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REVIEW

Interleukin-35: a Potential Therapeutic Agent for Autoimmune Diseases

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Abstract—Autoimmune diseases contain a large number of pathologies characterized by various factors that contribute to a breakdown in self-tolerance. Cytokine-mediated immunity plays an essential role in the pathogenesis of varieties of autoimmune diseases. Recent studies reveal that interleukin-35 (IL-35), a newly identified cytokine of IL-12 family, is implicated in the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), *etc*. In this review, we will discuss the biological features of IL-35 and summarize recent advances in the role of IL-35 in the development and pathogenesis of autoimmune diseases; the discoveries gained from these findings might translate into future therapies for these diseases.

KEY WORDS: interleukin-35; Th17 cells; Treg cells; therapeutic agent; autoimmune diseases.

INTRODUCTION

Autoimmune diseases are characterized by various factors that contribute to a breakdown in self-tolerance, that is, the ability of the immune system to effectively distinguish self from non-self and to refrain from attacking self. Autoimmune diseases include a broad spectrum of disorders, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), inflammatory bowel disease (IBD), idiopathic thrombocytopenic purpura (ITP), *etc*. Although significant progresses have been achieved in the development of approaches to the treatment of autoimmune diseases, the etiologies and pathogenesis of autoimmune diseases remain obscure. Numerous studies have revealed that some cytokines exert immunosuppressive roles in the development and pathogenesis of autoimmune diseases, such as IL-10 [19, 30, 56], TGF-β [36, 68], *etc*. Recently, the imbalance between Treg cells and Th17 cells has been considered as a new paradigm in the pathogenesis of autoimmune diseases, including SLE [55, 69], RA [41, 58], SSc [3], IBD [24, 35], ITP [66, 67], *etc*. As a newly identified cytokine of IL-12 family, IL-35 facilitates the propagation and optimally suppressive function of Treg cells [6, 9] and restricts the differentiation and function of Th17 cells [39, 59, 60]. These evidences illustrated that IL-35 is vitally associated with autoimmune diseases, and IL-35 may exert an effective immunosuppressive role in autoimmune diseases. In this review, we will discuss the biological features of IL-35 and summarize recent advances in the role of IL-35 in the development and pathogenesis of autoimmune diseases; the discoveries gained from these findings might translate into future therapies for these diseases.

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IL-35 AND ITS IMMUNOLOGICAL FUNCTIONS

IL-35 is a newly identified heterodimeric cytokine belonging to the IL-12 family, which contains IL-12, IL-23, IL-27, and IL-35. All the four members are consisted of an α chain (p19, p28, or p35) and a β chain (p40 or Epstein-Barr virus induced gene 3 (EBI3)). For instance, IL-12 consists of p35 and p40 subunits, while p19 combines with p40 forming IL-23 and p28 combines with EBI3 forming IL-27. The latest recognized member, IL-35, composes of p35 and EBI3 [8, 40]. Similarly, the receptors of IL-12 family also possess the chain-sharing feature [11, 18]. The IL-12Rβ1 subunit couples with IL-12Rβ2 structuring the receptors for IL-12 and binds IL-23R together forming the receptors for IL-23. IL-27 and IL-35 share the gp130 subunit to structure their own receptors, gp130 and IL-27Rα structure, the receptor for IL-27, and that for IL-35 is gp130 and IL-12Rβ2 [7, 8, 40]. In addition, IL-35 can also signal through IL-12 Rβ2-IL-12 Rβ2 and gp130-gp130 homodimers [7]. It is notable that all the receptors of IL-35 enable suppression the proliferations of T cells, but the homodimers are incapable of mediating the generation of IL-35 induced regulatory T cells (iTr35) cells compared with heterodimeric one [7].

