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COMMENT

Novel insights into the role of anti-inflammatory IL-38 in immunity against infection

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Since the identification and cloning of interleukin (IL)-38 in 2001, a plethora of studies have focused on its essential roles as a novel IL-1 family cytokine in both innate and acquired immunity. Based on the shared traits with IL-36 receptor antagonist (IL-36Ra) and IL-1 receptor antagonist (IL-1Ra), IL-38 constitutes one of the most important anti-inflammatory cytokines within the IL-36 subfamily and thus elicits its antagonistic effects through binding to IL-36 receptor (IL-36R) and to IL-R1 with a relatively low affinity [1, 2]. IL-38 has been proven to bind to X-linked interleukin-1 receptor accessory protein-like 1 (IL1RAPL1) [1], and IL1RAPL1 therefore serves as a novel receptor of IL-38. The IL-38 precursor has no signal peptide and is readily released once activated. Notably, released IL-38 requires N-terminal processing to be fully biologically active, and the natural N-terminus of IL-38 has not yet been identified, resulting in the use of several recombinant forms of IL-38 with distinct biological functions. Truncated IL-38 with amino acids 20-152 is widely considered anti-inflammatory [1], albeit the 1-152 amino acid full-length IL-38 is proinflammatory and has been reported to enhance the secretion of inflammatory IL-6 and CXCL8 from lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs) [1, 2], leading to diverse therapeutic potentials of IL-38 in different types of disease settings. The expression patterns of IL-38 are most prominent in epithelial barrier tissues and immune cells, suggesting a role of IL-38 in protecting these barriers from pathogens. Recent evidence highlighting the involvement of IL-38 in the development of infectious diseases derived from bacterial, virus and fungal infections, together with the proposed regulatory mechanisms, will be discussed in this correspondence.

The critical role of IL-38 in infectious diseases has received widespread attention based on the fact that IL-38 is a universal immunosuppressive cytokine and that infectious diseases are often accompanied by a dysregulated hyperimmune response. Beginning with our study of IL-38 in allergic asthma in 2016, we reported that IL-38 significantly inhibited proinflammatory IL-6, IL-1β, CCL5, tumor necrosis factor (TNF)-α, and Th1-related CXCL10 as well as antiviral interferon (IFN)-β expression in human primary bronchial epithelial cells upon stimulation by the viral RIG-like receptor (RLR) ligand poly (I:C) LyoVec [3], leading to our speculation that IL-38 modulates infectious diseases. Unlike the activities of IL-36 agonists IL-36α, IL-36β and IL-36γ, which enhance lung inflammation and exacerbate lung injury in pulmonary infections, we have proposed that IL-38 can counteract the biological activities of IL-36 signaling [4]. Although IL-38 was barely detectable in healthy lung tissue, it was enriched in viral-related TLR3 ligand poly(I:C)-induced lung injury, a model mimicking respiratory viral infections. Subsequently, IL-38 expression was found to be significantly increased in patients hospitalized with influenza A/B or SARS-CoV-2 infection, and its level was negatively correlated with disease severity [4]. A recent study demonstrated that circulating IL-38 levels were not affected by COVID-19, disease severity, age, sex, or comorbidity with chronic disease in each stratum, while obese patients showed lower IL-38 levels [5]. Otherwise, those parameters seem to have concurrent impacts on IL-38 levels, as female patients showed higher IL-38 levels than male patients in severe disease subgroups, and older patients showed higher IL-38 levels than patients <50 years old in critical disease subgroups [5]. Consistent with our studies, they showed that moderate disease patients tended to have higher IL-38 levels than severe or critical disease patients [5]. Sample size, as well different ELISA kits used in different studies, may account for the contradictory parts among the investigations. Apart from the lung, the liver is the second most studied organ for the role of IL-38 in viral infections. In chronic hepatitis B, Alaaraji SF detected lower levels of IL-38 in hepatitis B virus (HBV)-infected patients, and IL-38 was thought to be pathogenic in the context of HBV infection [6]. Wang et al., however, detected higher IL-38 levels in chronic hepatitis B individuals [7]. Although the exact mechanism of the phenomenon has not been elucidated, the baseline IL-38 concentrations were associated with persistent liver damage in untreated individuals, and a higher level of serum IL-38 at baseline was associated with a greater probability of virological response to telbivudine treatment. Therefore, baseline serum IL-38 levels in patients with CHB may serve as a biomarker for the outcome of ongoing liver injury and positive antiviral therapy [7]. The changes in IL-38 levels are suggested to play a role in HBV infection, based on these limited data. However, whether IL-38 is beneficial or detrimental may depend on the stage of the disease, the form and the dose of the applied IL-38, and in this regard, an animal model with IL-38 knockout mice will help elucidate the regulation of IL-38 in HBV infection. Of note, a recent article reported statistically higher IL-38 levels in treated hepatitis C virus

