

Commentary:**The role of the IL-18 system and other members of the IL-1R/TLR superfamily in innate mucosal immunity and the pathogenesis of inflammatory bowel disease: friend or foe?****Brian K. Reuter and Theresa T. Pizarro**

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Crohn's disease and ulcerative colitis are examples of inflammatory bowel disease (IBD), and are multifaceted chronic autoimmune disorders with unknown etiology; to date, there is no known cure. IBD is thought to occur as a result of an inappropriate immune response to environmental factors in a genetically predisposed host, and it has become increasingly clear that cytokines play an important role in this process. In recent years, several groups have provided evidence that IL-18 is significantly up-regulated during the course of chronic intestinal inflammation and appears to play a pivotal role in the pathogenesis of human IBD, particularly in Crohn's disease. IL-18 is a pleiotropic cytokine with several biological functions, but is most commonly associated with its ability to synergistically induce the expression of IFN- γ . However, although IL-18 has been extensively studied in both human IBD as well as in murine models of colitis, no definitive function of IL-18 during the initiation and perpetuation of chronic gut inflammation has been firmly established, and its precise role in the pathogenesis of IBD has yet to be determined. In light of the recent observation that the transcription factor interferon regulatory factor-1 has the ability to regulate the functional activity of IL-18, and concomitantly disease severity, in a murine model of colitis through altered expression of its endogenous inhibitor, IL-18-binding protein, this commentary will review what is currently known regarding the role of IL-18 in normal mucosal immunity and during the pathogenesis of IBD.

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Key words: IL-18 / Inflammatory bowel disease**1 Introduction**

Crohn's disease (CD) and ulcerative colitis are the best-known forms of inflammatory bowel disease (IBD), which is an organ-specific autoimmune disorder in which the gastrointestinal tract is damaged by bouts of uncontrolled, chronic mucosal inflammation and the subsequent processes of remodeling that occur during periods of remission. In recent years, the number of newly diagnosed cases of IBD has dramatically increased

worldwide, and it is now the second most common chronic inflammatory disorder behind rheumatoid arthritis [1]. The generally accepted dogma is that IBD is caused by an inappropriate immune response to environmental factors in genetically predisposed individuals. However, although the contribution of these three components (i.e. host immune system, environment and genetic predisposition) has been extensively studied, the precise etiology of IBD has yet to be determined and there is currently no known cure for this devastating disease.

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Abbreviations: **CD:** Crohn's disease **DSS:** Dextran sulfate sodium **IBD:** Inflammatory bowel disease **IEC:** Intestinal epithelial cell **IL-18BP:** IL-18-binding protein **IRF-1:** Interferon regulatory factor-1 **TLR:** Toll-like receptor **TNBS:** Trinitrobenzenesulfonic acid

Several lines of evidence support the concept that cytokines are important mediators of intestinal inflammation. A broad array of cytokines have been implicated in the pathogenesis of IBD, including the proinflammatory and Th1 cytokines IL-1, IL-2, IL-12, IFN- γ , TNF, IL-18 and,

more recently, TL1A and IL-23, as well as the anti-inflammatory and Th2 cytokines IL-4, IL-10 and IL-13 [2–5]. In CD, evidence has accumulated from both human studies and animal models to indicate that Th1 cytokines, in particular, are involved in the pathogenesis of this disorder. In fact, cytokine dysregulation is currently an important focus of both basic as well as clinical research in IBD, and many targeted therapies aimed at treating chronic intestinal inflammation are designed to alter aberrant cytokine function in order to restore normal mucosal immune responses and maintain gut homeostasis (e.g. treatment of steroid-refractory CD with anti-TNF). However, identification of the specific initiating factor(s) driving cytokine-mediated immune responses has not yet been fully elucidated and remains an important area of investigation.

The article by Siegmund et al. in the current issue of this journal [6] examines the role of interferon regulatory factor-1 (IRF-1) in mediating the inflammatory response using two murine models, in which colitis is chemically induced by dextran sulfate sodium (DSS) or trinitrobenzenesulfonic acid (TNBS). IRF-1 is a transcription factor normally associated with the activation of the IFN- β gene; however, it has been demonstrated to possess multiple biological functions, including blood cell homeostasis and hematopoietic cell-associated immune/inflammatory responses [7, 8]. A more causal link between IRF-1 and IBD may exist since IRF-1 has been shown to regulate a variety of cytokines and inflammatory mediators (e.g. IFN- γ , IL-12, nitric oxide and prostaglandins) that are known to play an important role in the pathogenesis of chronic intestinal inflammation [8, 9]. In fact, IRF-1 has been demonstrated to play a pivotal role in the regulation of IL-18 [9, 10], a cytokine recently shown to be up-regulated in IBD [11, 12].

