Interleukin-18

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Abstract: Interleukin-18 (IL-18), a recently described member of the IL-1 cytokine superfamily, is now recognized as an important regulator of innate and acquired immune responses. IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases, in a variety of cancers, and in the context of numerous infectious diseases. This short review will describe the basic biology of IL-18 and thereafter address its potential effector and regulatory role in several human disease states including autoimmunity and infection. IL-18, previously known as interferon-γ (IFN-γ)-inducing factor, was identified as an endotoxin-induced serum factor that stimulated IFN-γ production by murine splenocytes [1]. IL-18 was cloned from a murine liver cell cDNA library generated from animals primed with heat-killed *Propionibacterium acnes* and subsequently challenged with lipopolysaccharide [2]. Nucleotide sequencing of murine IL-18 predicted a precursor polypeptide of 192 amino acids lacking a conventional signal peptide and a mature protein of 157 amino acids. Subsequent cloning of human IL-18 cDNA revealed 65% homology with murine IL-18 [3] and showed that both contain an unusual leader sequence consisting of 35 amino acids at their N terminus. *J. Leukoc. Biol.* 73: 213–224; 2003.

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REGULATION OF INTERLEUKIN (IL)-18 GENE EXPRESSION

Little is yet understood of detailed regulation of IL-18 at the gene level. The murine IL-18 gene is composed of seven exons, of which one and two are noncoding. At least two distinct TATA-less IL-18 promoters have been identified. Promoter activity upstream of exon 2 acts constitutively, whereas an area upstream of exon 1 can be lipopolysaccharide (LPS)-activated [4]. Furthermore, as the 3′ untranslated region of human IL-18 mRNA lacks AUUUA destabilization sequences, these observations likely explain the constitutive expression of IL-18 mRNA in freshly isolated human peripheral blood mononuclear cells (PBMC), murine splenic macrophages, and nonimmune cells [5]. Additional studies have identified the transcription factors interferon (IFN) consensus sequence-binding protein (ICSBP) and PU.1 as being critical for the activation of the IL-18 promoter upstream of exons 1 and 2, respectively [6]. ICSBP and PU.1 are themselves up-regulated by IFN-γ [7–9]. IFN-γ stimulation of macrophages has also been shown to up-regulate IL-18 gene expression via ICSBP and activator protein-1 (AP-1) elements [10]. Nuclear factor (NF)-κB recognition sequences identified in the promoter region of IL-18 suggest the additional involvement of NF-κB in regulating IL-18 gene expression.

IL-18 EXPRESSION AND SYNTHESIS

Commensurate with a proposed role in a variety of early inflammatory responses, IL-18 has been identified in cells of haemopoietic and nonhaemopoietic lineages. Thus, IL-18 expression has been reported in macrophages, dendritic cells (DC), Kupffer cells, keratinocytes, osteoblasts, adrenal cortex cells, intestinal epithelial cells, microglial cells, and synovial fibroblasts [11–18]. However, without the enzymatic machinery necessary for IL-18 processing, expression of IL-18 mRNA or indeed pro-IL-18 protein should not necessarily infer the capacity to contribute biologic activity. The nature of native stimuli for IL-18 expression remains under investigation but includes at least LPS and FasL [19].

IL-18, like IL-1β, with which it shares structural homology, is produced as a 24-kD inactive precursor lacking a signal peptide (pro-IL-18). Pro-IL-18 is cleaved after Asp35 by the endoprotease IL-1β-converting enzyme (ICE; caspase-1) to generate a biologically active, mature 18-kD moiety [20, 21]. The importance of caspase-1 in IL-18 processing is highlighted by the lack of IFN-γ production by LPS-stimulated splenocytes from ICE-deficient mice [22] and protection of caspase-1-deficient mice from ischemic acute renal failure [23]. In humans, secretion of mature IL-18 by granulocyte macrophage-colony stimulating factor (GM-CSF)-treated macrophages infected with influenza or Sendai virus is abolished by caspase-1 inhibitors [24]. However, caspase-1 cleavage of pro-IL-18 is not exclusive, as recent reports indicate that proteinase 3 can also generate biological activity from pro-IL-18 [25]. In contrast, cleavage of pro-IL-18 or mature IL-18 at Asp71-Ser72 and Asp76-Asn77 by caspase-3 results in the generation of biologically inactive peptides [26]. We have recently observed that culture supernatants generated from human neutrophils cleave recombinant pro- and mature IL-18 into a number of

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distinct moieties (unpublished data). We identified that this activity resides in the serine proteases elastase and cathepsin G. The biological significance of the latter remains unclear but raises the fascinating possibility that neutrophil activation during early responses may critically regulate the capacity of IL-18 to contribute to the phenotype of subsequent adaptive immune responses.