Despite the structural similarities with each other, the cytokines of IL-12 family have different cellular sources and immunological functions. IL-12, IL-23, and IL-27 are secreted by activated antigen-presenting cells (dendritic cells, monocyte/macrophages, B cells) in reaction to bacteria, intracellular parasites, *etc.* [16, 32, 42]. Both IL-12 and IL-23 primarily act as proinflammatory cytokines, respectively, promoting Th1 and Th17 cells development [16, 23, 25]. IL-12 promotes T and NK cells secreting interferon (IFN)-γ, thus enhancing the differentiation of Th1 cells and the cytotoxicity of cytotoxic T cells (CTL) and inhibiting the propagation of Th2 cells [23]. IL-23 mainly links to the development of pathogenic IL-17-producing Th17 cells, which further promotes the production of various proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-6, *etc.* [25]. IL-27 possesses dual functions of proinflammatory and anti-inflammatory due to its roles in promoting Th1 cells propagation and IFN-γ production and restraining Th17 cells differentiation [44, 63, 64].

Unlike other IL-12 family members, IL-35 was reported to be secreted by regulatory T cells (Tregs) [6, 9], activated B cells [53], *etc.* Recently, the imbalance between Th17 cells and Treg cells has been considered as a new paradigm in the pathogenesis of autoimmune diseases, including SLE [55, 69], RA [41, 58], SSc [3], IBD [24, 35], ITP [66, 67], *etc.* IL-35 plays an essential role in promoting the optimally suppressive function of Treg cells *in vitro* and controlling homeostatic proliferation *in vivo* [9]. IL-35 could induce naive human or mouse T cells to differentiate into regulatory T cells (iTr35 cells), which maintain immunological self-tolerance exclusively via IL-35 rather than TGF-β or IL-10 [6]. Subsequently, IL-35 and iTr35 cells form a positive feedback cycle: IL-35 could elicit the propagation of iTr35 cells, and more iTr35 cells further secrete more IL-35 [6]. Massive studies supported that IL-35 could suppress Th17 differentiation and function, rather than TGF-β or IL-10 [39, 59, 60]. Therefore, IL-35 may exert an essential role in the balance between Th17 cells and Treg cells (Fig. 1). In addition, recent studies implied that IL-35-secreting B cells play a crucial role in the suppressive regulation of immunity, and IL-35 facilitates the differentiation of human B cells into Breg cells which secrete IL-35 and IL-10 [13, 33, 53, 57]. All these evidences indicate that IL-35 orchestrates a significant regulation in immune responses.

IL-35 IN AUTOIMMUNE DISEASES

Autoimmune diseases are characterized by the destruction, and the impaired function of tissues that are caused by an immune response in which abnormal antibodies are produced and attacking the body's own cells and tissues. There is a wide spectrum of autoimmune diseases such as SLE, RA, SSc, IBD, and ITP. These diseases involve various molecules, cells, and tissues, which are targeted by the autoimmune responses [45]. Although the critical details of the etiologies of these diseases are not yet

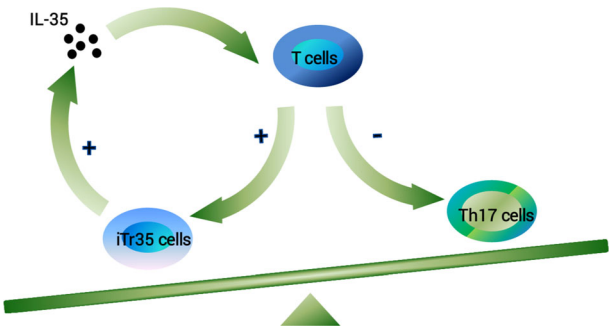

Fig. 1. IL-35 plays an essential role in the balance between Th17 cells and Treg cells. IL-35 could induce naive human or mouse T cells to differentiate into regulatory T cells(iTr35 cells), and more iTr35 cells further secrete more IL-35, forming a positive feedback cycle. In addition, IL-35 could suppress the differentiation and function of Th17 cells. Therefore, IL-35 may exert an indispensable role in the balance between Th17 cells and Treg cells.

fully understood and the clinical features of these diseases are not equal with each other, current findings have revealed that IL-35 is implicated in the development and pathogenesis of autoimmune diseases, including SLE [5], RA [20, 38, 39], SSc [10], IBD [14, 28, 60], and ITP [62] (Table 1).

