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Anti-Interleukin-18 Therapy in Murine Models of Inflammatory Bowel Disease

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Abstract
Interleukin (IL)-18 is a cytokine with a broad array of effector functions, the most prominent of which is to act synergistically with IL-12 in interferon-γ production and the induction of a strong T-helper-1-mediated immune response. In addition, IL-18 also upregulates the production of proinflammatory cytokines such as IL-1 and tumor necrosis factor-α. Analysis of IL-18-deficient mice revealed an important role of IL-18 in the activation of macrophages and natural killer cells in the context of infection with intracellular bacteria or parasites. In humans, it was reported that IL-18 is elevated at sites of inflammation in inflammatory bowel disease (IBD), particularly in Crohn's disease, suggesting a possible role for IL-18 in the development and persistence of IBD. In this review we summarize recent findings on the functional role of IL-18 in the pathogenesis of colitis with a special focus on murine models of IBD. The neutralizing mouse anti-mouse IL-18 antibodies generated in our group should facilitate the evaluation of the efficiency of therapeutic blockade of endogenous IL-18 in chronic mouse models of colitis besides the use of recombinant forms of the inhibitory IL-18-binding protein.

Introduction

The cytokine interleukin (IL)-18 was initially identified as a potent interferon-γ (IFN-γ)-inducing factor [1]. It was purified from the livers of mice treated with *Propionibacterium acnes* and subsequently challenged with lipopolysaccharide (LPS) to induce a toxic shock syndrome [2]. IL-18 is synthesized as a bioinactive 24-kD precursor protein that is cleaved by IL-1β-converting enzyme (ICE, caspase-1), leading to the release of the biologically active 18-kD protein. However, alternative caspase-1 independent processing by caspase-3 or -4, or proteinase-3 has also been postulated [3]. Production of IL-18 mRNA and the bioactive protein has been demonstrated for a wide range of cells including Kupffer cells, macrophages, T cells, B cells, osteoblasts, keratinocytes, dendritic cells, astrocytes and microglia [4, 5]. Besides this production by immune cells, expression of bioactive IL-18 has also been detected in nonimmune cells such as intestinal and airway epithelial cells [6, 7].

The most prominent function of IL-18 is to induce IFN-γ production from T, B and natural killer (NK) cells, in particular in the presence of IL-12 [2, 8]. In vitro, treatment of T cells with both IL-12 and IL-18 leads to strong IFN-γ production, but these T cells do not develop into T-helper-1 (Th1) cells without concomitant T-cell receptor engagement [8]. Stimulation of T cells with anti-CD3 and IL-18 results in IFN-γ production which can be abro-

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gated by the addition of anti-IL-12 or by using T cells from IL-12$^{-/-}$ mice [9]. Thus, IL-18 itself has no Th1-driving potential on its own. Besides T cells, also murine bone marrow-derived macrophages and dendritic cells produce high amounts of IFN-γ when stimulated with IL-12 and IL-18 and are thus not only a source of IL-12 and IL-18, but are also able to respond to their own secreted cytokines in an autocrine manner [10–12]. In addition to its IFN-γ-inducing activity, IL-18 has direct proinflammatory effects, being able to induce the synthesis of tumor necrosis factor-α (TNF-α), IL-1α, IL-1β and IL-6 by mouse peritoneal macrophages [13]. Recombinant human IL-18 stimulates production of TNF-α, IL-1β, IL-8, IL-6 or granulocyte-macrophage colony-stimulating factor (GM-CSF) by human T cells, monocytes, NK cells and chondrocytes [14–16]. Although these findings together led to the classification of IL-18 as a proinflammatory Th1-driving cytokine, recent data demonstrate that following T-cell receptor activation in the absence of IL-12, IL-18 can drive naïve CD4+ T-cells into Th0 and Th2 cells and stimulate the production of IL-4, IL-5 and IL-13 from these cells [17, 18].