IL-18 RECEPTOR (IL-18R) AND SIGNALING

Like that of IL-1, the IL-18R complex is a heterodimer containing an α (IL-1Rrp) chain responsible for extracellular binding of IL-18 and a nonbinding, signal-transducing β (AcPL) chain [27–29]. Both chains are required for functional IL-18 signaling [30]. IL-18R is expressed on a variety of cells including macrophages, neutrophils, natural killer (NK) cells, endothelial, and smooth muscle cells [31–34]. The IL-18R complex can be up-regulated on naïve T cells, T helper cell type 1 (Th1) cells, and B cells by IL-12 [28, 35]. IL-18Rα retention on the membrane of mature Th1 cells serves as a marker for the latter in humans and murine systems [36]. In contrast, T cell receptor (TCR) ligation in the presence of IL-4 results in down-regulation of the IL-18R [37]. Modulation of this complex is therefore likely to be functionally significant. Consistent with this, administration of anti-IL-18Rα antibody in vivo results in reduced LPS-induced mortality associated with a subsequent shift in balance from a Th1 to a Th2 immune response [36].

Upon binding of IL-18 to IL-18Rα, IL-18Rβ is recruited to form a high-affinity complex-inducing signaling pathways shared with other IL-1R family members. These involve recruitment of myeloid differentiation 88 and IL-1R-associated kinase (IRAK) to the receptor complex and their activation [38–40]. Thus, IL-18 shares downstream effector pathways with critical immune regulatory molecules such as Toll-like receptors, which are in turn implicated in regulating IL-18 expression [41, 42]. The signaling machinery therefore provides for numerous regulatory feedback loops, even at the single-cell level. Following activation, IRAK autophoshorylates, dissociates from the receptor complex, and interacts with the adaptor protein tumor necrosis factor receptor-associated factor 6 (TRAF6) [43, 44]. Phosphorylation of NF-κβ-inducing kinase and rapid induction of IκBα degradation allow NF-κB nuclear translocation [45, 46]. Recently, dominant-negative transfectants of IκBα have been shown to inhibit IL-18-dependent IκBα degradation, NF-κB activation, and IFN-γ expression by KG-1 cells [47]. In addition to IRAK/TRAF6 signaling, recent evidence suggests a role for mitogen-activated protein kinases (MAPK) in IL-18 signaling. Thus, activation of the MAPK p38, extracellular signal-regulated kinases (ERK) $p44^{erk\text{-}1}$ and $p42^{erk\text{-}2}$ by IL-18, was detected in a human NK cell line [48]. In Th1-type cells, IL-18 has also been shown to induce expression of GADD45β, which in turn binds MAPK-ERK kinase 4 (MEKK4). GADD45β also activated the MAPK p38. Moreover, GADD45β expression in naïve T cells results in selectively increasing cytokine but not TCR-induced IFN-γ production that could be abrogated by kinase-inactive MEKK4 or p38 inhibitors [49]. However, the ability of MEKK4 to activate p38 requires further clarification [50, 51]. In addition to IL-18-induced MAPK signaling, diminished NK cell activity and IFN-γ production by mice deficient in the transcription factor tyk-2 in response to IL-18 suggest that like IL-12, IL-18 may also signal via tyk-2 [52]. Cooperation between IL-12 and IL-18 signaling pathways extends further, as IL-12-induced signal transducer and activator of transcription-4 has been shown to enhance IL-18-induced AP-1 binding to and activation of the IFN-γ promoter [53, 54]. Recently, use of phosphatidylinositol-3 kinase and NF-κB inhibitors suggested that both were required for IL-18 induction of vascular cell adhesion molecule-1 (VCAM-1) on synovial fibroblasts [55]. Further studies in which the expression of IL-18R is systematically examined for correlation with IL-18-dependent signaling are ongoing in several laboratories.

