Interleukin-35 in autoimmune dermatoses: Current concepts

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Abstract: Interleukin-35 (IL-35) is a lately observed cytokine and is part of the IL-12 cytokine family. IL-35 includes two subunits, p35 and Epstein-Barr virus-induced gene 3, and activates subsequent signaling pathways by binding to receptors to mediate signal transduction, thereby modulating the immune regulatory functions of T cells, B cells, macrophages, and other immune cell types. Although there is currently limited research on the roles of IL-35 in human autoimmunity, many studies have demonstrated that IL-35 may mediate immunosuppression. Therefore, it plays an essential role in some autoimmune dermatoses, including systemic lupus erythematosus, psoriasis, systemic sclerosis, and dermatomyositis. We will introduce the structure and biological characteristics of IL-35 and summarize its effects on the occurrence and development of autoimmune dermatoses in this article. It is suggested that IL-35 is a possible target for therapy in the aforementioned diseases.

Keywords: dermatomyositis, interleukin-35, psoriasis, systemic lupus erythematosus, systemic sclerosis

1 Introduction

As described recently, interleukin-35 (IL-35) is a new member of the IL-12 cytokine family, which also contains IL-12, IL-23, and IL-27 [1,2]. The members of the IL-12 cytokine family are all heterodimers made up of an α-subunit and a β-subunit. IL-12 consists of p40 and p35 subunits, IL-23 consists of p40 and p19, while IL-27 is made up of p28 and Epstein-Barr virus-induced gene 3 (Ebi3) [1,3,4]. It is reported that p35 and Ebi3 form IL-35 [1,2]. The two subunits of IL-35 can both regulate the immune activities independently, while p35 is the main subunit of IL-35 that plays an immunological role, and Ebi3 can form a heterodimer with p35 to enhance its function [5]. Mouse IL-35 can be expressed by non-stimulated CD4+Foxp3+ regulatory T cells (Tregs) [1]. However, human IL-35 is undetectable in non-stimulated Tregs but can be produced by Tregs after activation [6–9]. IL-35 is inducible in regulatory B cells (Bregs) [10], tolerogenic dendritic cells [11], and placental trophoblast cells [12].

IL-35 receptors are heterodimers or homodimers that are composed of IL-12Rβ2, gp130, or IL-27Ra, including two heterodimers, IL-12Rβ2-gp130 and IL-12Rβ2-IL-27Ra, and two homodimers, IL-12Rβ2–IL-12Rβ2 and gp130–gp130 [13,14]. Among these subunits, IL-12Rβ2 is mainly expressed by activated natural killer cells and T cells [15,16]. Gp130 is expressed by most immune cells [17], and IL-27Ra is primarily expressed by activated CD8+ T cells, CD4+ T cells, and monocytes [18]. Once IL-35 binds to the corresponding receptors, signal transduction begins, and signal transducer and activator of transcription (STAT) family members and Janus kinase (JAK) family members are activated. In T cells, its signal transduction involves three receptors, including IL-12Rβ2-gp130, IL-12Rβ2–IL-12Rβ2, and gp130–gp130, and the process is mainly accomplished by activating STAT1 and STAT4 [13]. The two homodimeric receptors can only suppress T cell proliferation, while the IL-12Rβ2–gp130 heterodimeric receptor is able to reduce T cell multiplication and mediate the induction of a potent Treg subset, IL-35-induced regulatory T cells (iTr35) [13]. In Bregs, recombinant IL-35 activates...
STAT1 and STAT3 pathways by binding with IL-12Rβ2-IL-27Ra, which can produce two Breg subsets that secrete IL-35 (IL-35+ Bregs) and IL-10 (IL-10+ Bregs) (Table 1) [14,19]. These results show that IL-35 is capable of binding to different receptors in different cell types.

2 Biological functions of IL-35 in autoimmunity

IL-35 is significant in the progression of inflammation and immune reactions, and IL-35 can mediate immunosuppressive and immunoregulatory functions.

In mice, IL-35 expressed by T cells can reduce effector T cell (Teff) multiplication [1]. Furthermore, mouse recombinant IL-35, a functional heterodimer genetically engineered by combining p35 and Ebi3, can strengthen the inhibitory effect of CD8+CTLA-4 Tregs on the propagation of autologous T cells [20]. IL-35 inhibits CD8+ T cells by influencing cellular immunosuppressive regulation and external regulatory protein stimulation [21]. It has been reported that mesenchymal stem cells, transfected with lentivirus carrying IL-35, stimulate CD4+CD25+ Tregs proliferation and inhibit CD4+ T cell propagation. And in the supernatant of the coculture system containing these three cell subpopulations, the levels of IL-10 and IL-35 increased, while the secretion of IL-17 decreased compared to control groups with non-transfected mesenchymal stem cells or phosphate buffer saline (PBS) [22]. IL-35 inhibits the activation and differentiation of IL-17A+ T helper (Th17) cells [23,24]. To investigate the effect of IL-35 on Th17-related transcription factors, a study found that Th17-related transcription factors T-bet and retinoic acid receptor-associated orphan receptor (ROR)y T were significantly inhibited in mice treated with IL-35 [25]. Another study in PBMC showed that recombinant IL-35 regulates Th17 cell differentiation by inhibiting RORα and RORγ T transcription factors, and inhibits IL-17 mRNA transcription, thereby reducing IL-17 secretion [26].

Inducible costimulator-positive Tregs are able to produce IL-35 to suppress IL-17 production [23]. Tregs are in a position to reduce the propagation of Th17 cells; moreover, IL-35 strengthens the inhibition of Tregs [27]. Additionally, it was observed that IL-35 can maintain the Treg phenotype and inhibit Th17 cell differentiation [24] (Figure 1a). These findings suggest that IL-35 plays an important role in regulating the balance between Tregs and Th17 cells.