IL-18 exerts its functions by binding to the IL-18 receptor (IL-18R) complex which shows a remarkable similarity to the IL-1 receptor (IL-1R). In fact, the IL-18-binding subunit (IL-18Rα) was originally described as IL-1R-related protein (IL-1Rrp) [19]. IL-18Rα binds IL-18 with relatively low affinity, but recruitment of the second chain (IL-18Rβ), originally referred to as IL-1R accessory protein (IL-1RacP), leads to the formation of the high-affinity IL-18 receptor complex. As is the case for the IL-1R complex, both chains are required for IL-18 signalling [20, 21]. A small IL-18-binding protein (IL-18BP) has recently been described [22] and was shown to regulate IL-18 activity by preventing its interaction with the IL-18R. IL-18BP exists in several naturally occurring isoforms, four in humans and two in mice [23]. In addition, many members of the poxvirus family possess a homologue of the IL-18BP, which has been shown to inhibit the host response to viral infection [24].

While freshly isolated T and B cells do not express the IL-18R, culture of these cells in the presence of IL-12 upregulates the production of IL-18R mRNA and the expression of the high-affinity IL-18R [8]. As a consequence, only Th1 cells express high levels of IL-18R, whereas Th2 cells do not express IL-18R or IL-18R mRNA [8]. Since IL-18 has been shown to upregulate the IL-12Rβ2 subunit on T cells [9], the synergism of IL-12 and IL-18 in IFN-γ production lies at least in part in their reciprocal upregulation of each other's receptors. Since IL-18 alone shows no Th1-driving potential, the major effect of IL-18 on the differentiation of Th1 cells is mediated by its capacity to augment IL-12Rβ2 expression and thereby enhance IL-12-driven Th1 differentiation [9].

Functional Importance of IL-18 in vivo

Due to impaired IFN-γ production, IL-18 knockout mice display defective Th1-mediated immune responses and mount drastically altered immune responses to microbial infection [18, 25–27]. In particular, IL-18 plays a crucial role in the defense against intracellular bacteria and parasites by stimulation of TNF-α and nitric oxide production by macrophages besides induction of increased IFN-γ levels [18, 28]. IL-12 and IL-18 are both required for optimal cytolytic activity of NK cells and mice deficient for both cytokines possess barely detectable NK activity [25]. In contrast, elevated NK cell activity following injection of IL-18 improved resistance of mice to *Toxoplasma gondii* infection [29]. With regard to the pathogenesis of autoimmune and inflammatory diseases, increased production of IL-18 has been associated with the development of endotoxin-induced liver injury [30], endotoxin-mediated shock syndrome [27], allergic asthma [31], experimental allergic encephalomyelitis [32] and also rheumatoid arthritis, a common tissue-specific autoimmune disease. Elevated levels of IL-18 were observed in synovial fluid from patients with rheumatoid arthritis, leading to induction of TNF-α, GM-CSF, IFN-γ and nitric oxide production by synovial cells isolated from these patients [33]. Neutralization of endogenous IL-18 in a murine streptococcal cell wall arthritis model with polyclonal anti-IL-18 antibodies or recombinant human IL-18BP (rhIL-18BP) led to a significant reduction in disease symptoms and local proinflammatory cytokine production (TNF-α, IL-1β) [34, 35]. A recent study demonstrated expression of IL-18 and IL-18R in human atheroma, implying a role for IL-18 in atherogenesis [36].

Participation of IL-18 in the Pathogenesis of Inflammatory Bowel Disease

There is increasing evidence that an imbalance of the Th1/Th2 polarization in favor of a Th1 directed cytokine pattern is involved in the pathology of idiopathic inflammatory bowel diseases (IBD), particularly Crohn's disease (CD) [37–39]. The first evidence for a role of IL-18 in IBD came from two independent studies exploring the expres-

sion levels of IL-18 in the course of immunoinflammatory response in CD [40, 41]. Reverse-transcriptase polymerase chain reaction analysis demonstrated an increase in IL-18 mRNA transcripts in mucosal tissue and lamina propria mononuclear cells (LPMCs) of infiltrated mucosal areas from CD patients as compared to tissues from ulcerative colitis (UC) patients or from non-IBD controls [40]. Whereas the non-biologically active 24-kD IL-18 precursor could be detected by Western blot in mucosal tissue samples from CD and UC patients as well as in noninflamed controls, the mature, biologically active 18-kD form of IL-18 was abundantly detected in CD, weaker in UC but absent in non-IBD controls [40, 41]. Immunohistochemical examination of colonic mucosa obtained from CD patients revealed strong staining of the colonic epithelium, as well as scattered inflammatory cells in the lamina propria and submucosal lymphoid aggregates. IL-18-positive cells were morphologically identified as intestinal epithelial cells, tissue macrophages and dendritic cells [41].