FUNCTIONAL EFFECTS OF IL-18

Although originally identified as a factor capable of inducing IFN-γ production by murine splenocytes, the effector role of IL-18 is rapidly expanding. Consistent effects on lymphoid series, particularly Th1 lineage in combination with IL-12, have emerged [2]. Thus, IL-18 enhances T and NK cell maturation, cytokine production, and cytotoxicity [2, 35, 56, 57]. IL-18 also increases FasL on NK cells and consequent Fas-FasL-mediated cytotoxicity [58, 59]. IL-18-deficient mice have reduced NK cell cytolytic ability that can be restored by exogenous IL-18 [60]. However, together with IL-2, IL-18 coinduces IL-13 in murine T and NK cells and in the presence of TCR activation, induces T cell IL-4, IL-10, IL-13, and IFN-γ production [61, 62]. In isolation, IL-18 induces high immunoglobulin E expression by B cells and in combination with IL-2, anti-CD3, and anti-CD28, markedly enhances IL-4 production by $CD4^+$ T cells [63]. When cultured alone or in combination with IL-4, IL-18 is known to induce murine T cell Th2 differentiation. This however is dependent on genetic influences, as spleen cells from BALB/c and C56BL/6 strains of mice stimulated with anti-CD3 and IL-18 exhibit enhanced Th2 and Th1 responses, respectively [64]. Thus, IL-18 can promote Th1 or Th2 lineage maturation dependent on underlying genetic influences and the ambient cytokine milieu.

On non-T cell populations, IL-18, in conjunction with IL-3, induces IL-4 and IL-13 production by bone marrow-derived basophils [65]. Direct effects on macrophages and DC have also been observed. Stimulation of bone marrow-derived macrophages or splenic DC with IL-12 and IL-18 can induce IFN-γ production [66, 67]. Studies of knockout mice also reveal that IL-18 stimulation of peritoneal macrophages induces IL-6 production, independent of the intermediate induction of endogenous cytokines such as tumor necrosis factor α (TNF-α) or IL-1β [68]. Macrophages derived from rheumatoid arthritis (RA) synovial membrane but not peripheral blood monocytes respond directly to IL-18 with TNF-α production. Similarly, IL-18 promotes neutrophil activation, reactive oxygen intermediate synthesis, cytokine release, and degranulation [18, 31]. Recent studies suggest that IL-18 up-regulates intracellular adhesion molecule-1 (ICAM-1) and VCAM-1 expression on endothelial cells and synovial fibroblasts [55].

TABLE 1.

Knockout	Effect	Reference
IL-18	Increased susceptibility to:	
	Leishmania major infection	[85, 86]
	Cryptococcus neoforms infection	[87]
	Streptococcus pneumoniae infection	[88]
	Plasmodium berghei infection	[89]
	Mycobacterial infection	[90]
	Increased severity of septic arthritis	[85]
	Increased LPS-induced endotoxic shock in *P. acnes*-primed mice	[91]
	Reduced:	
	Septicaemia following *Staphylococcus aureus* infection	[85]
	Collagen-induced arthritis (CIA)	[92]
	2,4,6-T-nitrobenzene sulfonic acid (TNBS)-induced colitis	[93]
	IFN-γ in response to *L. monocytogenes* stimulation	[94]
	Severity of autoimmune encephalomyelitis	[95]
	LPS-induced shock	[96]
	Increased resistance to:	
	T Muris infection	[97]
	LPS-induced liver injury in *P. acnes* primed mice	[91]
	Impaired:	
	Microglial activation after influenza A infection	[98]
	Th1 response	[60]
	IFN-γ response to *Mycobacterium tuberculosis* infection	[99]
IL-18R	Defective NK activity, reduced IFN-γ production, impaired Th1 development	[28]

However, other nonhaemopoietic cell responses to IL-18 are likely with direct effects on chondrocytes and cartilage matrix degradation having been reported [69]. IL-18 has further been shown to inhibit osteoclast formation via T cell GM-CSF production [70]. Keratinocytes, traditionally thought to produce but not process IL-18 [71], have now been shown to secrete biologically active IL-18 when treated with dinitrochlorbenzene and proinflammatory mediators such as LPS [72]. In addition to keratinocytes, Langerhans cells (LC) also produce IL-18 [12], which in turn contributes to the regulation of LC migration [73].