2 Biology of IL-18

IL-18 was initially characterized as a novel IFN- γ -stimulating factor in mice infected with *Propionibacterium acnes* and subsequently challenged with a sublethal dose of LPS [13]. IL-18 has been identified in cells of both hemopoietic and non-hemopoietic cell lineages, including M ϕ , DC, Kupffer cells, keratinocytes, osteoblasts, adrenal cortex cells, intestinal epithelial cells (IEC), microglial cells, and synovial fibroblasts [11, 14–20]. Within the gut mucosa, our group and others have identified that IL-18 is primarily produced by IEC, tissue histiocytes (or M ϕ), and DC [11, 12, 21].

IL-18 is produced as a precursor molecule (pro-IL-18) and does not contain a signal peptide required for the cellular secretion of mature bioactive protein [22]. The IL-

1 β -converting enzyme (ICE or caspase-1) cleaves the 23.0 kDa precursor form of IL-18 after Asp35, thus generating the mature and bioactive 18.3 kDa IL-18 protein [23]. However, caspase-1 cleavage of pro-IL-18 is not exclusive, as recent reports have demonstrated that proteinase-3 can also generate biological activity from pro-IL-18 [24]. Secreted, mature IL-18 interacts with the IL-18R complex, which is a heterodimer consisting of an α chain [IL-1R-related protein (IL-1Rrp)], that is responsible for extracellular binding of IL-18 as well as IL-1, and a non-binding, signal-transducing β chain [accessory protein like (AcPL)]; both chains are members of the IL-1R family and are required for functional IL-18 signaling [25–28].

The IL-18R complex is expressed on a variety of cell types, including T and B lymphocytes, M ϕ , neutrophils, NK cells, endothelial cells, and smooth-muscle cells [29–32], and can be up-regulated on naive T cells, Th1 cells, and B cells by IL-12 [26, 33]. In contrast, TCR ligation in the presence of IL-4 results in down-regulation of the IL-18R complex [34]. Therefore, modulation of the IL-18R complex has obvious functional implications and in fact, although initially described as a Th1-polarizing cytokine, IL-18 has recently been shown to be a pleiotropic cytokine that can mediate both Th1- and Th2-driven immune responses [35, 36]. Therefore, in addition to its recognized ability to act as a co-stimulatory factor for IFN- γ production, IL-18 possesses several other biological activities that underscore its potential to serve as a key mediator in the pathogenesis of several chronic inflammatory disorders, including IBD. A summary of IL-18's biological properties relevant to IBD-related mucosal immunity is listed in Table 1.

Table 1. IL-18-mediated mucosal immune responses associated with IBD

Activity	References
Induction of IFN- γ (alone or in combination with IL-12)	[17, 18]
Activation of Th1 and Th2 responses	[35, 36, 76, 77]
Induction of IL-11	[49]
Endothelial cell activation	[78]
Up-regulation of adhesion molecule expression	[78]
Neutrophil activation and degranulation	[79]
CD4 ⁺ T cell, DC and neutrophil chemotaxis/migration	[80–82]
Induction of IL-8, IL-1 β and TNF	[83]

3 Differential role of IL-18 in acute versus chronic phases of gut inflammation

Several lines of evidence support the role of cytokine dysregulation in the pathogenesis of IBD; for example, in CD, it is widely thought that a pro-inflammatory Th1-polarized state prevails [37–40]. However, the specific initiating cytokine(s) driving Th1-mediated immune responses in CD has yet to be fully elucidated. Our group and others were the first to report that IL-18 is up-regulated in patients with IBD, particularly in CD [11, 12]. In fact, IL-18 is present in the serum of CD patients, and bioactive IL-18 expression, along with IL-18-induced cytokines, is increased in mucosal biopsies of patients with IBD compared with controls, in involved *versus* non-involved lesions, and in chronic advanced compared with early asymptomatic disease [11, 12, 21].

