

The role and mechanism of IL-35 in myasthenia gravis (Review)

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Abstract. Myasthenia gravis (MG) is a chronic autoimmune disorder characterized by impaired neuromuscular junction transmission, leading to fluctuating muscle weakness and fatigue. This condition is driven primarily by autoantibodies targeting the acetylcholine receptor at the neuromuscular junction. These antibodies are predominantly generated through a T-cell-dependent pathway, initiating immunomodulatory responses via complement activation. Cytokines and inflammatory mediators also play pivotal roles in the pathogenesis of MG. Recently, increasing attention has been given to the involvement of cytokines in autoimmune diseases. Interleukin-35 (IL-35), an immunoregulatory cytokine, is critical in various inflammatory and autoimmune conditions. It modulates immune responses by promoting Treg proliferation, enhancing their immunosuppressive functions, inhibiting Th17 cell differentiation, and reducing proinflammatory cytokine levels. IL-35 is thus pivotal in the onset and progression of MG. The present review outlines the key functions of IL-35 in MG pathogenesis and the impact of IL-35 on the treatment and prognosis of myasthenia gravis, explores its therapeutic potential, and assesses its prognostic value, offering insights into its mechanisms and implications for treatment.

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1. Introduction

Myasthenia gravis (MG) is a prototypical autoimmune disorder (1) characterized by fluctuating muscle weakness resulting from autoantibodies targeting acetylcholine receptors (AChRs) on the postsynaptic membrane. Of patients with MG, 40-60% initially present with ocular muscle weakness, classified as ocular MG (OMG), with 50-80% progressing to generalized MG (GMG) within a few years. Epidemiological data report an incidence of 0.3-2.8 cases of MG per 100,000 individuals, with a global prevalence exceeding 0.7 million (2,3). Of patients with MG ~80% have detectable anti-AChR antibodies, while a smaller subset produces antibodies against lipoprotein receptor-related protein 4 (LRP4). Anti-AChR antibodies are typically generated through T-cell- and B-cell-mediated pathogenic mechanisms, which activate the complement system (4,5). Cytokines also play pivotal roles in the abnormal activation of T-cell subsets, contributing to the progression of related autoimmune diseases (6-8). Current MG treatments include symptomatic management, thymectomy (9), plasma exchange and immunosuppressants such as tacrolimus (TAC). Recent therapies targeting various stages of MG progression have demonstrated varying levels of effectiveness (10). However, due to disease heterogeneity, ~15% of patients do not achieve optimal therapeutic outcomes (11,12). Thus, further exploration of biomarkers and a deeper understanding of MG pathogenesis are essential for developing novel and more effective therapies.

Interleukin-35 (IL-35) is an immunosuppressive and anti-inflammatory cytokine that is expressed primarily by regulatory T cells (Tregs). B cells have been reported to secrete IL-35 (13-15). IL-35 belongs to the IL-12 family and is a dimeric protein composed of IL-12p35 and EBI3. Its receptors, which are dimers, signal through the JAK-STAT pathway. Four types of IL-35 receptors have been identified, IL12Rβ2/IL27Rα, gp130/gp130, IL-12β2/IL12Rβ2 and IL-12Rβ2/gp130, with each triggering distinct signaling pathways. IL-35, which

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is released by Tregs and regulatory B cells (Bregs), plays a critical role in suppressing immune-inflammatory responses in autoimmune diseases, affecting a variety of conditions, including those of the digestive, musculoskeletal, and respiratory systems. IL-35 inhibits immune reactions by promoting Treg proliferation, enhancing their immunosuppressive function, suppressing Th17 differentiation, and reducing the levels of proinflammatory cytokines such as IL-17 (16,17). In a mouse model, Xie *et al.* (18) demonstrated that B cells lacking IL-35 expression fail to effectively recover from autoimmune diseases, such as inflammatory bowel disease (IBD). As with IL-10, IL-35 possesses potent immunosuppressive properties and acts as a key mediator among cytokines, increasing IL-10 levels. Additionally, IL-35 can upregulate cytokines involved in MG pathogenesis, including IL-1 β , IL-6, and IL-10, with IL-35 levels notably decreasing after treatment (19,20). However, the exact relationship between IL-35 and MG remains unclear. These findings indicate that IL-35 has anti-inflammatory effects on immune-mediated central nervous system diseases, including MG, and could serve as a potential therapeutic target. Consequently, understanding the role of IL-35 in the progression and treatment of MG is of considerable interest. The present review summarized the effect of IL-35 on MG pathogenesis, its potential therapeutic efficacy and its prognostic value, providing insights into its mechanisms and clinical implications for MG treatment.