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that is characterized by the breakdown of immune tolerance of B and T cells to body's own antigens and dysfunctions of multiple major organs, such as the kidney, skin, and blood vessels [37]. The etiology of SLE remains incompletely understood; it is generally thought to be caused by a combination of genetic and environmental factors [22]. Female MRL/lpr mice spontaneously develop severe autoimmune disease closely resembling human SLE and have been widely used as an experimental murine model of SLE [5, 12, 49, 54]. Recently, Cai *et al.* [5] demonstrated that compared with controls (phosphate-buffered saline (PBS)-treated MRL/lpr mice), IL-35-treated MRL/lpr mice got significant remissions of histopathology characteristics of lupus flare and nephritis. The plasma concentrations of proinflammatory cytokines (IFN-γ, TNF- α, IL-6, and IL-17A) were significantly decreased with IL-35 treatment, and the plasma concentrations of anti-inflammatory cytokines (IL-10 and IL-2) were significantly increased upon IL-35 treatment [5]. The messenger RNA (mRNA) expressions of Treg-regulated FoxP3, IL-35 subunit (p35 and EBI3), and soluble IL-35 receptor subunit (gp130 and IL-12Rβ2) of splenic and thymic cells in IL-35-treated MRL/lpr mice were significantly higher than that in PBS-treated MRL/lpr mice [5]. The Treg- related FoxP3 gene expression in IL-35-treated MRL/lpr mice was enhanced compared with that of PBS-treated MRL/lpr mice [5]. These *in vivo* results imply that IL-35 exerts an essentially immune regulatory role in SLE and provides a biochemical basis that IL-35 may act as an efficient therapeutic strategy for SLE.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic joint inflammatory disease with abnormal synovial hyperplasia and progressive destruction of cartilage and bone [34]. Although both clinical and basic scientific researches have attempted to determine the factors involved in the pathogenesis of this disease, the exact cause of RA is still unclear. Niedbala *et al.* [39] has illustrated that IL-35, *in vitro*, promotes the propagation of $CD4^+CD25^+$ T cells along with remarkable elevation of IL-10 levels and markedly restrains the differentiation of Th17 cells. *In vivo*, IL-35 significantly attenuated the synovial hyperplasia, cartilage, and bone erosion of collagen-induced arthritis (CIA) mice [39]. Compared with PBS-treated control mice, the frequency of IL-17-secreting spleen cells of IL-35-treated mice were significantly lower, whereas the serum levels of IL-10 in IL-35-treated mice were significantly higher [39]. Kochetkova *et al.* [20] verified that IL-35 markedly reduced the incidence of clinical symptoms of arthritis and attenuated the severity of synovial hyperplasia and bony destruction of CIA mice. Upon IL-35 treatment, the levels of IFN-γ and IL-17 in protected CIA mice were reduced by 4- and 5-fold, respectively. Recently, Nakano *et al.* [38] illustrated that compared with normal controls, the serum IL-35 levels of RA patients were significantly reduced. Moreover, serum IL-35 levels were significantly higher in patients with inactive RA *vs* active RA. There was a significant inverse correlation between serum IL-35 levels and the 28-joint DAS based on CRP (DAS28-CRP) in RA patients. Furthermore, recombinant IL-35 facilitated the function of natural Treg *in vitro* and restrained proinflammatory cytokines such as IL-17 and IFN-γ [38]. These findings suggest that IL-35 might suppress T cell activation

Table 1. IL-35 Expression in Autoimmune Diseases

Author	Study year	Disease	Parameter	Increase/decrease compared with controls
Nakano *et al.*	2015	RA	Serum levels of IL-35	Decrease
Dantas *et al.*	2015	SSc	Serum levels of IL-35	Increase
Li *et al.*	2014	IBD	Serum levels of IL-35	Decrease
Fonseca-Camarillo *et al.*	2015	IBD	Levels of IL-35 mRNA	Increase
Yang *et al.*	2014	Active ITP	Plasma levels of IL-35	Decrease
Yang *et al.*	2014	Active ITP	Levels of p35 mRNA	Decrease

IL-35 interleukin 35, *RA* rheumatoid arthritis, *SSc* systemic sclerosis, *IBD* inflammatory bowel disease, *ITP* idiopathic thrombocytopenic purpura

during the peripheral immune responses of RA and thereby exert a potential role in the treatment of RA.