As previously mentioned, IL-35 is capable of inducing iTreg generation, which produces IL-35 in both humans and mice [28]. Induced regulatory T cells are different from thymus-derived natural Treg cells in that they are produced by IL-10 or transforming growth factor-beta (TGF-β) induction [29,30]. However, iTreg cells mediate immunological suppression only through

**Table 1:** The expression, signaling pathways, and functions of IL-35

| Position | Receptor | STATs       | Function                        |
|----------|----------|-------------|---------------------------------|
| T cells  | gp130–gp130 | STAT1       | Suppressing T cell proliferation|
|          | IL-12Rβ2–IL-12Rβ2 | STAT4       | Suppressing T cell proliferation|
|          | IL-12Rβ2–gp130 | STAT1       | Suppressing T cell proliferation|
|          |           | STAT1, STAT4 | Inducing iTreg generation       |
| B cells  | IL-12Rβ2–IL-27Ra | STAT1, STAT3| Promoting conversion of IL-10+ Bregs and IL-35+ Bregs |
IL-35, rather than by proven cytokines, including TGF-β and IL-10 [28]. Unlike the currently known TGF-β-induced regulatory T cells, which express Foxp3, iTreg cells do not express Foxp3 [28,31]. Furthermore, iTreg cells and IL-35 generate a positive feedback loop in which IL-35 induces iTreg cell proliferation, and the produced iTreg cells produce additional IL-35 [28] (Figure 1a).

IL-35 also mediates the immunoregulatory function of Bregs. Recombinant IL-35 induces Bregs and promotes their conversion to IL-10+ Bregs and IL-35+ Bregs. As mentioned above, this process is accomplished by activating STAT1/STAT3 pathways through the IL-35 receptor, made up of IL-12Rβ2 and IL-27Rα [14,19]. Meanwhile, IL-10+ Bregs downregulate the propagation of CD19+ B cells, and IL-35+ Bregs show a suppressive function of the expansion of B220hi cells [14] (Figure 1b). Tonsil-derived mesenchymal stem cells from humans increase the number of IL-10-producing Bregs through Ebi3, thus reducing the immune response mediated by B cells in mice [32].

IL-35 does not directly affect the viability of human mast cell line cells, but significantly inhibits the viability of human mast cell line cells stimulated by phorbol 12-myristate 13-acetate and A23187 (calcium ionophore) [33]. In addition, IL-35 suppresses histamine release, IL-6 and IL-17 mRNA expression, and mitogen-activated protein kinase (MAPK) phosphorylation in human mast cell line cells stimulated by phorbol 12-myristate 13-acetate and A23187 [33]. All these facts show that IL-35 mediates significant functions in immunological regulation.

3 Roles of IL-35 in autoimmune dermatoses

3.1 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease with multiorgan and multisystem involvement, such as skin, kidney, and vascular dysfunctions. The imbalance of CD8+ T cells and CD4+ T cells in SLE patients results in continued B cell activation to produce various types of autoantibodies and result in the persistence of autoimmunity [34–36]. A study found that in Murphy Roths Large (MRL)/lpr mice, a spontaneous lupus-like disease model, the reduction of IL-10+ Breg cells and serum IL-10 were accompanied by reduced IL-35, prompting that IL-35 may be involved in the regulatory function of Breg cells from lupus [37]. The immune imbalance between Th17 cells and Tregs leads to the destruction of immune homeostasis, which is closely correlated with the development of SLE [38–40]. Therefore, it is speculated that IL-35 may help restore the immune balance of SLE patients by limiting the functions of Th17 cells and CD4+ T cells and promoting Treg proliferation. Research on juvenile SLE has shown that leukocyte-associated-immunoglobulin-like receptor 1 (LAIR1), one of the observably downregulated differential expression proteins in juvenile SLE patients, binds to the Src homologue 2 domain of protein tyrosine phosphatase non-receptor type 11 (PTPN11) through its two cytoplasmic tyrosine inhibitory motifs, and has a potential inhibitory effect on lymphocytes and leads to dephosphorylation of subsequent kinases. IL-35 leads to the inhibition of the JAK/STAT and MAPK signaling pathways by increasing LAIR1 levels. Then, it adjusts the LAIR1-PTPN11-JAK-STAT-fibroectin 1 interaction network, and ultimately, may mitigate juvenile SLE nephritis [41].