NATURAL ANTAGONISTS TO IL-18

The discovery, cloning, and characterization of IL-18-binding protein (IL-18BP), a constitutively secreted protein able to bind IL-18 with high-affinity, provide a potential mechanism whereby IL-18 activity could be regulated. Indeed, IL-18BP inhibits IL-18-induced IFN-γ and IL-8 production and NF-κB activation in vitro and LPS-induced IFN-γ production in vivo [74, 75]. Via inhibition of IL-18-induced IFN-γ production, recombinant IL-18BP has also been shown to augment PBMC prostaglandin production [76]. Recent studies have shown that IL-18BP expression itself may be augmented by IFN-γ [77] and is up-regulated in sepsis [78], endothelial cells, and macrophages during active Crohn[rsquo]s disease (CD) [79], suggesting the existence of an endogenous, IFN-γ-regulated feedback loop. Local levels of free IL-18 or IL-18 complexed with IL-18BP are therefore likely vital in determining net IL-18 biological activity. Generated as a result of alternative mRNA splicing, four human and two murine IL-18BP isoforms have been identified. Human IL-18BPa and IL-18BPc and murine IL-18BPc and IL-18BPd isoforms are capable of binding to and neutralizing IL-18 [80]. In addition to IL-18BP, a homologous protein p13 encoded by the *ectromelia poxvirus* has been shown to bind to and inhibit human IL-18 activity in vitro [81]. *Molluscum contagiosum* viral proteins MC53 and MC54 inhibit IL-18-induced IFN-γ production and NK cell activity in a similar manner as IL-18BP [82]. The recent discovery of IL-1H, a protein with sequence homology to IL-1ra, which is able to bind the IL-18R but not IL-1R [83], suggests the possible existence of another IL-18R antagonist, although functional data are awaited.

IL-18 AND HOST DEFENSE

IL-18 possesses broad and potent immunomodulatory properties. It is unsurprising therefore that it appears essential to host defences against a variety of infections. First identified in the livers of mice infected with *Propionibacterium acnes* and LPS [84], IL-18 is particularly effective during the clearance of intracellular bacteria, fungi, and protozoa, requiring the induction of host-derived IFN-γ, which in turn evokes effector pathways involving molecules such as nitric oxide (NO). IL-18 also plays a part in the clearance of viruses, partly through the induction of cytotoxic T cells with viral clearance being impaired in IL-18-deficient mice. Key effects of IL-18 or IL-18R deficiency are summarized in **Table 1**.

Bacterial infections

The intracellular pathogen *Mycobacterium avium* has been widely studied using a variety of murine strains including IL-18 and IL-18R-deficient mice. These studies show the requirement for a strong Th1 response and a critical role for

TABLE 2.

	Bacterial	Viral	Fungal	Protozoan
Potentially protective	*M. avium* [60, 90, 100]	HSV [108]	*Cryptococcus neoformans* [87, 130, 131]	*L. major* [85, 86, 134]
	M. tuberculosis [101]	Vaccinia [109]	*Aspergillus fumigates* [132, 133]	*Trypanosoma cruzi* [135, 136]
	Mycobacterium leprae [103, 120]	Murine adenovirus [122]		*Plasmodium* [89, 137, 138]
	Salmonella typhimurium [104]	Murine cytomegalovirus [122, 123]		
	Yersinia enterocolitica [105]	HPV [110]		
	Chlamydiae trachomatis [106]	HIV [112, 113]		
	Shigella flexneri [107]	FLV [114]		
	P. acnes [84]	Epstein-Barr virus [124]		
		Ectromelia [81]		
		Rubella [125]		
		Influenza A [98, 126]		
		Encephalomyocarditis virus [127]		
Potentially harmful	*M. tuberculosis* [102, 121]	HIV [111, 128]		
	M. leprae [65]	*Ebola* [129]		