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Table 1. Recent evidence and potential mechanisms of IL-38 in infections

Pathogenic diseases	Models	Expression and regulation of IL-38		References
Influenza	Human study and animal model	Increased IL-38 levels in patients with influenza, returned to baseline once recovered.	IL-38 counteracts the proinflammatory effects of IL-36α.	Gao et al. [4]
COVID-19	Human study and animal model	Increased IL-38 levels in patients with SARS-CoV-2, negatively correlated with disease severity.		
	Human study	IL-38 levels were not influenced by COVID-19, disease severity, sex, age, or chronic disease, while obese people showed lower IL-38 levels. These parameters have concurrent impacts on IL-38 levels.		Al-Bassam et al. [5]
Chronic hepatitis B	Human study	Decreased levels of IL-38 in HBV infection patients, and IL-38 was assumed to be pathogenic in the case of HBV infection.		Alaaraji [6]
	Human study	Increased IL-38 levels in chronic hepatitis B patients, and IL-38 served as a biomarker of liver damage and positive virological response to antiviral treatment.		Wang et al. [7]
HCV infection	Human study	Higher IL-38 levels in treated HCV infection patients and healthy individuals than in pretreated patients; patients with higher IL-38 levels showed reduced liver injury.		Fazeli et al. [8]
Fungal infection	Cell model	IL-38 inhibited *C. albicans*-induced Th17 cytokine production.		Van de Veerdonk et al. [2]
Sepsis	Human study and animal model	Elevated IL-38 levels correlated negatively with IL-6 and TNF-α levels in sepsis; exogenous IL-38 improved survival of septic mice.		Xu et al. [11]
	Animal model	Increased IL-38 levels were produced by CD4 + CD25 + Treg cells and, in turn, increased survival of septic mice by enhancing production of IL-10, TGF-β1 and Tregs.		Ge et al. [12]
	Animal model	IL-38 promoted M2 macrophage polarization, inhibited macrophage apoptosis, and suppressed NLRP3 inflammasome activation in macrophages.		Ge et al. [13]
ARDS	Human study, animal model and cell model	Elevated IL-38 levels in ARDS; IL-38 protected from ARDS by inhibiting the differentiation of Th17 cells.		Chai et al. [14]
Trained immunity	Human study, animal model and cell model	IL-38 prevented the proinflammatory epigenetic reprogramming in the subsequent response to secondary stimulation by LPS. SNP rs5896312 affected both plasma IL-38 levels and the inducibility of trained immunity in monocytes.		de Graaf et al. [15]

(HCV)-infected patients and healthy individuals than in pretreated patients and that patients with higher IL-38 levels had lower alkaline phosphatase (ALP) levels and thus reduced liver injury [8], thereby implying a reduction in the IL-38 response in HCV-induced inflammation. However, it is unclear whether the IL-38 response contributes to or reverses the progression of HCV infection, which warrants future studies in animal models (Table 1).

Early in 2012, van de Veerdonk F. L et al. elucidated that IL-38 may inhibit *C. albicans*-induced Th17 cytokine production, which is similar to the activity of IL-36Ra [2], and this phenomenon showed a bell-shaped concentration dependence, with concentrations above 250 ng/ml being ineffective, the underlying reasons for which remain to be addressed. As the inhibition of IL-36R by IL-36Ra reduced Aspergillus-induced IL-17 and IFN-γ responses [9], we believe that this evidence strongly suggests a positive regulation of IL-38 in fungal disease, possibly by antagonism of the IL-36R pathways to inhibit Th1 and Th17 responses. Later in 2016, a study showed that depletion of IL-38 in apoptotic cells provoked increased levels of inflammatory IL-6 and CXCL8 in cocultured human primary macrophages, subsequently inducing Th17 cell expansion at the expense of IL-10-producing regulatory T (Treg) cells [1]. Given that macrophages are always the frontline innate effector cells against pathogens during fungal invasion, these studies therefore provide evidence that IL-38 may suppress fungal infection-induced inflammation by downregulating the Th17 pathway (Table 1).