A study by Cai et al. in female MRL/lpr mice showed that in comparison with PBS-treated mice, nephritis and lupus diseases in IL-35-treated mice were obviously remisive [42]. The mRNA levels of Foxp3, IL-35 subunits (p35 and Ebi3), free subunits of IL-35 receptors (IL-12Rβ2 and gp130) from splenic and thymic cells were distinctly elevated in MRL/lpr mice after IL-35 therapy in comparison with PBS-treated MRL/lpr mice [42]. Subsequently, plasma concentrations of gp130 and IL-35 of MRL/lpr mice with mild disease, IL-12Rβ2 and gp130 of MRL/lpr mice with moderate disease, and IL-12Rβ2 of MRL/lpr mice with severe disease after IL-35 therapy were found to be elevated relative to those of PBS-treated MRL/lpr mice [42]. CD4+CD25+Foxp3+ Treg cells were upgraded in the spleen, thymus, and peripheral blood of lupus mice treated with IL-35, so that the CD4+CD25+Foxp3+ Treg/CD4+CD25− Teff ratio was obviously upregulated in all groups after IL-35 therapy, and the proportion of IL-10+ Bregs obviously increased with IL-35 treatment [42]. The percentage and absolute number of Tregs in the spleen, thymus, and peripheral blood of lupus mice treated with IL-35 overexpression plasmid were higher [41]. Concentrations of pro-inflammatory cytokines in plasma containing IL-17A, IL-6, interferon-gamma (IFN-γ), and tumour necrosis factor-alpha (TNF-α) were significantly decreased, and IL-10, an anti-inflammatory cytokine, was significantly increased in plasma of IL-35-treated mice [42]. It was also shown that SLE-related plasma antibodies (antineutel antibody and anti-double-stranded DNA antibody) concentrations were significantly reduced, demonstrating that IL-35 probably mediates the suppression of SLE through the immunoregulatory functions of Tregs and
Bregs [42]. A separated peripheral blood mononuclear cell (PBMC) culture experiment revealed lower IL-35 levels in active SLE patients relative to healthy controls (HCs) [43]. IL-35 levels in the serum of active SLE patients were lower than those of non-active SLE patients, and a negative relationship between SLE Disease Activity Index (SLEDAI)-2k scores and serum IL-35 levels was observed [44,45]. The proportion of CD4+ T cells expressing EB13 in peripheral blood of patients with active SLE was lower than that of HCs and inactive SLE patients and was negatively correlated with the SLEDAI score [45]. These results may indicate that IL-35 and Eb13-expressing CD4+ T cells may play a protective part in the development of SLE, and their IL-35 levels may be used as a measure of SLE activity. IL-35 levels in the serum of SLE patients with lupus nephritis were obviously lower than those of SLE patients without lupus nephritis, and IL-35 might be a possible biomarker for kidney damage related to SLE [44]. IL-35 levels in the plasma of newly diagnosed SLE patients were significantly decreased compared with HCs [46]. The decrease of IL-35 quantity in patients with newly diagnosed SLE probably occurs since the plasma concentrations of IL-35 and the number of circulating IL-35+ Bregs are both decreased [46]. The expression levels of gp130 on CD4+ helper T (Th) cell surface of patients with severe SLE were lower and were negatively related to SLEDAI, and the elevation of soluble forms of gp130 may be related to the reduced expression of gp130 on CD4+ T cell surface in patients with SLE [47]. The amounts of CD4+CD25+ Tregs were in smaller quantities in moderate and severe SLE patients in comparison with HCs [47]. Data in this research revealed that the proliferation of Tregs is related to the gp130 expression level on the surface of CD4+ Th cells, which may suggest that the decreased gp130 expression on CD4+ Th cells was associated with the downregulated population of Tregs [47].

However, contrary to the studies mentioned above, some researchers reported that IL-35 levels in serum of active SLE patients were higher than those in HCs [48,49]. Qiu et al. observed that higher levels of IL-35 in serum of active SLE patients were reduced after treatment with large doses of prednisone [48]. Therefore, we hypothesized that the reason for the difference in the serum IL-35 concentration compared with the aforementioned studies might partly be related to the fact that active SLE patients in those studies had been treated with glucocorticoids. IL-35 levels in serum were also found obviously increased in newly diagnosed SLE patients in comparison with HCs [50]. In another study, soluble gp130 and IL-35 levels in plasma were increased in newly diagnosed severe SLE patients in comparison with HCs, and Eb13 and p35 mRNA levels in PBMCs of severe SLE patients were also increased [47]. It is shown that the mRNA level of Eb13 and p35 in B cells had a considerable increasing trend in the SLE patients compared with HCs [50]. A study that examined subsets of CD3+CD4+, CD3+CD4+, CD3+CD4- lymphocytes in patients with SLE found no significant differences in the levels of IL-12Rβ2 and gp130 on the surface of the subsets studied [49]. However, the study did not distinguish between living and dead cells, nor did it distinguish Treg independently. The possible reasons for these different results may be that different samples, including PBMCs, plasma, and serum, were used, and only Chinese patients were studied [51]. Therefore, future experiments with larger sample size and experiments in other ethnic groups are needed for verification.

## 3.2 Roles of IL-35 in psoriasis

Psoriasis is a chronic disease featuring inflammation. Its characteristics include abnormal infiltration, activation of T cells, and excessive propagation of keratinocytes. The IL-23/IL-17 axis and Th17 cells are both significant in the pathological process of psoriasis [52–54]. The IL-35 levels in the serum of psoriasis vulgaris patients before treatment were prominently lower than those in HCs, but the IL-35 levels in serum were obviously upregulated with routine treatment [55]. Plasma IL-35 levels were lower in psoriasis patients in comparison with HCs [56,57]. The level of IL-35 in psoriatic skin biopsies was lower than that in the surrounding skin and normal controls [58]. The study of plaque psoriasis found that the skin lesion of plaque psoriasis patients gradually recovered after treatment with adalimumab; however, the plasma IL-35 level was lower in the adalimumab group [57]. The possible reason for the contrary result is that the observation time is short and the skin lesions in patients with adalimumab treatment are more severe. Expression levels of plasma IL-35 were negatively correlated with IL-17, IL-22, IL-23, TNF-α, and IFN-γ [56]. Meanwhile, its expression was positively related to the levels of IL-10 and TGF-β in psoriasis patients [56]. It is suggested that IL-35 may influence the pathogenesis of psoriasis by regulating the generation of cytokines related to Th17 cells or Tregs.

Similar results were seen in keratin 14-vascular endothelial growth factor A (VEGF-A)-transgenic mouse models. Compared with the control group, which was transfected with the pcDNA3.1 vector, the IL-6 and IL-17A levels in ear tissues and serum supernatant were
lower, there were fewer IL-17-secreting CD4+ T cells of lymph nodes and spleen, and the number of CD4+ T cells that secrete IL-10 in lymph nodes and spleen were increased in mice that were transfected with the plasmid coding human IL-35 (pIL-35) group [59]. The levels of classically inflammatory macrophages (M1) in ear tissues and spleens of the pIL-35 group were decreased, while the level of alternatively anti-inflammatory macrophages (M2) was increased, and the M1/M2 ratio was decreased. M1 and M2 are considered to be linked with the inflammatory response in wound healing [60]. IL-35 induced immunosuppression in keratin 14-VEGF-A-transgenic mice via the reduction of the local infiltration of macrophages and the decrease in the M1/M2 proportion [59]. The treatment of IL-35 improved the redness, scaliness, severe ear thickening and swelling, and other psoriatic lesions in psoriasis mice models [59,61]. Rather, it has been reported that no IL-35 was present in the serum in an experiment conducted in Brazilian psoriasis patients [62]. IL-35 may be a potential target to monitor or treat psoriasis, but more studies are needed to confirm its effects in psoriasis.