The emerging paradigm that IBD develops in two distinct phases is gaining support, and is substantiated by recent evidence demonstrating that in IL-10-knockout mice, lamina propria mononuclear cells from mice with early disease synthesize progressively greater quantities of IL-12 and IFN- γ , whereas production of both cytokines dramatically declines and returns to pre-disease levels in the late phase of colitis [41]. In contrast, IL-4 and IL-13 production increases progressively from pre- to early to late disease, indicating that different mediators and mucosal immune processes may be important in initiating, *versus* sustaining, chronic intestinal inflammation. In fact, recent data show that Th2 cytokines, such as IL-4, may possess pathogenic function during the chronic phase of ileitis in SAMP1/YitFc mice, a spontaneous model of CD-like enteritis [42]. This Th2 polarization appears to be particularly important for inflammation localized to the small intestine or terminal ileum, *versus* the colon, since treatment with anti-IL-4 abrogated ileal lesions in TCR-deficient recipients of IFN- γ -knockout CD4⁺CD45RB^{high} T cells, as well as ileitis in SAMP1/YitFc mice [42, 43].

To date, little is known regarding the role IL-18 plays in mediating Th2 immune responses during normal gut immunity or in chronic intestinal inflammation. Although previous studies have reported that IL-18 promotes the development of chronic gastrointestinal helminth infection by down-regulating the Th2 cytokines, IL-4 and IL-13 [44, 45], it has also been shown that IL-18 has the ability to polarize naïve CD4⁺ T cells to produce both IL-4 and IL-13 [46]. Therefore, although initially described as a Th1-polarizing cytokine, IL-18 is able to mediate both Th1- and Th2-driven immune responses, which may play differential roles in early/acute phases compared with later/chronic phases of IBD.

Further lines of evidence support the paradigm that IBD develops in two distinct phases and that key mediators involved in the pathogenesis of IBD may, in fact, possess very different functions depending on the stage of disease. Our group and others have recently generated data that support the role of IL-18 as a protective factor during the early, acute phase of mucosal immune responses when IEC are the primary source of IL-18 [47, 48], and that this protective effect may occur through an IL-11-dependent mechanism [49]. Specifically, we have shown that IL-18R is present on IEC and that IL-18 has the ability to act in an autocrine manner by potently inducing epithelium-derived IL-11. In turn, IL-11 has the ability to promote IEC proliferation, protect clonogenic stem cells within the intestinal crypts, and inhibit IEC apoptosis, with a net effect of epithelial repair and restitution of barrier function [50].

This novel function for IL-18 contrasts with the established pathogenic role that IL-18 is believed to play in the more chronic phases of Th1-mediated inflammation. However, another indication that IL-18 may possess functionally different, and perhaps dichotomous, roles in IBD is the observation that a dramatic shift in the cellular source of IL-18 occurs as the severity of disease increases in patients with CD, from IEC to lamina propria M ϕ and DC [11]. In the early phase of IBD, epithelial activation can occur as a result of innate immune responses to environmental factors, such as commensal or pathogenic bacteria, which may in turn result in the up-regulation of IL-18 from IEC. In the later, more chronic and severe phase of disease, infiltration of macrophages, lymphocytes and other immune cells occurs and provides both the cellular source as well as the cytokines (e.g. IL-18 and IL-12) to mount a prototypic Th1-mediated immune response. In fact, IL-18 has been shown to be a potent proliferative factor and inducer of IL-2R α on intestinal mucosal lymphocytes, specifically from CD patients [21].

The shift in the expression pattern of IL-18 has also been confirmed during the course of ileitis in SAMP1/YitFc mice, in which we recently reported that the mouse IL-18 gene is located within an interval on chromosome 9 that confers genetic susceptibility to disease (terminal ileitis) [51]. Further evidence that IL-18 plays an important role in chronic intestinal inflammation is provided by *in vivo* model systems demonstrating that IL-18 is increased during active gut inflammation [40, 52, 53], and that neutralization, or targeted gene deletion, of IL-18 results in amelioration of chemically or immunologically mediated colitis [53–56]. However, the temporal and spatial expression of IL-18 in regard to the cellular source, as well as the presence (or absence) of specific IL-18R-bearing effector cells, may explain the observed

dichotomous effects of IL-18 during the acute, early phases, *versus* the later, chronic stages, of IBD. A schematic representation regarding the potential relevance of the IL-18 system to the pathogenesis of CD is shown in Fig. 1.