2. Overview of IL-35

The following is an overview of the molecular structure and sources of IL-35, the composition and function of IL-35 receptors and the mechanism of IL-35 signal transduction.

Molecular structure and sources of IL-35. IL-35 is a recently identified heterodimeric cytokine critical for the regulatory functions of Tregs. As a member of the IL-12 cytokine family, IL-35 comprises an α chain (p19 or p28) and a β chain [p40 or Epstein-Barr virus-induced gene 3 (EBI3)] (21). It is part of a group of five major heterodimeric cytokines: IL-12, IL-23 (p19 and p40), IL-27 (p28 and EBI3) and the recently proposed IL-39 (p19 and EBI3) (22). Specifically, IL-35 consists of IL-12p35 and EBI3, which form the IL-12p35/IL-27EBI3 dimer. EBI3, a glycoprotein containing a signal peptide with a repeated Alu sequence, plays a role in cellular pathways and maternal immune tolerance during embryogenesis. EBI3 is also expressed in Hodgkin lymphoma cells, as well as in acute and chronic myeloid leukemia cells (23). While IL-12p35 is involved in promoting inflammatory responses, IL-35 serves to regulate and suppress inflammation. As shown in Fig. 1, IL-35 regulates the functions of various immune cells (such as Tregs, Bregs, macrophages, T cells, and B cells) to regulate the body's immunity. IL-35 is secreted by various immune cells, including tolerogenic dendritic cells (DCs), myeloid-derived suppressor cells, tumor-associated macrophages, neutrophils and natural killer (NK) cells, and primarily by IL-10/IL-35-secreting subsets of Bregs. As an inhibitory cytokine, IL-35 suppresses T-cell proliferation and induces the generation of iTr35 cells to control inflammatory responses (24).

The expression of IL-35 is linked to forkhead box P3 (Foxp3)+ Tregs, with the EBI3 subunit being highly expressed

in murine CD4+ Foxp3+ Tregs but absent in activated effector CD4+ T cells. Upon activation, Tregs generate distinct effector subsets (IL-35-producing and IL-10-producing) to maintain immune tolerance (25). iTr35, a newly identified subset of Tregs that are non-Foxp3 dependent, is induced by IL-35 from murine T cells (26). iTr35 cells release IL-35, promote Treg proliferation, block effector T-cell activation, and convert effector cells into iTr35 cells. The differentiation and function of iTr35 cells are driven primarily by IL-35 and these cells do not rely on Foxp3 or mediate immune suppression via IL-10 or TGF- β (26). Studies have highlighted Bregs as another significant source of IL-35 (27-29). Upon B-cell receptor activation, Toll-like receptor 4 (TLR4) binds to CD40, triggering the transcription of EBI3 and p35, leading to IL-35 secretion (27). Additional research using TLR-deficient B cells demonstrated that TLR4 and CD40L costimulation is necessary for the transformation of B cells into Breg subsets (i35-Bregs) capable of secreting IL-35 (30). In addition to Tregs and Bregs, IL-35 may also be produced by other immune cells, such as immature DCs, CD8+ T cells, and certain tumor cells (31-35). Under inflammatory conditions, additional tissues may have the potential to secrete IL-35. While IL-27 is produced primarily by activated antigen-presenting cells (APCs), IL-35 is secreted predominantly by activated Tregs (36).

IL-35 plays a critical role in modulating immune responses by inhibiting effector T-cell differentiation and IL-17 production, exerting a negative immunoregulatory effect that helps maintain immune homeostasis (37). It promotes the generation of Bregs and the secretion of IL-10 by Breg subsets, further contributing to its immunosuppressive effects (38). Activated B cells can produce both IL-35 and IL-10, mediating negative immune regulation (38). In combination with TGF- β , IL-35 also suppresses immune reactions by stimulating the proliferation of CD4+CD25+ T cells and enhancing IL-10 expression, which inhibits inflammatory responses (38). Studies in mouse models have shown that B cells deficient in IL-35 expression fail to recover from T-cell-induced autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) (39). These findings highlight the potent immunoregulatory functions of IL-35 in various conditions, including cancer, autoimmunity and infection. As summarized in Table I (40-45), IL-35 plays a pivotal role in inflammatory and autoimmune diseases, positioning it as a potential therapeutic target for these disorders (22,32).