IL-18 in expulsion of the pathogen [60, 90, 100]. The contribution of IL-18 during a protective Th1 response is further demonstrated in human studies on patients with *M. tuberculosis* infection who displayed a decreased ability to produce IL-18 and IFN-γ in response to antigen compared with healthy PPD-responsive controls [101]. Similarly, Kinjo and colleagues [99] have recently demonstrated impaired IFN-γ production in IL-18-deficient mice following infection. However, patients with advanced disease appear to have raised plasma IL-18 levels [102]. In leprosy, the Th1/Th2 balance is key to disease outcome, but currently data on IL-18 are conflicting. In resistant tuberculoid leprosy (TL), protective IFN-γ production is associated with increased IL-18 mRNA expression within lesions, and monocytes from TL patients show increased IL-18 mRNA expression following in vitro challenge with bacterial antigen [103]. Furthermore, such in vitro challenge of T and NK cells of TL patients resulted in increased IFN-γ production compared with cells from patients with susceptible lepromatous leprosy (LL). However Yoshimoto et al. [65] have shown that serum IL-18 levels were much higher in an LL cohort. IL-18 could therefore promote the development of the Th2 response, characteristic of LL. Further in vivo studies have shown the importance of IL-18 in the protective immune response to a number of bacterial infections including *salmonella, yersinia, chlamydiae,* and *shigella* [104–107].

Viral infections

In addition to inducing IFN-γ, IL-18 activates $CD8^+$ T cells crucial for viral clearance. IL-18 is protective in a murine model of Herpes simplex virus (HSV) infection [108]. Exogenous administration of IL-18 before infection results in upregulated IFN-γ-dependent NO production, leading to improved survival. In an in vivo model of *vaccinia* infection, IL-18 administration reduces pock formation [109]. Clearance of neurovirulent influenza A-infected neurons by microglial/macrophage cells is impaired in the brains of IL-18-deficient mice [98]. Down-modulation of IL-18-induced immune responses by human papilloma virus (HPV) oncoproteins may contribute to viral pathogenesis or carcinogenesis. This may arise via HPV binding to the IL-18R, thus preventing IL-18 induction of IFN-γ [110].

Although an early report suggested that IL-18 increased human immunodeficiency virus (HIV)-1 production in a chronically infected monocytic cell line [111], recent studies predict a protective role with IL-18 inhibiting HIV-1 production by peripheral blood cells [112]. In vaccine studies, the coinjection of DNA encoding IL-18 modulates the specific immune response toward a protective Th1 type [113]. Similarly, feline leukemia virus (FLV) DNA vaccine efficacy is enhanced by coadministration with IL-18 expression vectors [114].

Finally, immunomicroarray analysis reveals that T cells infected with HHV-6 respond by inducing a type 1 immune response. Thus, IL-18 production may play a significant role in the development and progression of diseases associated with HHV-6, including pediatric, hematologic, transplant, and neurologic disorders [115]. In contrast, in vitro HHV-6 infection of LPS-treated PBMC down-regulated IL-18 production, suggesting that the down-regulation of a cytokine involved in the induction of antiviral IFN-γ is a strategy used by the virus to evade a host response [116].

In a murine model of viral myocarditis, there was a reduction in heart weight/body weight ratio in IL-18-treated mice and TNF-α mRNA expression in myocardium [117]. IL-18 reduced severity of viral myocarditis by inducing cardiac expression of IFN-γ and increasing NK activity [118]. In a coxsackie myocarditis model, the proinflammatory response involving IL-18 contributes to pathology seen in connective tissues in the chronic stages of disease [119]. Other viral syndromes in which IL-18 is documented are shown in **Table 2**.

Fungal infections

IL-18 in synergy with IL-12 promotes the antifungal response to *C. neoformans* by inducing IFN-γ from NK cells and NO from macrophages [130, 139]. Thus, IL-18 administration during *C. neoformans* infection results in an increase in IFN-γ by NK and T cells with a down-regulation of IL-4 production [130,

139, 140]. IL-18 appears effective even in the absence of IL-12 [87].

In a chronic fungal asthma model, IL-18 promotes innate responses, preventing the development of severe fungus-induced asthmatic disease [132]. In caspase-1-deficient mice, IL-18 restores defective Th1 responses during *Candida albicans* infection [133].