4 Role for IL-18 in intestinal innate immunity

Another important issue to consider is the role that IL-18 plays in intestinal innate immunity and how the IL-18 system contributes to both the maintenance of normal gut homeostasis, as well as to the initiation and perpetuation of chronic intestinal inflammation characteristic of IBD. In fact, increasing evidence suggests that aberrations in intestinal innate immune responses may be one of the central mechanisms by which IBD pathogenesis occurs. This is particularly important in light of the recent and compelling finding that CARD15/

NOD2, an intracellular pathogen-recognition receptor (PRR) that activates NF- κ B in response to peptidoglycan, has been identified as the first disease-susceptibility gene in human CD [57]. Toll-like receptors (TLR) are also considered to be PRR, and recent investigation has focused on characterizing their expression on IEC as well as their potential role in the pathogenesis of IBD. TLR and the IL-18R are members of the IL-1R/TLR superfamily, and are structurally characterized by a cytoplasmic intracellular Toll/IL-1R (TIR) domain and extracellular domains containing leucine-rich repeats. Both the IL-18R and TLR have the ability to signal through the MyD88/IRAK signaling cascade, which ultimately leads to activation of NF- κ B [35, 58].

Although it is unclear if the intestinal epithelium plays a role in adaptive immune responses, it has become increasingly evident that IEC are crucial for early mucosal innate responses, particularly by responding appropri-

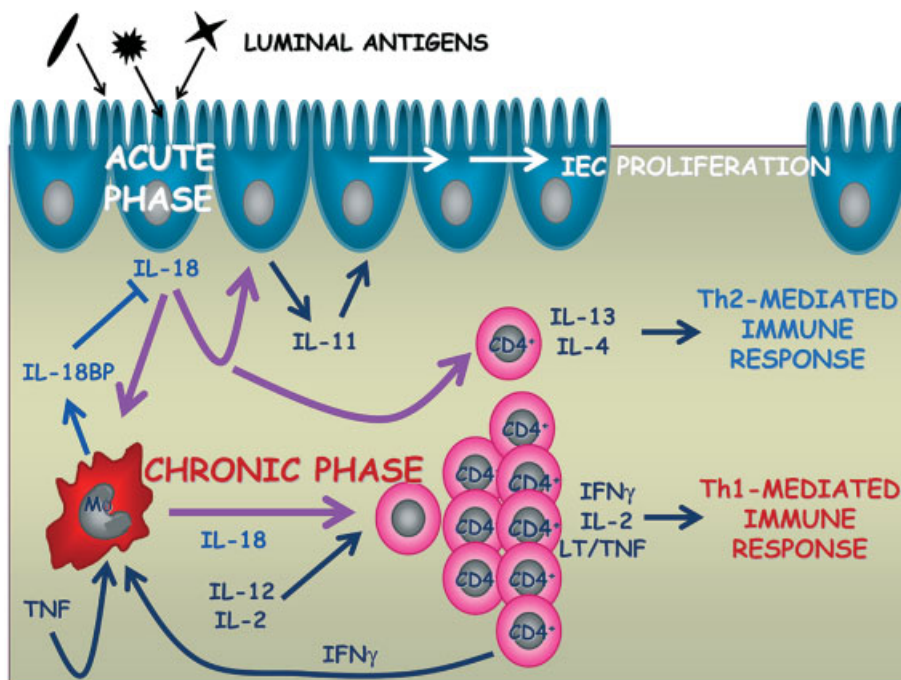


Fig. 1. Working hypothesis for the role of IL-18 in CD. IBD is the result of dysregulated immune responses in a genetically susceptible host to environmental factors, such as luminal antigens, that have the ability to activate the intestinal epithelium. Early during this acute phase, IEC are a potent source of IL-18, which can act in an autocrine manner to produce IL-11. In turn, IL-11 has the ability to promote IEC proliferation, protect clonogenic stem cells, and inhibit IEC apoptosis, with a net effect of epithelial repair and restitution of barrier function. Later in the disease process and in the presence of IL-18R-bearing immune cells, IL-18 has the ability to synergize with proinflammatory cytokines, including IL-2 and IL-12, to induce production of IFN- γ and mediate Th1-type immune responses, such as those observed in the chronic stages of CD. The resulting production of IFN- γ also increases the production of IL-18BP from M ϕ and endothelial cells, which in turn can bind IL-18 to down-regulate further stimulation of the inflammatory response. The transcription factor IRF-1 appears to be critically involved in suppressing the functional bioactivity of IL-18 by regulating the expression of IL-18BP (IRF-1 increases its levels) as well as caspase-1 (IRF-1 decreases its levels). Recent evidence also suggests that Th2-mediated immune responses may be responsible for mediating ileum-specific inflammation, particularly during the later phases of chronic intestinal inflammation. IL-18 may play a role in initiating Th2 responses by polarizing naive CD4⁺ T cells to produce both IL-4 and IL-13, further contributing to the pathogenesis of ileitis, characteristic of CD.