Kanai et al. [42] reported a significant increase in serum IL-18 concentrations in CD patients which correlated with disease activity. IL-18+ cells could hardly be detected in normal and inflamed UC colonic mucosa whereas in inflamed colonic mucosa of CD patients many IL-18-producing mononuclear cells, identified mainly as myeloid cells by CD68 staining, had infiltrated the lamina propria. Proliferative responses of freshly isolated lamina propria lymphocytes from CD patients to recombinant human IL-18 were significantly increased compared to lamina propria lymphocytes from UC patients or normal control mucosa [42].

Recently, there were several reports indicating a pathogenic role for IL-18 in murine models of IBD. Administration of IL-12 and IL-18 to BALB/c mice induced weight loss, diarrhea and inflammation of the small and large intestine in an IFN-γ-dependent manner [43]. However, administration of high doses of IFN-γ failed to replace the action of IL-12 and IL-18, suggesting the participation of additional factors induced by IL-12 and IL-18 in the described pathology. Surprisingly, no pathogenic role for TNF-α could be detected in this model.

Therapeutic Effect of IL-18 Blockade in Mouse Models of IBD

At present, the efficiency of IL-18 blockade in the prevention or therapy of IBD has been tested by pharmacological application of IL-18BP [44], xenogenic neutralizing IL-18-specific antibodies [45, 46], and IL-18 antisense mRNA [47] using chemically induced mouse models of colitis or a transfer colitis model. In addition, the susceptibility of IL-18$^{-/-}$ and Caspase-1$^{-/-}$ mice to colitis development has been examined by some groups. The outcome of these studies is summarized below.

Siegmund et al. [46] analyzed the role of IL-18 in the dextran sulfate sodium (DSS)-induced colitis model. Administration of DSS induced colitis in BALB/c and C57BL/6 mice within 4–10 days and increased IL-18 concentrations in colon cultures. Confocal laser microscopy of colon sections revealed colonic epithelial cells as the main source of IL-18. Neutralization of IL-18 with an anti-IL-18 rabbit antiserum led to a dose-dependent reduction in the clinical score (comprising stool consistency and rectal bleeding) but not to significant changes in weight loss. The latter is in agreement with our own observations that weight loss induced by feeding with 3.5% DSS was similar in IL-18$^{-/-}$ mice and C57BL/6 wild-type controls (unpublished data). Regarding the influence of anti-IL-18 treatment on cytokine production, Siegmund et al. [46] observed a reduced amount of IFN-γ and TNF-α in the colon of anti-IL-18-treated mice. In addition, spontaneous ex vivo synthesis of TNF-α, IFN-γ and IL-18 in colon organ cultures was reduced after anti-IL-18 treatment in BALB/c and C57BL/6 mice [46]. Using the same model, this group also examined ICE (caspase-1)-deficient mice for susceptibility to intestinal inflammation [48]. Remarkably, ICE-KO mice showed a stronger protection from acute DSS colitis than could be achieved by either anti-IL-18 treatment or IL-1 blockade with IL-1Ra. Even in chronic DSS colitis (30 days), DSS-fed ICE-KO mice were mostly protected from disease development. These findings indicate that ICE deficiency results in a more profound resistance to colitis development than blockade of either IL-1 or IL-18. However, inhibition of these cytokines alone also resulted in a partial protection.