The current research on the source of IL-35 in MG is extremely weak and relies mainly on animal models for speculation, lacking human evidence, cell tracing analysis, and clinical translation studies. In the future, multiomics studies targeting the immune microenvironment of patients with MG are needed to clarify the production of cells, regulatory mechanisms and therapeutic potential of IL-35.

IL-35 receptor. The receptors for IL-35 signaling, although distinct, partially overlap with those of other members of the IL-12 family (46). IL-35 signals through four primary receptor types: IL12R β 2/IL27R α and glycoprotein 130 (gp130)/gp130. The functional IL-35 receptor comprises IL12R β 2, gp130 and possibly IL-27R α . IL-35 transduces immunosuppressive signals primarily via the IL-12R β 2:gp130 receptor complex. The p35 subunit binds to IL-12R β 2, while the EBI3 subunit binds to

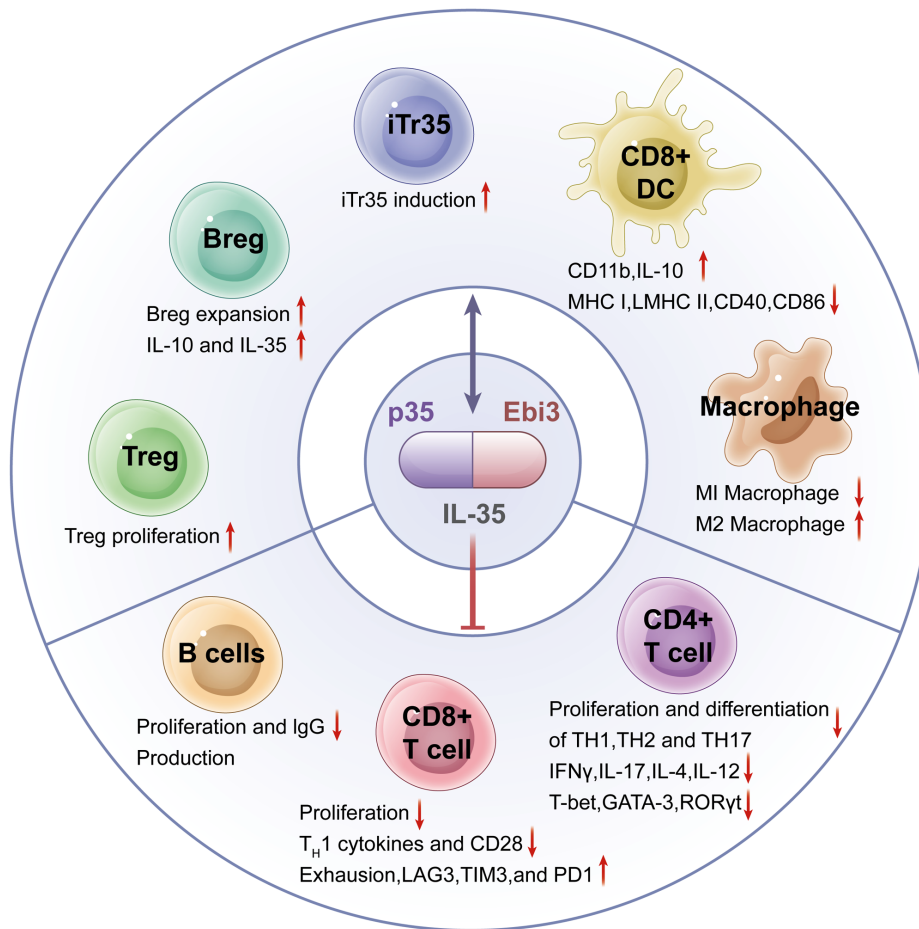


Figure 1. IL-35 producer and effector cells. IL-35 modulates immune responses by regulating the functions of various immune cells (such as Tregs, Bregs, macrophages, T cells, and B cells). i) Regulation of immune cells: Tregs: IL-35 promotes Treg proliferation and enhances their immunosuppressive function. Breg: IL-35 induces Breg expansion and increases the secretion of the anti-inflammatory cytokines IL-10 and IL-35. iTr35: IL-35 induces the generation of iTr35 cells, which exhibit a CD11b⁺ phenotype, secrete IL-10, and downregulate antigen-presenting molecules (MHC I/II, CD40/CD86), further suppressing immune responses. ii) Regulation of macrophage polarization: IL-35 inhibits the activation and function of M1 macrophages. IL-35 promotes the differentiation of M2 macrophages (anti-inflammatory type), enhancing tissue repair and immunosuppression. iii) Inhibition of B-cell proliferation and antibody (IgG) production: IL-35 reduces humoral immune responses by attenuating B-cell activity. iv) Effects on CD8⁺ T cells: IL-35 increases the number of CD8⁺ T cells by inhibiting apoptosis or promoting cell survival. IL-35 reduces the proliferative capacity and secretion of Th1-type cytokines (such as IFN- γ). Increased expression of exhaustion markers (LAG-3, TIM-3, and PD-1) leads to T-cell exhaustion. v) Effects on CD4⁺ T cells: IL-35 inhibits the differentiation and proliferation of Th1, Th2, and Th17 subsets. IL-35 reduces the expression of related cytokines (IFN- γ , IL-17, IL-4 and IL-12) and key transcription factors (T-bet, GATA-3, and ROR γ t). p35 + Ebi3: These two subunits of IL-35 together form the functional cytokine IL-35. IL-35: The core regulatory