ately to luminal antigenic challenge. PRR, including the TLR, on the gut epithelium are critical in this interaction, and differences in their expression have been demonstrated in patients with IBD [59]. In fact, CD has been associated with specific genetic polymorphisms of CARD15/NOD2 that result in a gene product with impaired ability to activate NF- κ B in response to peptidoglycan of both Gram-positive and Gram-negative bacteria [60]. More recently, it has been reported that mice deficient in TLR4, MyD88 or *Tir8* are more susceptible to DSS-induced colitis, and that the potential mechanisms involved may be due to impaired epithelial restitution due to decreased levels of IL-11 [49, 61, 62].

These findings are similar to those we have observed in IL-18-deficient mice that were given DSS, which were found to be more susceptible to colitis than their wild-type littermates [47, 49]. IL-18-deficient mice were defective in their ability to properly repair and reconstitute ulcerated epithelium, and there was an absence of IL-11 in reconstituting epithelium compared with wild-type mice in which epithelium-derived IL-11 was potently up-regulated following DSS administration. These data further support the concept that IL-18 may be functionally protective in early, and perhaps innate, immune responses upon intestinal challenge with commensal or pathogenic bacteria, and in the acute phase of colitis. However, further investigations are needed to define the precise role of the IL-18 system, including the IL-18 ligand, IL-18BP, and the IL-18R complex, in intestinal innate immunity and its contribution to the pathogenesis of IBD.

5 Regulation of IL-18 function by IRF-1 during experimental colitis

In addition to IL-18, an endogenous, naturally occurring inhibitory protein exists and functions to regulate the excessive and deleterious effects of IL-18 [63]. This inhibitory protein was first isolated from human urine and sera of mice with endotoxic shock, and was termed IL-18BP [63, 64]. To date, six naturally occurring isoforms of IL-18BP have been identified (four human, i.e. IL-18BP_a, IL-18BP_b, IL-18BP_c and IL-18BP_d; two murine, i.e. IL-18BP_c and IL-18BP_d) and result from mRNA (exon) splicing [65]. Not all variants possess the ability to neutralize IL-18. Human IL-18BP_a and IL-18BP_c, and murine IL-18BP_d are able to neutralize both human and mouse IL-18 at equimolar concentrations and with high affinity [65]. In active human CD, endothelial cells and M ϕ were found to be the major source of IL-18BP within the gut submucosa, and unbound IL-18BP_a and 18BP_c and inactive IL-18BP_d, but not IL-18BP_b, were detected in active CD and control patients [66].

The IL-18BP gene is located on chromosome 11q13 and its activation has been demonstrated to be regulated by at least two IFN- γ -induced transcription factors: (1) IRF-1 and (2) CCAAT/enhancer binding protein β (C/EBP β) [10, 65]. IRF-1 appears to modulate IL-18 production and activity via modulation of caspase-1 (IRF-1 decreases its mRNA and protein expression) and IL-18BP (IRF-1 induces its mRNA); both effects result in attenuation of mature/bioactive IL-18 as well as its downstream biological effects [9]. Therefore, the resulting induction of IFN- γ production by IL-18 serves as a negative feedback loop to sequester further IL-18 production via an IRF-1-dependent pathway.

In fact, one of the most intriguing findings of Siegmund et al [6] relates to the increased expression of IL-18 and attenuated expression of its natural, endogenous inhibitor IL-18BP in IRF-1-deficient mice exposed to DSS. On the basis of the current literature, and previous findings concerning the role of IRF-1 in autoimmune diseases and inflammatory events, Siegmund et al. initially hypothesized that IRF-1 deficiency would result in attenuated disease severity in both models of colitis studied (i.e. DSS and TNBS). However, the authors unexpectedly observed that IRF-1-deficient mice displayed dramatically increased susceptibility to both inducing agents. In an attempt to determine the mitigating factors that were involved in enhanced disease severity, Siegmund et al. examined various mediators known to be regulated by IRF-1; their studies revealed the following: (1) no alteration of the Th1 *versus* Th2 cytokine profile and inflammatory mediators (nitric oxide, prostanooids and STAT3 signaling) existed comparing IRF-1-deficient and wild-type mice challenged with DSS/TNBS, (2) IRF-1-deficient mice displayed significantly lower IFN- γ levels and fewer TCR $\gamma\delta$ intraepithelial lymphocytes (IEL) in colonic tissue compared with wild-type mice in response to DSS administration, and as mentioned earlier, (3) colitic IRF-1-deficient mice possessed significantly reduced mRNA and protein levels of IL-18BP compared with wild-type mice.