Systemic Sclerosis

Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by a complex interaction of vascular injury, immune system activation, and fibrosis. Of the autoimmune connective tissue diseases, SSc has the highest case specific mortality rate, with half of patients dying from pulmonary or cardiac causes [4, 50]. SSc is divided into two subtypes, limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc), based on the extent of skin involvement [26]. Currently, the etiology of SSc remains incompletely understood. Giovannetti *et al.* [15] revealed that increased Treg cell frequency in SSc patients was correlated with clinical phenotypes and clinical progression parameters. Moreover, elevated Treg frequency was also associated with interstitial lung disease and low carbon monoxide diffusing capacity in SSc patients [17]. Recently, Dantas *et al.* [10] reported that serum IL-35 levels were significantly higher in SSc patients, but there was no significant difference in serum IL-35 levels between lcSSc and dcSSc patients. Furthermore, IL-35 levels were higher in SSc patients with lung fibrosis than those without fibrosis [10]. Considering that Treg cells are the main source of IL-35 [6, 9], these results imply that IL-35 may play an indispensable role in the pathogenesis of SSc. However, further studies are required to clarify its potential utility as a serological biomarker and a new therapeutic target for the disease.

Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBDs), including ulcerative colitis (UC) and Crohn's disease (CD), that are chronic inflammatory disorders of unknown etiology, various genetic, environmental and immunologic factors have been proposed [47, 61]. Wirtz *et al.* [60] reported that compared with IL-27p28$^{-/-}$ (lacking IL-27 only) mice or control mice, EBI3$^{-/-}$ (lacking both IL-27 and IL-35) mice were more susceptible to spontaneous colitis, and their survival times were markedly shorter. In IL-35-treated experimental colitis mice, the mucosal levels of IFN-γ, IL-6, IL-17, and messenger RNA expression of T-bet and ROR-γt (transcription factors of Th1 and Th17, respectively) were dramatically restrained [60]. Li *et al.* [28] illustrated that compared with healthy controls, the serum levels of IL-35 were significantly declined in active UC patients and active CD patients. Moreover, there was a negative correlation between serum IL-35 levels and Mayo score in UC patients. Photomicrographs of immunostaining for IL-35 showed that IL-35 was expressed in infiltrating immune cells from UC patients and CD patients, whereas normal colon tissue from healthy controls had no IL-35 production [28]. There was a significant overexpression of Ebi3 and p35 mRNA in endoscopic specimens in both UC patients and CD patients. Additionally, a significant increase of IL-35 was also observed in inflamed mucosa of UC and CD patients [28]. Fonseca-Camarillo *et al.* [14] reported that the levels of IL-35 (EBI3) mRNA of colonic mucosa in active UC patients were significantly higher compared with inactive UC patients. And the percentage of CD20^{+}/IL-35^{+} B cells was higher in active CD patients compared with non-inflamed colonic tissue [14]. These evidences from both animal models and human studies give a new insight that IL-35 might exert an essential role in the pathogenesis and development of IBD.

Idiopathic Thrombocytopenic Purpura

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease characterized by bleeding disorder. Its pathogenesis involves both abnormal platelet destruction and impaired platelet propagation. Previous studies revealed that autoantibodies and cytotoxic T cells are implicated in the pathogenesis of ITP [43, 52]. Recent studies illuminated that inefficient production and functional defect of Treg cells were connected with the breakdown of immunologic tolerance in ITP patients [2, 31, 51, 65]. As a key immunosuppressive cytokine of Treg cells, the plasma IL-35 levels displayed a significantly positive correlation with platelet counts in active ITP patients, whereas the plasma levels of other immunosuppressive cytokines produced by Treg cells, such as TGF-β and IL-10, had no correlation with platelet counts in ITP patients [62]. Furthermore, compared with inactive ITP patients or healthy controls, the plasma levels of IL-35 in active ITP patients were significantly reduced. Similarly, the mRNA expression levels of P35 of peripheral blood mononuclear cells (PBMCs) in active ITP patients were reduced compared with inactive ITP patients or healthy controls [62]. Decreased IL-35 levels and its associations with platelet counts in ITP patients indicate that IL-35 may be involved in the development and pathogenesis of ITP.