3.3 Systemic sclerosis

Systemic sclerosis (SSc), an autoimmune connective tissue disease, develops due to abnormal activation of fibroblasts, which produce excessive collagen, resulting in skin and internal visceral fibrosis.

Macrophages, T cells, B cells, and other immune cells are implicated in SSc [63]. The role of IL-35 in the pathogenesis of SSc is unclear. Results of a study on SSc showed that IL-35 was elevated in patients compared to controls, and IL-35 inhibited CD4+ T lymphocyte proliferation and induced Treg differentiation [64]. IL-35 can inhibit type I collagen expression in normal fibroblasts and SSc dermal fibroblasts, and reduce the stability of collagen mRNA in normal fibroblasts [65]. These effects of IL-35 were realized by subunit Ebi3, while another subunit p35 was found to have no such effect in the study [65]. Skin injection of Ebi3 improved skin fibrosis in SSc mice models, further suggesting that IL-35 may have potential therapeutic effects to improve fibrosis during the pathogenesis of SSc [65].

However, other studies suggested that IL-35 promotes collagen production during the pathogenesis of SSc. According to the injury level of the skin, SSc is classified as either diffuse cutaneous SSc or limited cutaneous SSc [66]. These two kinds of SSc patients had higher IL-35 concentrations in serum than that in HCs, while no differences were found in serum IL-35 production between the two types of patients, and patients with lung fibrosis had higher IL-35 levels relative to those without fibrosis [67]. In another study, there was significant upregulation of p35, Ebi3, and IL-35 in lesioned skin from SSc patients in comparison with HCs at both the protein and the mRNA levels [68]. IL-35 expresses and releases increasingly under stimulation of TGF-β, and IL-35 can induce resting fibroblasts to differentiate into myofibroblasts, thereby increasing the release of collagen protein [68]. It has also been observed that IL-35 levels in the serum of SSc patients had a negative relationship with disease duration, and the results of capillary microscopy showed that early SSc patients have higher serum IL-35 levels in comparison with active or late SSc patients [68]. Likewise, Tang et al. observed that the IL-35 levels in the serum of SSc patients were obviously higher than those of the HCs, but after 3 months of treatment, the IL-35 levels in the serum of SSc patients were reduced primarily [69]. It can be inferred that the elevation of serum IL-35 was correlated with the early skin and pulmonary fibrosis of SSc, results that are opposite to the anti-inflammatory effects of IL-35 in other mentioned diseases. Yayla et al. also discovered higher concentrations of IL-35 in SSc patients’ serum, but they found that it was negatively related to C-reactive protein (CRP), Medsger disease severity score, and modified Rodnan skin score [70]. However, IL-35 levels were not different between the SSc patients with or without lung fibrosis in this study; therefore, more studies need to be performed to verify these results. In a word, IL-35 might be one of the serologic biomarkers indicating the inflammatory status of SSc.

3.4 Dermatomyositis

Dermatomyositis (DM) is an autoimmune disease involving the skin and striated muscle. There are different lymphocyte subsets that accumulate in different regions of the muscle in DM patients [71]. The membrane attack complex is composed of B cells and C5-9 complement bodies. T cells, macrophages, dendritic cells, and B cells are all possibly relevant to the pathogenesis of DM [71]. However, there are still few studies exploring the relationship between DM and IL-35. It has been observed that serum concentrations of IL-35 of DM patients are overexpressed relative to HCs [72–74]. Surprisingly, the results of the research revealed that IL-35 levels in serum were not correlated to peripheral blood lymphocyte subgroup counts in idiopathic inflammatory myopathy.
| Disease | Source | Control group | Sample | Findings | Function | Ref. |
|---------|--------|---------------|--------|----------|----------|------|
| SLE     | MRL/lpr mice | PBS-treated MRL/lpr mice | Peripheral blood, splenic, and thymic cells | Tregs increased in IL-35-treated MRL/lpr mice | Suppress inflammation | [41] |
|         |        |               |        | Nephritis and lupus diseases in IL-35-treated MRL/lpr mice were remissive | | [42] |
|         |        |               |        | Splenic and thymic cells | Foxp3, p35, Ebi3, and free gp130 and IL-12Rβ2 increased in IL-35-treated MRL/lpr mice | [42] |
|         |        |               |        | Plasma | IL-35, gp130, and IL-12Rβ2 increased in IL-35-treated MRL/lpr mice | [42] |
|         |        |               |        | Peripheral blood, splenic, and thymic cells | CD4+CD25+Foxp3+ Tregs and IL-10+ Bregs increased in IL-35-treated MRL/lpr mice | [42] |
|         |        |               |        | Plasma | IFN-γ, TNF-α, IL-6, and IL-17A decreased, and IL-10 increased in IL-35-treated MRL/lpr mice | [42] |
|         |        |               |        | Plasma | Antinuclear antibody and anti-double-stranded DNA antibody decreased in IL-35-treated MRL/lpr mice | [42] |
| Human   | Healthy individuals | PBMC supernatants | IL-35 decreased in active SLE patients | | Suppress inflammation | [43] |
|         |        |               |        | Plasma | IL-35 decreased in newly diagnosed SLE patients | | [46] |
|         |        | PBMCs | IL-35+ B cells decreased in newly diagnosed SLE patients | | Suppress inflammation | [46] |
|         |        | Serum | IL-35 decreased in active SLE patients | | | [44,45] |
|         |        | Serum | IL-35 levels were negatively correlated with SLEDAI-2 k | | | [44,45] |
|         |        | Serum | IL-35 decreased in SLE patients with lupus nephritis | | | [44] |
| Healthy individuals | Serum | IL-35 increased in active SLE patients | Promote inflammation | | [48,49] |
|         |        | Serum | IL-35 increased in newly diagnosed SLE patients | Promote inflammation | | [50] |
|         |        | B cells | P35 and Ebi3 increased in SLE patients | Promote inflammation | | [50] |
|         |        | Plasma | IL-35 and soluble gp130 increased in severe SLE patients | Promote inflammation | | [47] |
|         |        | PBMCs | P35 and Ebi3 increased in severe SLE patients | Promote inflammation | | [47] |
|         |        | Th cells | Gp130 decreased in severe SLE patients | | | [47] |
|         |        | Th cells | Gp130 levels were negatively correlated with SLEDAI | | | [47] |
|         |        | PBMCs | CD4+CD25+ Tregs decreased in severe and moderate SLE patients | | | [47] |
| Psoriasis | Human | Healthy individuals | Serum | IL-35 decreased in patients with psoriasis vulgaris | Suppress inflammation | [55,56] |