molecule that induces an immunosuppressive microenvironment through multiple pathways. '↑' indicates upregulation or promotion. '↓' indicates downregulation or inhibition. iTr35, inducible inhibitory T cells; Tregs, regulatory T cells; Bregs, Regulatory B cells; DC, dendritic cell; MHC-II, major histocompatibility complex class II; LAG-3, lymphocyte activation gene 3; TIM-3, T cell immunoglobulin and mucin domain-3; PD1, programmed cell death 1; T-bet, T-box expressed in T cells; GATA-3, GATA binding protein 3; ROR γ t, transcription factors retinoic acid related orphan receptor gamma transcription; EBI3, Epstein-Barr virus-induced gene 3; IFN- γ , interferon-gamma; IL, interleukin; Th, T-helper type; P35, cyclin-dependent kinase 5 regulatory subunit 1.

gp130, leading to receptor dimerization, JAK-STAT activation, and the formation of either heterodimers (STAT1-STAT4) or homodimers (STAT1-STAT1). However, IL-35 can also signal through two types of homodimeric receptors formed by gp130 and IL-12R β 2, namely, gp130:gp130. The IL-12R β 2 subunit is typically expressed by activated NK and T cells, with some expression on DCs and B cells, whereas gp130 is expressed on nearly all cell types. The expression level of IL-12R β 2 determines the functional scope of IL-35 signaling. IL-35 can initiate signaling not only by binding to the IL-12R β 2:gp130 heterodimeric receptor but also by engaging gp130:gp130 homodimers (47). As shown in Fig. 2, unlike other cytokines in the IL-12 family, IL-35 can signal through homodimeric receptors. Specifically, it can interact with IL-12R β 2:IL-12R β 2 homodimers, initiating signal transduction via the STAT1 or

STAT4 pathway, respectively. However, since only one pathway is activated in this case, the immunoregulatory function of IL-35 is partially compromised (47). Full immunosuppressive activity, including the induction of iTr35 cells, requires binding to the IL-12R β 2:gp130 heterodimer and simultaneous activation of both the STAT1 pathway and the STAT4 pathway (48). While homodimers can suppress T-cell proliferation, they are less effective than heterodimers in regulating the activity of IL-35-induced Tregs (iTr35 cells) (47). At present, research on the role of the IL-35 receptor in MG is still in its early stages, and its specific mechanism of action, receptor expression regulation, and clinical translational potential are not yet clear.

IL-35 signal transduction. A key function of the IL-12 family of cytokines is their mediation of activity through the

Table I. IL-35 immunosuppressive functions: Key mechanisms and applications.