The effect of blockade of endogenous IL-18 in the trinitrobenzene sulfonic acid (TNBS) colitis model was assessed in two independent studies by Ten Hove et al. [44] and Kanai et al. [45]. Ten Hove et al. [44] treated BALB/c mice with TNBS in the presence or absence of the rhIL-18BP isoform a (rhIL-18BPa). Only the highest dose of rhIL-18BPa with daily injections of 200 μg resulted in partial protection from colitis development with some decrease in colon weight, caudal lymph node cellularity and the percentage of splenic CD4+CD69+ cells. Histological analyses of colons revealed a reduction in the number of cells infiltrating the mucosa and the absence of ulcerations in mice treated with rhIL-18BPa, although

inflammation was still present. While a reduction in TNF-α, IL-1β and IL-6 production in colonic homogenate cultures of rhIL-18BPa-treated mice was observed, no differences in IFN-γ levels were detected. In line with the finding that IFN-$\gamma^{-/-}$ or IFN-$\gamma R^{-/-}$ mice were not protected from development of TNBS colitis [49], these authors propose that the protective effect of IL-18-blockade in colitis development is not mediated through inhibition of IFN-γ production but rather through the reduction of other proinflammatory cytokines such as TNF-α.

In an independent study Kanai et al. [45] determined the role of mucosal macrophages and IL-18 in TNBS colitis in C57BL/6 wt and IL-$18^{-/-}$ mice. Colitis was induced after priming the mice with TNBS-bovine serum albumin (TNBS-BSA) in the presence of complete Freund's adjuvant and subsequent induction of acute colitis by rectal administration of 10 mg TNBS in 40% ethanol. Following this treatment, increased IL-18 levels were detected in splenocytes, LPMCs and in colonic tissue homogenates. Upon administration of saporin-conjugated anti-Mac-1 antibodies and depletion of macrophages, intestinal inflammation and weight loss were attenuated. In addition, mice treated with rabbit anti-IL-18 antibodies (1 mg/mouse on days 0, 1, 3 and 5) were protected from weight loss, had reduced colon thickening, and a decreased histological scoring. In this case, anti-IL-18 treatment resulted in a reduction in IFN-γ production from phorbol myristate acetate/ionomycin-stimulated LPMCs to levels seen in non-TNBS-treated mice. The same result was also obtained when IL-$18^{-/-}$ mice were used for TNBS treatment and compared to wild-type mice.

Finally, Wirtz et al. [47] employed a different strategy of local IL-18 blockade by rectal administration of an adenovirus containing antisense IL-18 RNA (Ad-asIL-18). Colitis was induced by transfer of CD62L+CD4+ cells from normal BALB/c mice into BALB/cSCID mice. Administration of Ad-asIL-18 was performed in reconstituted SCID mice after the onset of disease symptoms (10% weight loss) over a period of 6 days and resulted in an immediate reduction in mucosal IL-18 contents and improvement of colitis scores as assessed by coloscopy and histology.

Taken together, the above reports indicate that neutralization of IL-18 in acute colitis models or in short-term treatment regimens (IL-18 anti-sense mRNA) causes an improvement in colitis pathology. However, complete protection could in most cases not be obtained and it remains to be shown whether IL-18 blockade is also beneficial in long-term treatment of chronic colitis.

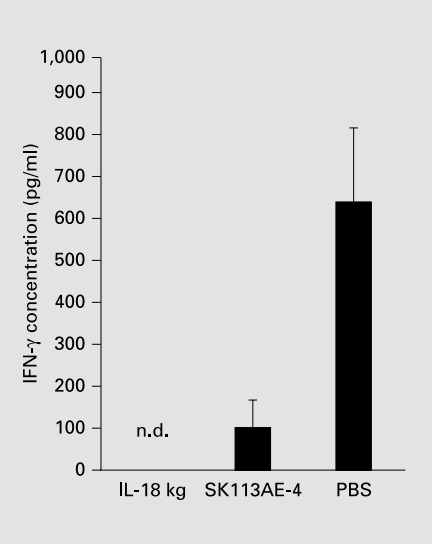

Fig. 1. In vivo neutralization capability of anti-IL-18 antibody SK113AE-4. C57BL/6 mice were injected intraperitoneally with 500 μg of SK113AE-4 monoclonal antibody or phosphate-buffered saline (PBS). Twelve hours later, the same mice and, in addition, IL-$18^{-/-}$ mice were injected intraperitoneally with 500 μg LPS. Six hours after LPS treatment, mice were bled and serum IFN-γ levels were determined by ELISA.