(Continued)
| Disease | Source | Control group | Sample | Findings | Function | Ref. |
|---------|--------|---------------|--------|----------|----------|------|
| SS, Human | Healthy individuals | Serum | IL-35 increased in SSc patients, inhibited CD4+ T lymphocyte proliferation, and induced Treg differentiation | Suppress inflammation | [64] |
| SS, Human | Healthy individuals | Serum | IL-35 increased in SSc patients | Promote inflammation | [67–70] |
| SS, Human | Healthy individuals | Serum | IL-35 levels were negatively correlated with disease duration, modified Rodnan skin score, Medsger disease severity score, and CRP | | [68,70] |
| SS, Human | Healthy individuals | Skin | IL-35 increased in SSc patients, and upregulated differentiation of myofibroblasts | | [68] |
| SSc mice model | Balb/c mice injected with PBS | Skin | Skin fibrosis was improved in mice skin injected with Ebi3 | Suppress inflammation | [65] |
| DM Human | Healthy individuals | Serum | IL-35 increased in DM patients | Suppress inflammation | [72–74] |
| DM Human | Healthy individuals | Serum | IL-35 levels were negatively correlated with disease duration | | [72,74] |
| DM Human | Healthy individuals | Serum | IL-35 levels were positively correlated with ESR, CRP, visual analogue scale, creatine kinase, and LDH | | [73] |
| Recurrent patients | Serum | IL-35 increased in untreated DM patients | | [73] |
| Patients in remission | Serum | IL-35 increased in active DM patients | | [73] |
| Healthy individuals | PBMCs | IL-35 increased in PBMCs stimulated by lipopolysaccharide from DM patients | | [73] |

SLE – systemic lupus erythematosus, SSc – systemic sclerosis, PBMC – peripheral blood mononuclear cell, SLEDAI-2k – systemic lupus erythematosus disease activity index-2k, pIL-35 – plasmid coding human IL-35 sequence, CRP – C-reactive protein, DM – dermatomyositis, ESR – erythrocyte sedimentation rate, LDH – lactate dehydrogenase.
patients [72]. The results of previous experiments showed that the populations of Tregs in the peripheral blood of DM patients were decreased in comparison to HCs [75,76]. There were also decreases in populations of CD3+ cells, CD3+CD8+ cells, and CD3+CD4+ cells in active DM patients compared with patients with inactive DM and HCs [77]. These might mean that the source of IL-35 may come from non-T cell sources but not peripheral blood T cells.

Higher serum expression of IL-35 was found in active DM patients than in remissive individuals [73]. It is observed that serum levels of IL-35 were the highest in DM patients whose disease durations were shorter than 6 months, while the lowest serum frequency of IL-35 was in DM patients with disease duration longer than 12 months compared with HCs. Moreover, the IL-35 frequency in the serum of untreated patients was also higher than in those who relapsed [73]. Additionally, the serum concentrations of IL-35 in idiopathic inflammatory myopathy patients were negatively correlated with the disease course [72,74]. Serum IL-35 quantity had a positive relationship with erythrocyte sedimentation rate (ESR), CRP, lactate dehydrogenase (LDH), creatine kinase, and visual analogue scale [73]. As these measures are correlated with disease activity, and creatine kinase and LDH are the indicators to estimate muscle damage, therefore, serum IL-35 may be a potential biomarker to assess disease activity or muscle damage of DM. Exogenous human recombinant IL-35 downregulated IL-17 and TNF-α production in PBMCs stimulated by lipopolysaccharide from DM patients compared with HCs [73]. These findings suggest that IL-35 may have the effect of immune suppression on DM. However, the possibility that elevated IL-35 probably mediates a pro-inflammatory role in DM patients cannot yet be ruled out. The function of IL-35 in DM pathogenesis still needs to be explored in the future. The experimental results of the above-mentioned diseases in each article are summarized in Table 2.

4 Conclusions

IL-35 acts as a significant inhibitory cytokine in the immunity system, modulating dysfunctional B cells and T cells and regulating various immune-related inflammatory factors. For this reason, IL-35 is important in autoimmune dermatosis. In SLE and psoriasis, a large amount of evidence supports a protective immunosuppressive effect of IL-35; however, it may promote fibrosis, which may suggest the development of inflammation in SSc. IL-35 might play an immunosuppressive role in DM, but this hypothesis remains to be verified. Therefore, its role in immune regulation may vary greatly in different autoimmune diseases, and more studies are needed in the future to elucidate its function of the occurrence and development of different diseases. Although IL-35 plays different roles in the immune regulation of different diseases, it can still be regarded as a treatment target for autoimmune diseases. If IL-35 expression is decreased in a disease, recombinant IL-35 can be used for treatment. If IL-35 expression is significantly upregulated in some diseases, treatment can be performed by reducing the expression of IL-35. With further research on IL-35 in the future, IL-35 is expected to be a new therapeutic target for autoimmune diseases.