Polymorphisms in the IL-38 gene have been observed to correlate with serum levels of C-reactive protein (CRP), which may reflect a role for IL-38 in systemic and acute inflammatory responses, particularly those caused by bacterial infections [10]. In 2018, IL-38 levels were found to be elevated in adults with sepsis in an inverse relationship with proinflammatory IL-6 and TNF-α levels, and exogenous IL-38 improved survival in both LPS- and cecal ligation and puncture (CLP)-induced septic mice [11]. This result was further confirmed by Ge Y *et al*., in 2020, who also demonstrated that increased IL-38 levels in sepsis may be produced by CD4 + CD25 + Treg cells and, in turn, increase the survival of septic mice by enhancing the production of IL-10, TGF-β1 and immunosuppressive Tregs in a positive feedback [12]. Moreover, IL-38 promoted macrophage polarization toward the anti-inflammatory M2 phenotype, inhibited macrophage apoptosis, and suppressed NLRP3 inflammasome activation in macrophages [13]. Therefore, IL-38 may be a novel therapeutic target for the dysregulation of cellular signaling pathways during sepsis. Consistent with this hypothesis, IL-38 also protected against acute respiratory distress syndrome (ARDS) driven by LPS and CLP by inhibiting the development of Th17 cells [14]. This evidence has revealed the value of IL-38 immunotherapy in infectious diseases, and translational exploration in humans would be preferable for the proposed applications of IL-38 immunotherapy to antagonize inflammation in this regard (Table 1).

A recent study by de Graaf, Dennis M et al. showed that IL-38 may block β-glucan-induced phosphorylation of the Akt/mTOR signaling pathway and therefore prevent proinflammatory epigenetic reprogramming to abrogate β-glucan-induced trained immunity and the subsequent response to secondary stimulation by LPS [15]. The above result was confirmed by analyzing a cohort of healthy subjects in which SNP rs5896312, adjacent to the gene encoding IL-38, affected both plasma IL-38 levels and the inducibility of trained immunity in monocytes [15]. These findings are consistent with the anti-inflammatory properties of IL-38. As there is a positive role of trained immunity in providing protection against sustained long-term infections as well as secondary infections and autoimmunity, especially for IL-38, which may produce long-term immunosuppressive effects by modulating trained immunity, whether its protective or deleterious role in

infectious diseases that rely on trained immunity may need further investigation (Table 1).

Overall, the general immunosuppressive function of IL-38 in inflammatory infectious disease strongly suggests that it is an attractive candidate as a potential target. Nevertheless, the biology of IL-38 in infections has not been extensively explored, leaving several key questions unanswered. For example, we still do not know which form of IL-38 is the natural variant present in humans or how this protein is produced in response to pathogens. It also remains to be addressed whether IL-38 can regulate pathogen elimination before acting as a protective factor in resolving inflammation. The feedback mechanism regarding why a lower IL-38 concentration has a more pronounced effect is also elusive. In addition, the necessity of IL-38 processing in vivo and how the antagonistic or even agonistic effects of different forms of IL-38 can be regulated are still not well understood. We also have some insights into the role of IL-38 in mucosal immunity. Upon pathogen attack, successful host immunity against invading pathogens relies on a complete and intact barrier as the frontline of the inflammatory response. Our previous studies have shown that IL-38 can directly attenuate bronchial epithelial cell hyperresponsiveness via distinct intracellular signaling pathways [3], which may provide some crucial clues for the future study of IL-38 in barrier tissue. We look forward to future studies, such as those on intestinal, respiratory, and epidermal tissues, to reveal the important functions of IL-38 in various human diseases.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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