Protozoan infections

Murine models suggest that susceptibility and resistance to infection with *L. major* depend on the production of IL-4 and IFN-γ, respectively [141, 142]. Several groups have reported the protective role of IL-18 during infection. Using IL-18-deficient mice, Wei and colleagues [85] reported increased susceptibility. Neither IL-12 nor IL-18 alone induced wound healing, whereas in synergy, footpad swelling was inhibited through a NO-dependent pathway, and mice were protected from further reinfection [86]. Neutralizing anti-IL-18 antibody treatment markedly reduced protection. It is interesting that although IL-18-deficient mice on a resistant background do develop larger lesions during early disease, compared with wild-type littermates, this eventually resolves [86, 134]. Therefore, although IL-18 appears to control early disease, it is not obligate for host immunity and the required development of a Th1 phenotype [86, 134]. Protection from *T. muris* infection is associated with a strong Th2 response. IL-18-deficient mice are resistant to chronic nematode infection, and administration of exogenous IL-18 to normally resistant strains results in chronic disease. IL-18 directly suppresses the antigen-specific IL-13- and IL-4-protective response, independent of IFN-γ production [97].

In severe combined immunodeficiency (SCID) mice, IL-18 augments NK cell-mediated immunity to *Toxoplasma gondii* [143]. Similarly, IL-18 promotes healing in IL-12-deficient mice, which have reduced capacity a priori to produce IFN-γ [144]. In contrast, extensive liver damage and lymphoid degeneration during lethal infection with high virulence strains associated with a strong Th1-type response are reported. High levels of serum IL-18 are detected, and neutralizing anti-IL-18 antibodies can increase survival times [145]. Resistance to *T. cruzi* requires the development of a successful IFN-γ response, which correlates with increased expression of IL-12 and IL-18 [135]. Finally, high levels of IL-18 are detected in mice infected with *P. berghei*, and neutralizing anti-IL-18 antibodies shortens survival times [137]. IL-18 is also implicated in host defense by inducing IFN-γ production during blood-borne stages of disease [89]. Serum IL-18 rises in patients with uncomplicated *Plasmodium falciparum* malaria who mount an effective Th1 response [138].

IL-18 IN AUTOIMMUNE AND INFLAMMATORY DISEASES

IL-18 expression and effector function has now been described in inflammatory diseases across a broad range of tissues. We have focused on recent key examples of a proinflammatory role for IL-18. Activities in additional disease states, including a role in cancer, have been discussed recently elsewhere [19, 146].

Inflammatory arthritis

IL-18 is present in synovial membrane of patients with RA [18, 147, 148] and with psoriatic arthritis (unpublished observations). Pro-IL-18 (24-kD) predominates, although mature IL-18 is consistently detected. IL-18 expression is localized in $CD14^+$ and $CD68^+$ macrophages and in fibroblast-like synoviocytes (FLS) in situ. IL-18R (α and β) chains are detected ex vivo, on up to 40% of synovial $CD3^+$ lymphocytes and on 20% of synovial $CD14^+$ macrophages and in vitro on FLS. IL-18BP may also be present in substantial concentrations [149–151]. These data clearly indicate that IL-18 and its receptor system are present in inflammatory synovitis. Its functional activities include promotion of cytokine release (particularly TNF-α, GM-CSF, and IFN-γ). Marked synergy with IL-12 and IL-15 is observed in this respect. IL-18 acts not only through lymphocyte activation but also through direct effects on macrophages. IL-18 expression is in turn up-regulated in FLS by IL-1β and TNF-α, suggesting the existence of positive feedback loops linking monokine predominance in RA with innate cytokine production and Th/c1 cell activation in synovial immune responses. IL-18 induces NO release by RA SM in vitro, which as NO inhibits caspase-1 activity, provides a further potential regulatory loop. IL-18 possesses prodegradative effects in articular cartilage. IL-18 reduces chondrocyte proliferation; upregulates inducible NO synthase, stromelysin, and cycloxygenase-2 (COX-2) expression; and increases glycosaminoglycan release in vitro. Such activities may be IL-1β-independent, although contradictory data have also emerged [152]. IL-18 further promotes synovial chemokine synthesis and angiogenesis [153, 154]. Finally, IL-18 effects are not necessarily detrimental. IL-18 inhibits osteoclast maturation through GM-CSF production by T cells, thereby retarding bone erosion [70]. Suppression of COX expression may also be mediated through IFN-γ production with consequent effects on prostanoid-mediated local inflammation.