Generation of Isogeneic Anti-Murine IL-18 Antibodies for Chronic Therapy

All of the available IL-18 or IL-18 receptor-neutralizing antibodies have been generated by immunization across species barriers, mostly in rabbits or rats. However, the feasibility of long-term therapeutic studies depends on the availability of isogeneic reagents to avoid immunological rejection of the injected protein. For this reason, we have recently generated neutralizing monoclonal mouse anti-mouse IL-18 antibodies [50] using IL-18-deficient mice [27] for immunization. The use of this immunization protocol enabled us to circumvent the problem of immunological tolerance against endogenous proteins. In addition, we have made use of synthetic oligodeoxynucleotide-containing CpG motifs [51] as a potent adjuvant to stimulate the production of strong, high-affinity immune responses. With the combination of immunostimulatory DNA as an adjuvant and the use of IL-18-deficient animals for immunization, an unusually strong and highly specific humoral immune response to recombinant murine IL-18 could be obtained. This strategy enabled us to identify a panel of isogeneic IL-18-binding and neutralizing antibodies.

To assess the in vivo neutralizing capacity of the antibodies, we used a high-dose LPS model in mice and determined serum IFN-γ levels 5 h after treatment with a single dose of LPS (500 μg). Figure 1 shows the neutralizing capacity of anti-IL-18 mAb SK113AE-4 injected 12 h before LPS treatment. This treatment resulted in a reduction in serum IFN-γ levels by 85% compared to phosphate-buffered saline-treated wild-type mice, while no IFN-γ was detectable in IL-18$^{-/-}$ mice, in agreement with the data of Hochholzer et al. [27].

With the availability of this reagent it is now possible to test the efficiency of IL-18 blockade in chronic mouse models of IBD. We haven chosen to use the transfer colitis model originally established by Powrie et al. [52] for this purpose because it represents a Th1-dominated chronic model of IBD with some similarities to CD. Preliminary data from these studies indicate that a reduction in the severity of colitis symptoms can be achieved through application of the antibody over a period of 3–6 weeks, similar to attenuated disease development in IL-18$^{-/-}$ recipients. However, these data need to be confirmed with larger numbers of animals and in additional mouse models of colitis.

Conclusion

The present data on the therapeutic efficiency of IL-18 blockade in murine colitis models suggest that IL-18-neutralizing reagents are promising candidates for the development of alternative treatment regimens of IBD and potentially other inflammatory diseases. Although anti-TNF treatment is presently used successfully in clinics, a substantial proportion of IBD patients do not respond to this treatment. Thus, it will be important to also assess the efficiency of combination therapies using anti-TNF reagents together with anti-IL-18 and/or anti-IL-12. In light of the increased susceptibility of patients treated with TNF-neutralizing agents to bacterial infection, e.g. recurrence of latent tuberculosis [53], it is, however, necessary to balance the efficiency of newly developed anti-inflammatory reagents with residual protection to microbial infections. Therefore, novel alternative treatment strategies in IBD should also be evaluated with regard to their effect on the functionality of the innate immune system.