**Abbreviation list**

| Abbreviation | Description |
|--------------|-------------|
| Breg | regulatory B cell |
| CRP | C-reactive protein |
| DM | dermatomyositis |
| Ebp3 | Epstein-Barr virus-induced gene 3 |
| ESR | erythrocyte sedimentation rate |
| HC | healthy control |
| IFN-γ | interferon-gamma |
| IL-10 Breg | regulatory B cells secreting IL-10 |
| IL-35 Breg | regulatory B cells secreting IL-35 |
| IL-10 | interleukin-35 |
| iTril | IL-35-induced regulatory T cells |
| JAK | Janus kinase |
| LAIR1 | leukocyte-associated-immunglobulin-like receptor 1 |
| LDH | lactate dehydrogenase |
| M1 | inflammatory macrophages |
| M2 | anti-inflammatory macrophages |
| MAPK | mitogen-activated protein kinase |
| MRL | Murphy Roths Large |
| PBMC | separated peripheral blood mononuclear cell |
| PBS | phosphate buffer saline |
| pIL-35 | plasmid coding human IL-35 |
| PTPN11 | protein tyrosine phosphatase non-receptor type 11 |
| ROR | receptor-related orphan receptor |
| SLE | systemic lupus erythematosus |
| SLEDAI | systemic lupus erythematosus disease activity index |
SSc  systemic sclerosis
STAT  signal transducer and activator of transcription
Teff  effector T cell
TGF-β  transforming growth factor beta
Th cell  T helper cell
TNF-α  tumour necrosis factor-alpha
Treg  regulatory T cell
VEGF  vascular endothelial growth factor

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References

[1] Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature. 2007;450(7169):566–9. doi: 10.1038/nature06306.

[2] Devergne O, Birkenbach M, Kieff E. Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. Proc Natl Acad Sci U S A. 1997;94(22):12041–6. doi: 10.1073/pnas.94.22.12041.

[3] Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, et al. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J Exp Med. 1989;170(3):827–45. doi: 10.1084/jem.170.3.827.

[4] Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000;13(5):715–25. doi: 10.1016/s1074-7613(00)00070-4.

[5] Dambuza IM, He C, Choi JK, Yu CR, Wang R, Mattapallil MJ, et al. IL-12p35 induces expansion of IL-10 and IL-35-expressing regulatory B cells and ameliorates autoimmune disease. Nat Commun. 2017;8(1):719. doi: 10.1038/s41467-017-00388-4.

[6] Bardel E, Larousserie F, Charlot-Rabiega F, Coulomb-L’Hermine A, Devergne O. Human CD4 + CD25 + Foxp3 + regulatory T cells do not constitutively express IL-35. J Immunol. 2008;181(10):6898–905. doi: 10.4049/jimmunol.181.10.6898.

[7] Collison LW, Pillar MR, Chaturvedi V, Vignali DA. Regulatory T cell suppression is potentiated by target T cells in a cell contact, IL-35- and IL-10-dependent manner. J Immunol. 2009;182(10):6121–8. doi: 10.4049/jimmunol.20083646.

[8] Gutteck K, Reinhold D. Stimulated human peripheral T cells produce high amounts of IL-35 protein in a proliferation-dependent manner. Cytokine. 2013;64(1):46–50. doi: 10.1016/j.cyto.2013.04.037.

[9] Allan SE, Song-Zhao GX, Abraham T, McMurchy AN, Levings MK. Inducible reprogramming of human T cells into Treg cells by a conditionally active form of FOXP3. Eur J Immunol. 2008;38(12):3282–9. doi: 10.1002/eji.200838373.

[10] Shen P, Rocchi T, Lambropoulou V, O’Connor RA, Stervbo U, Hilgenberg E, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. Nature. 2014;507(7492):366–70. doi: 10.1038/nature12979.

[11] Dixon KO, van der Kooij SW, Vignali DA, van Kooten C. Human tolerogenic dendritic cells produce IL-35 in the absence of other IL-12 family members. Eur J Immunol. 2015;45(6):1736–47. doi: 10.1002/eji.201445217.

[12] Mao H, Gao W, Ma C, Sun J, Liu J, Shao Q, et al. Human placental trophoblasts express the immunosuppressive cytokine IL-35. Hum Immunol. 2013;74(7):872–7. doi: 10.1016/j.humimm.2013.04.010.

[13] Collison LW, Delgoffe GM, Guy CS, Vignali KM, Chaturvedi V, Fairweather D, et al. The composition and signaling of the IL-35 receptor are unconventional. Nat Immunol. 2012;13(3):290–9. doi: 10.1038/ni.2227.

[14] Wang RX, Yu CR, Dambuza IM, Mahdi RM, Dolinska MB, Sergeev YV, et al. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. Nat Med. 2014;20(6):633–41. doi: 10.1038/nm.3554.

[15] Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. J Exp Med. 1997;185(5):817–24. doi: 10.1084/jem.185.5.817.

[16] Perrett D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G. Differentiation of human NK cells into NK1 and NK2 subsets. J Immunol. 1998;161(11):5821–4. https://www.ncbi.nlm.nih.gov/pubmed/9834059.

[17] Saltow Yoshida K, Hibi M, Taka T, Kishimoto T. Molecular cloning of a murine IL-6 receptor-associated signal transducer, gp330, and its regulated expression in vivo. J Immunol. 1992;148(12):4066–71. https://www.ncbi.nlm.nih.gov/pubmed/1602163.

[18] Dietrich C, Candon S, Ruemmele FM, Devergne O. A soluble form of IL-27Ralpha is a natural IL-27 antagonist. J Immunol. 2014;192(11):5382–9. doi: 10.4049/jimmunol.1303435.