Authors, year	Function	Mechanism	Target cells/pathways	Therapeutic applications	(Refs.)
Collison <i>et al</i> , 2010	Inhibits pro-inflammatory cytokines	Downregulates Th1 (IFN- γ ↓), Th17 (IL-17↓), and Tfh (IL-21↓) via STAT1/STAT3 signaling	Th1/Th17/Tfh cells	MS, RA, SLE	(40)
Zhang <i>et al</i> , 2023	Expands regulatory cells	Induces Treg proliferation (Foxp3↑) and generates iTREG35 (Foxp3 ⁺ IL-10 ⁺ IL-35 ⁺)	Tregs, iTREG35 cells	MG, IBD	(41)
Li <i>et al</i> , 2024	Suppresses B-cell activation	Silences plasma cell differentiation (<i>Prdm1</i> methylation via STAT3/DNMT1)	B cells, plasma cells	SLE, MG	(42)
Vignali <i>et al</i> , 2024	Metabolic regulation	Inhibits mTORC1 in Th17 (glycolysis↓); enhances Treg oxidative phosphorylation	Immunometabolic pathways	Type 1 diabetes	(43)
Wang <i>et al</i> , 2024	Localized delivery	Nanoparticle-targeting (such as lymph nodes/thymus) boosts local IL-35 levels	Tissue-specific niches	Thymoma-associated MG	(44)
Bluestone <i>et al</i> , 2024	CRISPR-enhanced secretion	Genome editing (<i>Ebi3/Il12a</i> knock-in) amplifies Treg-derived IL-35	Engineered Tregs	Refractory autoimmunity	(45)

MS, multiple sclerosis; mTORC1, mammalian target of rapamycin complex 1; MG, myasthenia gravis; STAT, signal transducer and activator of transcription; DNMT1, DNA methyltransferase 1; CRISPRs, clustered regularly interspaced short palindromic repeats; PRDM1, PR domain zinc finger protein 1; IFN- γ , interferon- γ ; iTREG35, inducible regulatory T cells 35; Th, helper T cell; EB13, Epstein-Barr virus induced gene 3; IL12A, IL-12A gene; RA, rheumatoid arthritis; mTORC1, mechanistic target of rapamycin complex 1; IBD, inflammatory bowel disease; SLE, systemic lupus erythematosus.

activation of the JAK-STAT signaling pathway (49). Each member of the IL-12 family induces the activation of specific STAT family members, which results in distinct but sometimes overlapping gene transcription patterns. Upon activation of the IL-35 receptor, JAK family members are activated, initiating signal transduction (49). Phosphorylated JAKs subsequently phosphorylate STAT1 and STAT4, members of the STAT family. These phosphorylated STATs then translocate to the nucleus, where they regulate the expression of the p35 and EB13 genes, promoting IL-35 expression (50). The activation of JAKs by the IL-12R β 2 and gp130 receptor subunits primarily involves JAK1 and JAK2, respectively, which act on downstream STAT4 and STAT1 molecules. The binding of IL-35 to a specific homodimeric receptor activates a corresponding signaling branch. Thus, the IL-12R β 2:IL-12R β 2 homodimer predominantly mediates the JAK1-STAT4 signaling pathway, whereas the gp130:gp130 homodimer primarily activates the JAK2-STAT1 signaling pathway (Fig. 3).

In Tregs, IL-35 transduces signals via either heterodimeric receptors (IL-12R β 2/gp130) or homodimeric receptors (gp130/gp130), leading to the activation of STAT4, either

concurrently or independently (51). When IL-35 binds to its corresponding receptor, only one signaling branch is activated (either STAT1 with gp130:gp130 or STAT4 with IL-12R β 2:IL-12R β 2 homodimers), which inhibits T-cell proliferation (52). This results in partial loss of its immunosuppressive activity. However, signaling through the IL-12R β 2:gp130 heterodimeric receptor can also induce the production of iTREG35 cells, which fully exert their immunosuppressive functions (Fig. 3).

IL-35 can bind to a heterodimeric receptor composed of IL-12R β 2, activating downstream STAT1 and STAT3 signaling molecules. This activation induces the production of both IL-10 and IL-35 and promotes the proliferation of Bregs (28). These findings demonstrate that IL-35 regulates its biological functions through different receptors and STAT molecules in various cell types (Fig. 3).