During the acute course of DSS administration, it was observed that IRF-1-deficient mice contained significantly fewer TCR $\gamma\delta$ cells in colonic tissue than wild-type mice did, which may be due to defects in the maturation and proper development of NK1.1⁺ T cells, NK cells and IEL cells, found to be dependent on IRF-1 [67]. In fact, it has been previously reported that TCR $\gamma\delta$ -deficient mice display an increased susceptibility to DSS-induced colitis, and a protective role for TCR $\gamma\delta$ cells in this model has been postulated to be mediated by the ability of TCR $\gamma\delta$ cells to generate KGF, as well as other mediators involved in the epithelial repair process [68, 69]. However, on the basis of the data shown by Siegmund et al. — that

DSS colitis is more severe in IRF-1-deficient, compared with TCR $\gamma\delta$ -deficient mice — the paucity of TCR $\gamma\delta$ cells in IRF-1-deficient mice does not solely account for the altered disease severity seen with DSS-induced colitis.

In addition, the role of IFN- γ in murine models of IBD is still unclear. It has been shown that the transfer of CD4⁺CD45RB^{high} T cells from IFN- γ -deficient mice to congenic severe combined immunodeficiency (SCID) mice fails to produce the typical wasting disease indicative of colitis [70]. Conversely, the colitis associated with IL-10 deficiency or TNBS administration is IFN- γ -independent, since anti-IFN- γ monoclonal antibody administration, or mice possessing functionally inactivated IFN- γ R1 develop disease to an equivalent degree as controls animals do [71, 72].

In the current article by Siegmund et al. [6], levels of IFN- γ in the colons of DSS-treated IRF-1-deficient mice were significantly decreased compared with controls; however, disease severity was significantly augmented. In addition, they demonstrated that administration of DSS to IFN- γ -deficient mice resulted in a colitis of equivalent severity to that seen in wild-type animals. These data suggest that IFN- γ is not likely a critical mediator involved in the pathogenesis of DSS-induced colitis. In recent clinical studies, preliminary findings have indicated that anti-IFN- γ therapy, using humanized monoclonal anti-IFN- γ antibodies, may not be effective in inducing remission in patients with refractory CD [73]. However, given the important role of IFN- γ in the initiation or acute phase of chronic intestinal inflammation in several experimental models of IBD [42, 74], a possible role for IFN- γ therapy in maintenance, remission, prevention of flares, or in pediatric IBD cases, should not be excluded. Further clinical studies are necessary to fully understand the role anti-IFN- γ therapy may play in the future treatment of IBD.

As previously described, IRF-1 has the ability to regulate IL-18 function at two different levels — by decreasing caspase-1 expression and increasing IL-18BP expression. Although caspase-1 expression was not measured, Siegmund et al. [6] report a significant decrease in IL-18BP mRNA levels in DSS-treated IRF-1-deficient mice compared with wild-type controls. Furthermore, administration of exogenous IL-18BP appeared to ameliorate colitis in DSS-treated IRF-1 deficient mice. However, since IL-18 levels were not found to be significantly different between IRF-1 and wild-type control mice challenged with DSS, and since wild-type mice produce IL-18BP, it is unclear if the effects of exogenous IL-18BP administration is due to IL-18 neutralization or if IL-18BP has other anti-inflammatory effects that result in amelioration of colitis. It is also possible that, like IL-18, the

roles of IL-18BP, IRF-1 and even IFN- γ may be different in early, acute phases compared with later, chronic stages of IBD.

Since DSS administration resulting in colitis represents a model of epithelial injury and repair, and can occur in the absence of T and B lymphocytes [75], further studies are needed, particularly in animal models that are not dependent on chemical induction and recapitulate the more chronic aspects of IBD, to answer these questions. As such, whether IL-18BP and/or IRF-1 represent potential therapeutic strategies for IBD and other autoimmune diseases is yet to be determined.

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