IL-18 has been targeted in several arthritis models in vivo. Upon challenge with type II collagen (CII) in complete Freund's adjuvant (CFA), IL-18-deficient mice on a DBA/1 background exhibit reduced incidence and severity of arthritis. Ex vivo analysis determined both cellular and humoral responses to CII were suppressed [92, 155]. Moreover, administration of recombinant IL-18 can replace the requirement for CFA in CII-induced erosive arthritis in DBA/1 mice [155]. Neutralization of IL-18 in vivo using specific antibodies or IL-18BP effectively reduces developing and established rodent arthritis in streptococcal cell wall and CIA models [152, 156]. Such effects may operate independent of IFN-γ [152]. A feature of both models is suppression, not only of inflammation but also of matrix destruction. These data strongly suggest that the net effect of IL-18 expression is proinflammatory, at least in the context of antigen-driven, articular inflammation. Clinical studies to test this hypothesis in RA are awaited.

Insulin-dependent Diabetes Mellitus (IDDM)

Nonobese diabetic (NOD) mice, which spontaneously develop insulitis as a result of β-islet destruction, are a useful model for

human IDDM. IL-18 mRNA is up-regulated in NOD mice treated with the diabetes-inducing agent cyclophosphamide, and the murine IL-18 gene maps to the *Idd2*-susceptibility locus, suggesting a potential role in the predisposition to IDDM [157]. In a transgenic NOD model where the TCR from $CD4^+$ diabetogenic T cells is overexpressed, IL-18 as well as IL-12 and TNF-α levels are raised [158]. Surprisingly, however, the administration of exogenous IL-18 to diabetes-susceptible mice delayed the onset of disease, presumably by interfering with the Th1/Th2-immune balance within the pancreas [159]. The future development of IL-18-deficient NOD mice should help clarify these issues. In support of a pathogenic role for IL-18 in human disease, IL-18 serum levels are increased during the early subclinical stages of IDDM [160].

Multiple Sclerosis (MS)

MS is characterized by myelin sheath inflammation, demyelination, and impaired nerve function [161]. Experimental autoimmune encephalomyelitis (EAE) is a murine model of MS in which the induction of myelin basic protein (MBP)-specific $CD4^+$ T cells secreting cytokines, particularly IFN-γ and TNF-α, results in limb paralysis. There is evidence for IL-18 involvement in the disease process. High levels of IL-18 mRNA are found in the brains and spinal columns of EAE rats at onset and during the disease [162, 163]. In an alternate model of autoimmune encephalomyelitis, IL-18-deficient mice mount a defective, autoreactive Th1 response and are resistant to disease [95]. Up-regulation of the IL-12R by IL-18 results in enhanced IFN-γ production and exacerbated disease. In further support of IL-18 involvement in MS, administration of a neutralizing anti-IL-18 antibody partially protects animals from disease and reduces the Th1-dominant anti-MBP T cell response [162]. Furthermore, the brains taken from patients with demyelinating MS exhibit up-regulation of IL-18 and IFN-γ mRNA with the accompanying accumulation of Th1-specific T cells [164]. Finally, caspase-1-deficient mice exhibit decreased disease severity, and cells taken from MS patients have elevated caspase-1 levels [165, 166].

Gastrointestinal system

Parallels exist between effector mechanisms in inflammatory bowel disease (IBD) and RA. Elevated IL-18 expression is reported in IBD, particularly CD [16, 167–169]. Thus, IL-18 is present in serum of CD patients, and bioactive IL-18 is detected in CD mucosal biopsies. Moreover, in mucosal explant cultures, IL-18 antisense suppresses IFN-γ expression, indicating a direct relationship between IL-18 expression and Th1 effector function [167]. IL-18BP isoforms are similarly up-regulated in CD mucosa in epithelial cells and macrophages, and IL-18/IL-18BP complexes are detected in tissues together with free, mature IL-18 [79].

In vivo model systems indicate that in vitro observations are of importance. Studies using several gene-targeted murine strains suggested that IL-18 promotes colonic inflammation via IFN-γ-dependent but NO-, Fas-L-, and TNF-α-independent pathways [170]. ICE-deficient mice exhibit reduced severity of dextran sulfate (DS)-induced colitis associated with reduced IL-18 expression [171]. DS colitis is ameliorated by anti-IL-18 antibody [172] or by IL-18BP:Fc protein [173]. The latter effectively suppresses DSS colitis in vivo associated with a reduction in mucosal cytokine, chemokine, and metalloproteinase gene expression [173]. Similarly, colitis is associated with increased, local IL-18 expression [168], and IL-18-deficient mice fail to develop disease [93]. IL-12p40-deficient mice develop increased severity of TNBS-induced colitis associated with enhanced IL-18 expression, suggesting interactions between IL-12 and IL-18 in colonic mucosa [174]. Finally, transfer of $CD62L^+$ $CD4^+$ T cells into SCID mice induces CD-like mucosal inflammation associated with high IL-18 expression. Administration of adenovirus containing IL-18 antisense effectively reduces inflammation in this model [175]. Together, these data strongly implicate IL-18 as an important mediator of gastrointestinal inflammation.