The combination of IL-35 with its corresponding receptor activates STAT4 in T cells, which then inhibits the MAPK and NF- κ B pathways. This reduces proinflammatory responses and inhibits the maturation of monocyte-derived DCs (53). As shown in Figs. 3-4 and Table II (54-58), IL-35 plays a critical role in maintaining immune homeostasis by modulating

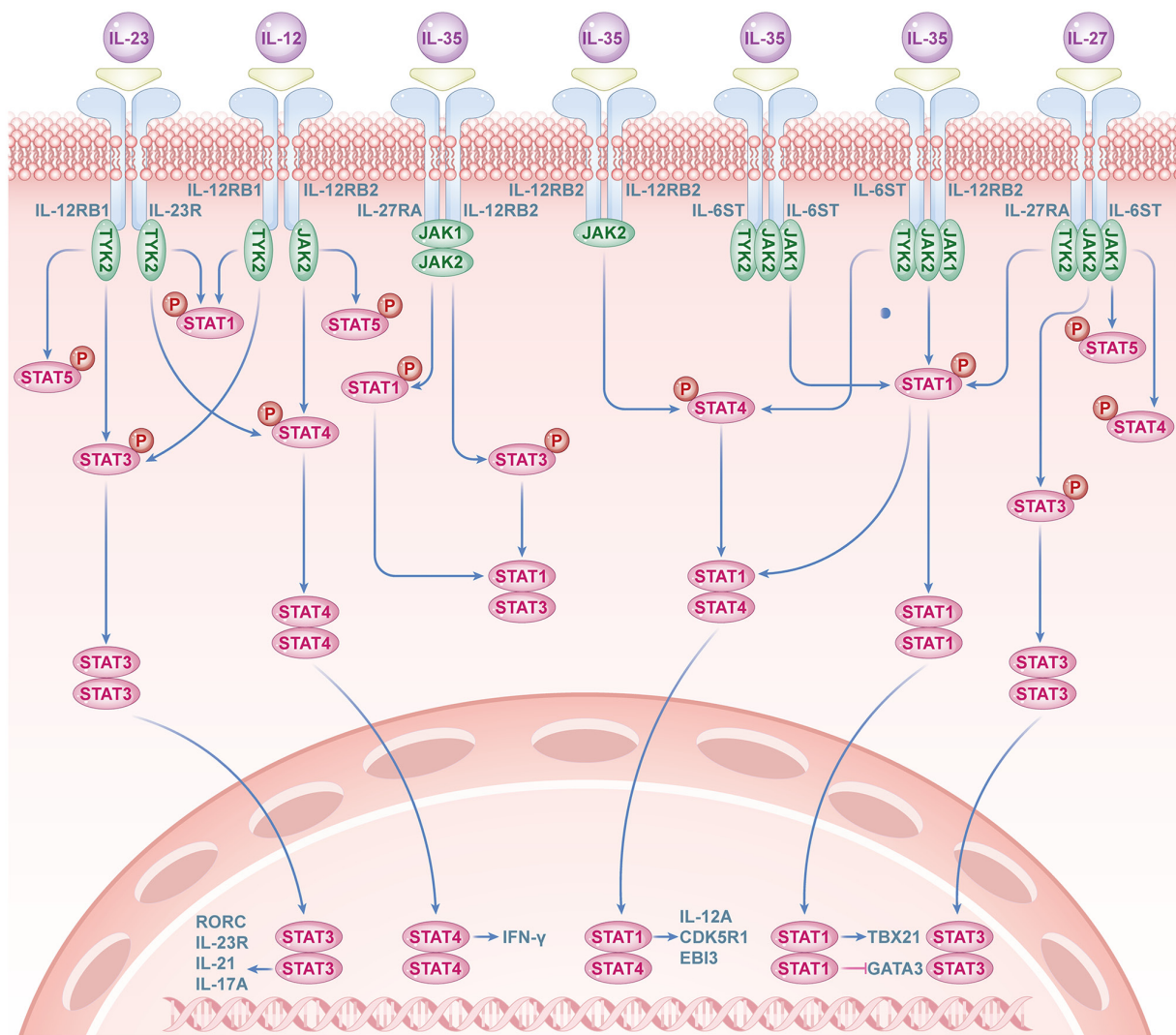


Figure 2. The IL-12 cytokine family and its heterodimeric receptors. The IL-12 family members are heterodimers formed by different combinations of an α subunit (p19, p28, p35) and an α β subunit (p40, Ebi3): IL-12 (p35/p40), IL-23 (p19/p40), IL-27 (p28/Ebi3), and IL-35 (p35/Ebi3). The α subunits share the four-helix bundle structure characteristic of the IL-6 superfamily, whereas the β subunits are structurally related to the extracellular domains of type I cytokine receptors. IL-12, IL-23, IL-27, and IL-35 can stimulate binding to their respective receptors to activate JAK/STAT signaling. Activated JAK phosphorylates and dimerizes STAT, and the dimerized STAT is transferred to the nucleus to regulate the transcription and expression of genes, stimulating further inflammatory release. IL-12 and IL-23 function primarily as pro-inflammatory/pro-stimulatory cytokines, amplifying inflammatory signals and being crucial for immune balance and inflammatory responses. IL-27 has dual pro- and anti-inflammatory properties: it promotes Th1 differentiation while inhibiting Th17 cell development. Since IL-27 signals through a receptor complex involving gp130, it is also often classified within the IL-6 family. IL-35, which is secreted mainly by regulatory T cells, has anti-inflammatory functions. The upward arrow ($\uparrow$) indicates an increase; the downward arrow ($\downarrow$) indicates a decrease. TYK2, tyrosine kinase 2; JAK1, Janus kinase 1; RORC, RAR-related orphan receptor C; STAT, signal transducer of activation; CDK5R1, cyclin-dependent kinase 5 regulatory subunit 1; EBI3, Epstein-Barr virus-induced gene 3; TBX21, T-box transcription factor 21; GATA3, GATA binding protein 3; IFN, interferon; P, phosphorylation.