IL-35 SERVES AS A POTENTIAL THERAPEUTIC AGENT FOR AUTOIMMUNE DISEASES

Due to its immunosuppressive roles in inflammation and autoimmunity, IL-35 may have promise as a potential therapeutic agent for autoimmune diseases. Understanding the specific mechanisms of IL-35 in autoimmune diseases, together with the knowledge on the capacity of current treatment strategy to target this process, may open a door to novel therapeutic options for autoimmune diseases. In fact, studies in animal models of several autoimmune diseases have yielded encouraging results. Upon IL-35 treatment, significant remissions of histopathology characteristics of lupus flare and nephritis were observed in MRL/lpr mice [5]; alleviations in synovial hyperplasia, cartilage and bone erosion, and fibroblast-like synoviocytes growth were seen in CIA mice [29, 39]; and mucosal levels of proinflammatory cytokines in colitis mice, such as IFN-γ, IL-6, and IL-17 were significantly decreased [60]. These evidences illuminated that IL-35 may serve as a promising therapeutic agent for autoimmune diseases.

THE PROSPECTIVE AND THE LIMITATION OF IL-35 IN CLINICAL APPLICATION

Since its suppressive activity in autoimmune diseases has been established in numerous reports *in vitro* and *in vivo*, IL-35 may act as a therapeutic agent for these diseases in clinical application. More recently, studies have explored the application of IL-35 in human and animal models. Kochetkova *et al.* reported that oral *Escherichia coli* colonization factor antigen I fimbriae can stimulate IL-35 and attenuate the inflammation and joint destruction in CIA mice by suppressing Th1 and Th17 cell responses [21]. Mesenchymal stem cells (MSCs) that can differentiate into cells of different lineages and possess the potent function of immune regulatory have recently emerged as promising cellular vehicles for potential clinical applications [46, 48]. In a study by Amari *et al.*, human Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs) were isolated and transduced with lentiviral particles harboring murine IL-35; the cells successfully secreted a high level of murine IL-35, which managed to inhibit $CD4^+$ T cell proliferation, and enhanced the frequency of Treg cells [1]. Li *et al.* reported that adenovirus-mediated delivery of IL-35 gene can alleviate allergic airway inflammation in experimental asthma, and the adenovirus expressing IL-35 elevated the numbers of $CD4^+CD25^+Foxp3^+$ Treg cells [27]. These results suggest that overexpressing IL-35 provides a useful approach for basic research on therapy for autoimmune disorders, which may be translated into clinical application.

Despite emerging evidence that IL-35 exerts a remarkable role in autoimmune diseases and the application of IL-35 in these diseases has already made significant progress, it is still too early to ensure the efficacy of this cytokine in clinical application. Most studies were based on animal experiments, and the specific mechanisms of these diseases were not involved, which may not be applicable to humans. Furthermore, the clinical features of autoimmune diseases are not equal with each other, and the causes of these diseases are miscellaneous, only as these take all the possible influencing factors into consideration, and can IL-35 exert as an efficient and safe therapeutic agent for autoimmune diseases.

CONCLUSION

Although much remains to be elucidated concerning the role of IL-35 in the etiology and pathogenesis of autoimmune diseases, a solid basis of data from *in vitro* and *in vivo* is now accumulating to support the therapeutic effect of IL-35 in autoimmune diseases. IL-35 promotes the propagation and suppressive function of Treg cells and restricts the differentiation and function of Th17 cells; IL-35 treatment significantly alleviates the histopathology characteristics and the plasma concentrations of proinflammatory cytokines in SLE model. Serum IL-35 level is inversely associated with disease activity of RA patients. Treg cell frequency is correlated with clinical phenotypes and clinical progression parameters in SSc patients. Plasma levels of IL-35 are negatively correlated with disease activity of IBD patients and positively correlated with platelet counts in active ITP patients. Given the correlations of IL-35 with clinical and histological markers of inflammation, promoter of IL-35 therefore might be useful for attenuating inflammation-related symptoms in autoimmune diseases. However, interventions for promoting IL-35 should be considered not only the advantageous effects but also the risk of potential deleterious consequences for the host. Furthermore, the causes of autoimmune diseases are miscellaneous, and several studies were based on *in vitro* experiments, which just elucidated typical symptoms of these diseases and the specific mechanisms of those diseases that were not involved. Therefore, further studies, especially in human systems, are required to clearly explore the immunosuppressive role and therapeutic benefits of IL-35 in autoimmune diseases.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

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