. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1996;275:77.
 99. Puren AJ, Fantuzzi G, Netea M, Dinarello CA. Interleukin-18 and endotoxin synergize for interferon- γ production in whole blood cultures. *J Infect Dis* 1998. In press.
 100. Bresnahan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheum* 1998. In press.
 101. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *Lancet* 1994;344:1105-10.
 102. Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med* 1997;337:141-7.
 103. Antel JP, Becher B, Owens T. Immunotherapy for multiple sclerosis: from theory to practice. *Nat Med* 1996;2:1074-5.
 104. Reznikov L, Fantuzzi G, Tsang M, Novick D, Kim S-H, Rubinstein M, et al. Soluble IL-1R α enhances but IL-18 binding protein reduces the activity of IL-18. *J Interferon Cytokine Res* 1999. In press.
 105. Vanham G, Toossi Z, Hirsch CS, Wallis RS, Schwander SK, Rich EA, et al. Examining a paradox in the pathogenesis of human pulmonary tuberculosis: immune activation and suppression/anergy. *Tuber Lung Dis* 1997;78:145-58.