[19] Liu J, Chen X, Hao S, Zhao H, Pang L, Wang L, et al. Human chorionic gonadotropin and IL-35 contribute to the
maintenance of peripheral immune tolerance during pregnancy through mediating the generation of IL-10(+) or IL-35(+) Breg cells. Exp Cell Res. 2019;383(2):111513. doi: 10.1016/j.yexcr.2019.111513.

[20] Olson BM, Jankowska-Gan E, Becker JT, Vignali DA, Burlingham WJ, McNeel DG. Human prostate tumor antigen-specific CD8+ regulatory T cells are inhibited by CTLA-4 or IL-35 blockade. J Immunol. 2012;189(12):5590–601. doi: 10.4049/jimmunol.1201744.

[21] Li X, Dong Y, Tu K, Wang W. Proteomics analysis reveals the interleukin-35-dependent regulatory mechanisms affecting CD8(+)-T cell functions. Cell Immunol. 2020;348:104022. doi: 10.1016/j.cellimm.2019.104022.

[22] Guo H, Zhao N, Gao H, He X. Mesenchymal stem cells overexpressing interleukin-35 propagate immunosuppressive effects in mice. Scand J Immunol. 2017;86(5):389–95. doi: 10.1111/sj.12613.

[23] Whitehead GS, Wilson RH, Nakano K, Burch LH, Nakano H, Cook DN. IL-35 production by inducible costimulator (ICOS)-positive regulatory T cells reverses established IL-17-dependent allergic airways disease. J Allergy Clin Immunol. 2012;129(1):207–15. e1–5 doi: 10.1016/j.jaci.2011.08.009.

[24] Singh K, Kadesjo E, Lindroos J, Hjort M, Lundberg M, Espevik T, et al. Interleukin-35 administration counteracts established murine type 1 diabetes–possible involvement of regulatory T cells. Sci Rep. 2015;5:12633. doi: 10.1038/srep12633.

[25] Wirtz S, Billmeier U, McHedlidze T, Blumberg RS, et al. Establishment IL-35 costimulator (ICOS) dependence for natural Treg cell generation. J Immunol. 2012;189(12):6001–10. doi: 10.4049/jimmunol.1203459.

[26] Okada K, Fujimura T, Kikuchi T, Aino M, Kamiya Y, Izawa A, Okada K, Fujimura T, Kikuchi T, Aino M, Kamiya Y, Izawa A, Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S, Horwitz DA. Establishing IL-35 costimulator (ICOS) dependence for natural Treg cell generation. J Immunol. 2012;189(12):6001–10. doi: 10.4049/jimmunol.1203459.
[56] Ye Z, Jiang Y, Sun D, Zhong W, Zhao L, Jiang Z. The plasma interleukin (IL)-35 level and frequency of circulating IL-35(+)
regulatory B cells are decreased in a cohort of Chinese patients with new-onset systemic lupus erythematosus. Sci Rep. 2019;9(1):13210. doi: 10.1038/s41598-019-49748-2.

[57] Cai Z, Wang CK, Kam NW, Dong J, Jiao D, Chu M, et al. Abrupt expression of regulatory cytokine IL-35 in patients with systemic lupus erythematosus. Lupus. 2015;24(12):1257–66. doi: 10.1177/096123315585815.

[58] Qiu F, Song L, Yang N, Li X. Glucocorticoid downregulates expression of IL-12 family cytokines in systemic lupus erythematosus patients. Lupus. 2013;22(10):1011–6. doi: 10.1177/096123313498799.

[59] Mohd Shukri ND, Farah Izati A, Wan Ghazali WS, Che Hussin CM, Wong KK. CD3(+)/CD4(+)/gp130(+)/ T cells are associated with worse disease activity in systemic lupus erythematosus patients. Front Immunol. 2021;12:675250. doi: 10.3389/fimmu.2021.675250.

[60] Abbasifar M, Kamibay H, Hasani M, Rahnama A, Saeed-Afsari P, Khorramdelazar H. Assessing the expression of immunosuppressive cytokines in the newly diagnosed systemic lupus erythematosus patients: a focus on B cells. BMC Immunol. 2020;21(1):58. doi: 10.1186/s12865-020-00388-3.

[61] Su LC, Liu XY, Huang AF, Xu WD. Emerging role of IL-35 in autoimmune diseases. Autoimmun Rev. 2018;17(7):665–73. doi: 10.1016/j.autrev.2018.01.017.

[62] Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol. 2008;128(5):1207–11. doi: 10.1038/sj.jid.5502123.

[63] Haider AS, Lowes MA, Suarez-Farinas M, Zaba LC, Cardinale I, K hatcherian A, et al. Identification of cellular pathways of “type 1,” Th17 T cells, and TNF- and inducible nitric oxide synthase-producing dendritic cells in autoimmune inflammation through pharmacogenomic study of cyclosporine A in psoriasis. J Immunol. 2008;180(3):1913–20. doi: 10.4049/jimmunol.180.3.1913.

[64] Lee E, Trecipichio WL, Oestreicher JL, Piltman D, Wang F, Chaman F, et al. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. J Exp Med. 2004;199(1):125–30. doi: 10.1084/jem.20030451.

[65] Chen J, Du J, Han Y, Wei Z. Correlation analysis between interleukin-35, IL-36gamma, CCL27 and psoriasis vulgaris. J Dermatol Treat. 2021;32(6):621–4. doi: 10.1080/09546634.2019.1689226.

[66] Li T, Gu M, Liu P, Liu Y, Guo J, Zhang W, et al. Clinical significance of decreased interleukin-35 expression in patients with psoriasis. Microbiol Immunol. 2018;62(7):454–61. doi: 10.1111/1348-0421.12605.