Pulmonary disease

A prominent role in pulmonary inflammation is suggested by studies in human tissues and in rodent models. The effects of IL-18 in airway inflammation are not easily predicted, as it can promote Th1 and Th2 responses. In general, it appears that IL-18 is primarily a negative regulator of Th2-mediated airways hyper-reactivity (AHR) but can promote pulmonary granuloma formation and subsequent lung parenchymal damage. Thus, IL-18 expression is enhanced in pulmonary infiltrates in sarcoidosis patients [176], whereas reduced levels are found in asthmatic subjects [177]. IL-18 is detected at significantly reduced levels in bronchoalveolar lavage fluid and in alveolar macrophage cultures derived from asthmatic donors compared with healthy controls. IL-18-deficient mice challenged with ovalbumin exhibit marked eosinophilia together with exaggerated lung damage compared with controls. IL-18 administration reverses such effects [178]. In the same model, IL-12 administration reduces the severity of AHR, eosinophilia, and T cell infiltration associated with increased IL-18R expression, suggesting that IL-18 could promote resolution of local inflammation induced by an ongoing Th2 response [179]. Commensurate with this, adenoviral delivery of IL-18 in established ovalbumin-induced AHR reduces AHR, IL-4 production, mucus expression, and eosinophilia [180]. IL-18 similarly suppresses immune complex-mediated changes in lung vascular permeability, whereas IL-18 neutralization increases inflammatory parameters [181]. However, IL-18 increases eosinophil IL-8 release in vitro [182] and may also increase local eosinophil accumulation in vivo, in part via eotaxin release in the cockroach allergen-induced model [183]. These data together with the in vitro effects on Th2 maturation suggest that the precise effect of IL-18 may depend on the kinetics and nature of specific pulmonary antigen responses.

Other inflammatory disease states

IL-18 expression has been described in a number of additional disease states. It is found together with a functional receptor in human atheroma tissues, predominantly in macrophages. IL-18 induces IL-6, IL-8, ICAM-1, and matric metalloproteinase expression in vascular smooth muscle cells, endothelial cells, and macrophages [33]. Unexpectedly, IL-18 together with IL-12 also promoted IFN-γ expression in smooth muscle cells.

Enhanced IL-18 expression has also been detected in acute and chronic hepatitis, systemic lupus erythematosis, psoriasis, and adult onset Still[rsquo]s disease [71, 72, 151, 184–187]. Very high serum levels of IL-18 are detected in the latter, which are equivalent to those detected in neoplastic and haemophagocytic syndromes in which cytokine dysregulation has been related to systemic clinical features such as fever and lymphadenopathy and with disease activity. However, there exist few clinical studies that clearly implicate IL-18 in disease pathogenesis, and many data remain circumstantial.

CONCLUSION

Data generated thus far indicate that IL-18 contributes to host defense and to inflammation through synergism in a cascade of cytokines associated with innate responses, including IL-12 and IL-15. Important questions remain. In particular, the means whereby IL-18 synthesis is regulated, and subsequent release of cytokine is mediated are poorly understood. Similarly, regulation of IL-18 bioactivities in vivo in the context of high levels of IL-18BP and other native inhibitors requires clarification. Finally, the position of IL-18 in the functional hierarchy of proinflammatory cytokines in chronic inflammation is not fully resolved, although there is consensus that it plays a critical, early role. Nevertheless, IL-18 appears able to modulate inflammation at multiple checkpoints, acting not only on initiation and expansion of putative autoreactive Th/c1 responses but also via direct effects on multiple cellular targets, including macrophages, lymphocytes, and target host tissue cells—endothelial cells and fibroblasts. As such, it deserves consideration as a therapeutic target.

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