different target cells and effector pathways. Failure to achieve effective immunosuppression can lead to the development of immune-related diseases.

Inflammatory signaling pathways are often stimulated under inflammatory conditions, leading to the release of proinflammatory factors. During inflammation, the interaction of TLRs, TNF receptors, or IL-18 receptors with their ligands rapidly activates MyD88. This in turn activates TRAF6 or enzymes such as IRAK-1, promoting the expression of NF- κ B and increasing the production of inflammatory factors (59). The transcription factors c-Fos and c-Jun, which are activated by MAPKs, form AP-1 (60), which can increase the levels of inflammatory cytokines (60). Additionally, the I κ B inhibitor is affected by IKK through ubiquitination, leading to NF- κ B release and its translocation to promote inflammatory

responses (59). Together, these two pathways contribute to the production of proinflammatory factors such as IL-1, IL-6, and interferon (IFN).

In addition to stimulating proinflammatory cytokines, NF- κ B also plays a pivotal role in increasing the levels of IL-35 (composed of EBI3 and p35 subunits) and IL-37, as shown in Fig. 5 (53,61). IL-35 has been found to inhibit the p38 MAPK pathway in various inflammatory diseases (62). IL-37 can inhibit the MAPK pathway (63), thus suppressing inflammatory responses. In summary, both IL-35 and IL-37 control inflammation by inhibiting the production of inflammatory mediators, with a preference for blocking NF- κ B activation over MAPK signaling.

Th1 and Th17 cells play essential roles in preventing cancer development and pathogen invasion, whereas Tregs