[67] Zdanowska N, Owczarzyk-Saczonek A, Czerwinska J, Nowakowski J, Kozera-Zywczak A, Owczarek W, et al. Adalimumab and methotrexate affect the concentrations of regulatory cytokines (interleukin-10, transforming growth factor-beta1), and interleukin-35) in patients with plaque psoriasis. Dermatol Ther. 2020;33(6):e14153. doi: 10.1111/dth.14153.

[68] Owczarzyk-Saczonek A, Czerwinska J, Orylska M, Placek W. Evaluation of selected mechanisms of immune tolerance in psoriasis. Postepy Dermatol Alergol. 2019;36(3):319–28. doi: 10.5114/ada.2019.85641.

[69] Zhang J, Lin Y, Li C, Zhang X, Cheng L, Dai L, et al. IL-35 decelerates the inflammatory process by regulating inflammatory cytokine secretion and M1/M2 macrophage ratio in psoriasis. J Immunol. 2016;197(6):2131–44. doi: 10.4049/jimmunol.1600446.

[70] Zheng XF, Hong YX, Feng GJ, Zhang GF, Rogers H, Lewis MA, et al. Lipopolysaccharide-induced M2 to M1 macrophage transformation for IL-12p70 production is blocked by Candida albicans mediated up-regulation of EB13 expression. PLoS One. 2013;8(5):e63967. doi: 10.1371/journal.pone.0063967.

[71] Wang Y, Yao M, Zhang J, Shi G, Cheng L, Lin Y, et al. IL-35 recombinant protein reverses inflammatory bowel disease and psoriasis through regulation of inflammatory cytokines and immune cells. J Cell Mol Med. 2018;22(2):1014–25. doi: 10.1111/jcmm.13428.

[72] Cardoso PR, Lima EV, Lima MM, Rego MJ, Marques CD, Pitta Ida R, et al. Clinical and cytokine profile evaluation in Northeast Brazilian psoriasis plaque-type patients. Eur Cytokine Netw. 2016;27(1):1–5. doi: 10.1684/ecn.2016.0371.

[73] Sakka A, Bogdanos DP. Systemic sclerosis: new evidence re-enforces the role of B cells. Autoimmun Rev. 2016;15(2):155–61. doi: 10.1016/j.autrev.2015.10.005.

[74] Yang C, Lei L, Pan J, Zhao C, Wen J, Qin F, et al. Altered CD4+ T cell and cytokine levels in peripheral blood and skin samples from systemic sclerosis patients and IL35 in CD4 + T cell growth. Rheumatology (Oxford). 2022;61(2):794–805. doi: 10.1093/rheumatology/keab359.

[75] Kudo H, Wang Z, Jinnin M, Nakayama W, Inoue K, Honda N, et al. EB13 downregulation contributes to type I collagen overexpression in scleroderma skin. J Immunol. 2015;195(8):3565–73. doi: 10.4049/jimmunol.1402362.

[76] LeRoy EC, Medsger Jr. TA. Criteria for the classification of early systemic sclerosis. J Rheumatol. 2001;28(7):1573–6. https://www.ncbi.nlm.nih.gov/pubmed/11469464.

[77] Dantas AT, Goncalves SM, Pereira MC, Goncalves RS, Marques CD, Rego MJ, et al. Increased IL-35 serum levels in systemic sclerosis and association with pulmonary interstitial involvement. Clin Rheumatol. 2015;34(9):1621–5. doi: 10.1007/s00296-015-3006-y.

[78] Tomcik M, Zerr P, Palumbo-Zerr K, Storkanova H, Hulejova H, Spirovic M, et al. Interleukin-35 is upregulated in systemic sclerosis and its serum levels are associated with early disease. Rheumatology (Oxford). 2015;54(12):2273–82. doi: 10.1093/rheumatology/kev260.

[79] Tang J, Lei L, Pan J, Zhao C, Wen J. Higher levels of serum interleukin-35 are associated with the severity of pulmonary fibrosis and Th2 responses in patients with systemic sclerosis. Rheumatol Int. 2018;38(8):1511–9. doi: 10.1007/s00296-018-4071-8.

[80] Yaya ME, Torgutalp M, Okatan IE, Yurteri EU, Kucuksahin O, Dincer ABK, et al. Serum interleukin 35 levels in autoimmune dermatoses. Br J Dermatol. 2018;179(6):1256–62. doi: 10.1111/bjd.15607.

[81] Yun L, Ge Y, Yang H, Peng Q, Lu X, Zhang Y, et al. The clinical utility of serum IL-35 in patients with polymyositis and
dermatomyositis. Clin Rheumatol. 2016;35(11):2715–21. doi: 10.1007/s10067-016-3347-1.

[73] Jiang Q, Li Y, Xia L, Shen H, Lu J. Interleukin-35: a serological biomarker for patients with polymyositis/dermatomyositis. J Interferon Cytokine Res. 2019;39(11):720–5. doi: 10.1089/jir.2019.0063.

[74] Mann H, Krystufkova O, Zamecnik J, Hacek J, Hulejova H, Filkova M, et al. Interleukin-35 in idiopathic inflammatory myopathies. Cytokine. 2020;137:155350. doi: 10.1016/j.cyto.2020.155350.

[75] Antiga E, Kretz CC, Klembt R, Massi D, Ruland V, Stumpf C, et al. Characterization of regulatory T cells in patients with dermatomyositis. J Autoimmun. 2010;35(4):342–50. doi: 10.1016/j.jaut.2010.07.006.

[76] Banica L, Besliu A, Pistol G, Stavaru C, Ionescu R, Forsea AM, et al. Quantification and molecular characterization of regulatory T cells in connective tissue diseases. Autoimmunity. 2009;42(1):41–9. doi: 10.1080/08916930802282651.

[77] Wang DX, Lu X, Zu N, Lin B, Wang LY, Shu XM, et al. Clinical significance of peripheral blood lymphocyte subsets in patients with polymyositis and dermatomyositis. Clin Rheumatol. 2012;31(12):1691–7. doi: 10.1007/s10067-012-2075-4.