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References

1 Nakamura K, Okamura H, Wada M, Nagata K, Tamura T: Endotoxin-induced serum factor that stimulates gamma interferon production. Infect Immun 1989;57:590–595.
2 Okamura H, Tsutsi H, Komatsu T, et al: Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 1995;378:88–91.
3 Fantuzzi G, Dinarello CA: Interleukin-18 and interleukin-1 beta: Two cytokine substrates for ICE (caspase-1). J Clin Immunol 1999;19:1–11.
4 Stoll S, Jonuleit H, Schmitt E, et al: Production of functional IL-18 by different subtypes of murine and human dendritic cells (DC): DC-derived IL-18 enhances IL-12-dependent Th1 development. Eur J Immunol 1998;28:3231–3239.
5 Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H: Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev 2001;12:53–72.
6 Takeuchi M, Nishizaki Y, Sano O, Ohta T, Ikeda M, Kurimoto M: Immunohistochemical and immuno-electron-microscopic detection of interferon-gamma-inducing factor ('interleukin-18') in mouse intestinal epithelial cells. Cell Tissue Res 1997;289:499–503.
7 Cameron LA, Taha RA, Tsicopoulos A, et al: Airway epithelium expresses interleukin-18. Eur Respir J 1999;14:553–559.
8 Yoshimoto T, Takeda K, Tanaka T, et al: IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. J Immunol 1998;161:3400–3407.
9 Chang JT, Segal BM, Nakanishi K, Okamura H, Shevach E: The costimulatory effect of IL-18 on the induction of antigen-specific IFN-gamma production by resting T cells is IL-12 dependent and is mediated by up-regulation of the IL-12 receptor beta2 subunit. Eur J Immunol 2000;4:1113–1119.
10 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;187:2103–2108.
11 Fukao T, Matsuda S, Koyasu S: Synergistic effects of IL-4 and IL-18 on IL-12-dependent IFN-gamma production by dendritic cells. J Immunol 2000;164:64–71.
12 Munder M, Mallo M, Eichmann K, Modolell M: Direct stimulation of macrophages by IL-12 and IL-18 – A bridge built on solid ground. Immunol Lett 2001;75:159–160.
13 Netea MG, Kullberg BJ, Verschueren I, Van Der Meer JW: Interleukin-18 induces production of proinflammatory cytokines in mice: No intermediate role for the cytokines of the tumor necrosis factor family and interleukin-1beta. Eur J Immunol 2000;30:3057–3060.
14 Horwood NJ, Udagawa N, Elliott J, et al: Interleukin 18 inhibits osteoclast formation via T cell production of granulocyte macrophage colony-stimulating factor. J Clin Invest 1998;101:595–603.
15 Puren AJ, Fantuzzi G, Gu Y, Su MS, Dinarello CA: Interleukin-18 (IFNgamma-inducing factor) induces IL-8 and IL-1beta via TNFalpha production from non-CD14+ human blood mononuclear cells. J Clin Invest 1998;101:711–721.
16 Olee T, Hashimoto S, Quach J, Lotz M: IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses. J Immunol 1999;162:1096–1100.
17 Xu D, Trajkovic V, Hunter D, et al: IL-18 induces the differentiation of Th1 or Th2 cells depending upon cytokine milieu and genetic background. Eur J Immunol 2000;30:3147–3156.

18 Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H: Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 2001;19: 423–474.
19 Parnet P, Garka KE, Bonnert 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;271:3967–3970.
20 Born TL, Thomassen E, Bird TA, Sims JE: Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. J Biol Chem 1998;273:29445–29450.
21 Debets R, Timans JC, Churakowa T, et al: IL-18 receptors, their role in ligand binding and function: Anti-IL-1RAcPL antibody, a potent antagonist of IL-18. J Immunol 2000;165: 4950–4956.
22 Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M: Interleukin-18 binding protein: A novel modulator of the Th1 cytokine response. Immunity 1999;10:127–136.
23 Kim SH, Eisenstein M, Reznikov L, et al: Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci USA 2000; 97:1190–1195.
24 Xiang Y, Moss B: IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. Proc Natl Acad Sci USA 1999;96:11537–11542.
25 Takeda K, Tsutsui H, Yoshimoto T, et al: Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity 1998;8:383–390.
26 Wei XQ, Leung BP, Niedbala W, et al: Altered immune responses and susceptibility to *Leishmania major* and *Staphylococcus aureus* infection in IL-18-deficient mice. J Immunol 1999; 163:2821–2888.
27 Hochholzer P, Lipford GB, Wagner H, Pfeffer K, Heeg K: Role of interleukin-18 (IL-18) during lethal shock: Decreased lipopolysaccharide sensitivity but normal superantigen reaction in IL-18-deficient mice. Infect Immun 2000;68: 3502–3508.
28 Swain SL: Interleukin 18:tipping the balance towards a T helper cell 1 response. J Exp Med 2001;194:F11–F14.
29 Cai G, Kastelein R, Hunter CA: Interleukin-18 (IL-18) enhances innate IL-12-mediated resistance to *Toxoplasma gondii*. Infect Immun 2000;68:6932–6938.
30 Sakao Y, Takeda K, Tsutsui H, et al: IL-18-deficient mice are resistant to endotoxin-induced liver injury but highly susceptible to endotoxin shock. Int Immunol 1999;11:471–480.
31 Wild JS, Sigounas A, Sur N, et al: IFN-gamma-inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. J Immunol 2000;164:2701–2710.
32 Shi FD, Takeda K, Akira S, Sarvetnick N, Ljunggren HG: IL-18 directs autoreactive T cells and promotes autodestruction in the central nervous system via induction of IFN-gamma by NK cells. J Immunol 2000;165:3099–3104.
33 Gracie JA, Forsey RJ, Chan WL, et al: A proinflammatory role for IL-18 in rheumatoid arthritis. J Clin Invest 1999;104:1393–1401.
34 Joosten LA, van De Loo FA, Lubberts E, et al: An IFN-gamma-independent proinflammatory role of IL-18 in murine streptococcal cell wall arthritis. J Immunol Methods 2000;165:6553–6558.
35 Plater-Zyberk C, Joosten LA, Helsen MM, et al: Therapeutic effect of neutralizing endogenous IL-18 activity in the collagen-induced model of arthritis. J Clin Invest 2001;108: 1825–1832.
36 Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schonbeck U: Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: Implications for atherogenesis. J Exp Med 2002;195: 245–257.
37 Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL: Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity 1994;1:553–562.
38 Targan SR, Murphy LK: Clarifying the causes of Crohn's. Nat Med 1995;1:1241–1243.
39 Parronchi P, Romagnani P, Annunziato F, et al: Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. Am J Pathol 1997;150: 823–832.
40 Monteleone G, Trapasso F, Parrello T, et al: Bioactive IL-18 expression is up-regulated in Crohn's disease. J Immunol 1999;163:143–147.
41 Pizarro TT, Michie MH, Bentz M, et al: IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: Expression and localization in intestinal mucosal cells. J Immunol 1999;162:6829–6835.
42 Kanai T, Watanabe M, Okazawa A, et al: Interleukin 18 is a potent proliferative factor for intestinal mucosal lymphocytes in Crohn's disease. Gastroenterology 2000;119:1514–1553.
43 Chikano S, Sawada K, Shimoyama T, et al: IL-18 and IL-12 induce intestinal inflammation and fatty liver in mice in an IFN-gamma dependent manner. Gut 2000;47:779–786.
44 Ten Hove T, Corbaz A, Amitai H, et al: Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice. Gastroenterology 2001; 121:1372–1379.
45 Kanai T, Watanabe M, Okazawa A, et al: Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease. Gastroenterology 2001;121:875–888.
46 Siegmund B, Fantuzzi G, Rieder F, et al: Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. Am J Physiol Regul Integr Comp Physiol 2001;281:R1264–R1273.
47 Wirtz S, Becker C, Blumberg R, Galle PR, Neurath MF: Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA. J Immunol 2002;168:411–420.
48 Siegmund B, Lehr HA, Fantuzzi G, Dinarello CA: IL-1 beta-converting enzyme (caspase-1) in intestinal inflammation. Proc Natl Acad Sci USA 2001;98:13249–13254.
49 Camoglio L, te Velde AA, de Boer A, ten Kate FJ, Kopf M, van Deventer SJ: Hapten-induced colitis associated with maintained Th1 and inflammatory responses in IFN-gamma receptor-deficient mice. Eur J Immunol 2000;30:1486–1495.
50 Lochner M, Wagner H, Classen M, Forster I: Generation of neutralizing mouse anti-mouse IL-18 antibodies for inhibition of inflammatory responses in vivo. J Immunol Methods 2002;259:149–157.
51 Lipford GB, Heeg K, Wagner H: Bacterial DNA as immune cell activator. Trends Microbiol 1998;6:496–500.
52 Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL: Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int Immunol 1993;5:1461–1471.
53 Keane J, Gershon S, Wise RP, et al: Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001;345:1098–1104.