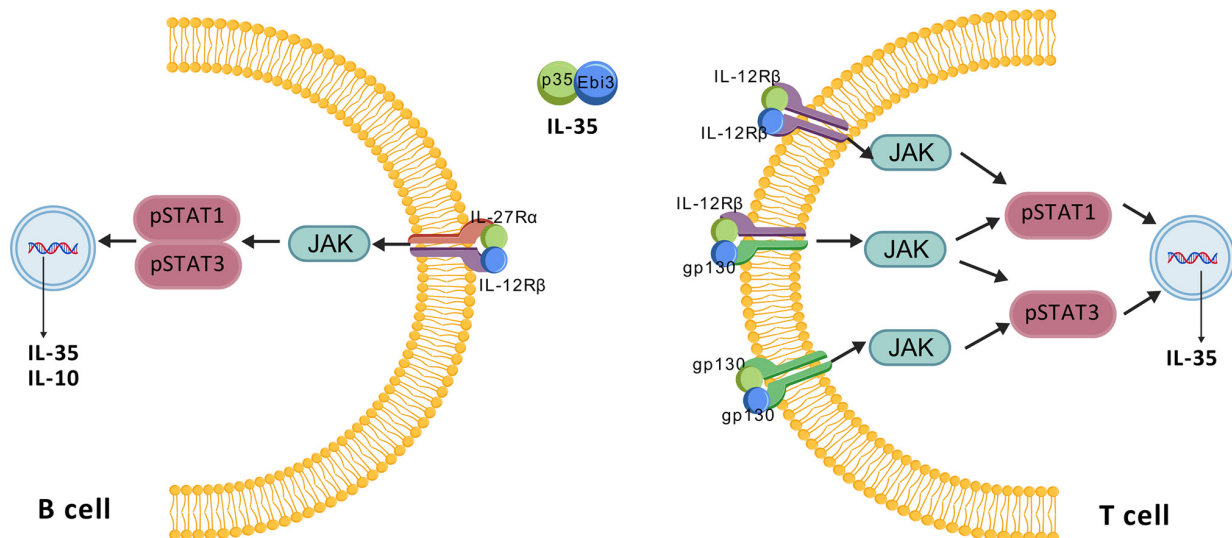


Figure 3. IL-35 signaling pathways in B lymphocytes and T lymphocytes. IL-35 signaling pathway in B cells: IL-35 binds to the IL-12R β 2/IL-12R β 1 receptor on the B-cell surface, activating JAK, which subsequently leads to the phosphorylation of STAT1 and STAT3 (forming pSTAT1 and pSTAT3). Phosphorylated STAT proteins translocate into the nucleus, regulate gene expression and prompt B cells to produce IL-35 and IL-10, which participate in immune regulation. IL-35 signaling pathway in T cells: IL-35 binds to receptors on the T-cell surface, including the IL-12R β 2/IL-27R α , IL-12R β 2/gp130 and gp130/gp130 homodimers. This activates JAK, leading to the phosphorylation of STAT1 and STAT4 (forming pSTAT1 and pSTAT4). Phosphorylated STAT proteins enter the nucleus, driving the expression of IL-35-related genes in T cells and exerting immunoregulatory functions (such as suppressing excessive immune responses). STAT, signal transducer of activation; p, phosphorylation; JAK, Janus kinase.

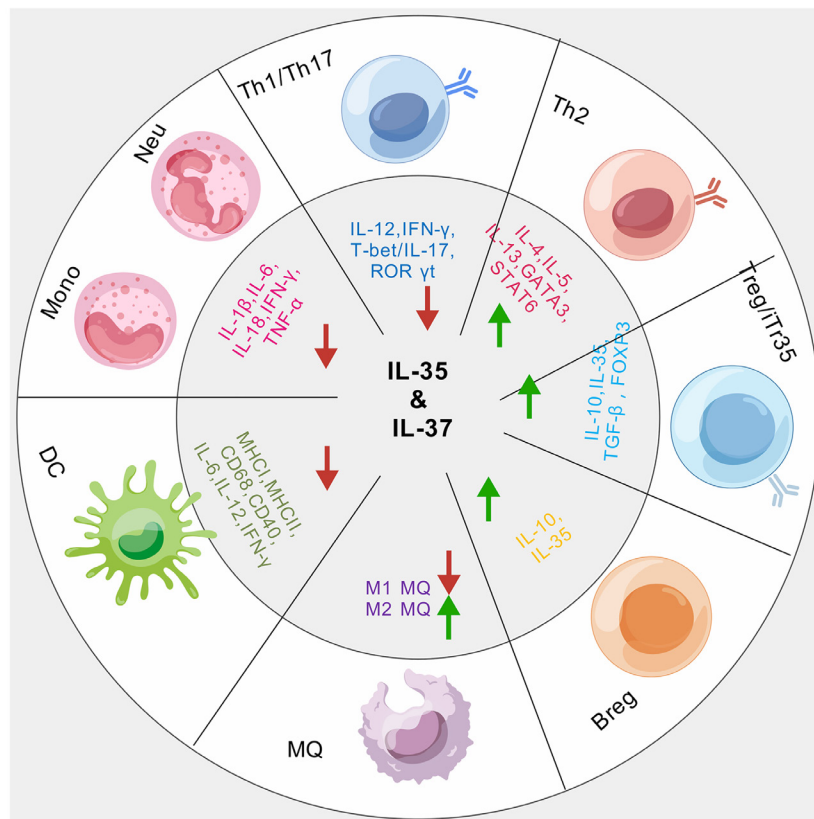


Figure 4. Anti-inflammatory effects of IL-35 and IL-37 on immune system cells. IL-35, which is derived primarily from Tregs and Bregs, exerts its anti-inflammatory effects mainly through the regulation of adaptive immune cells. In T cells, IL-35 potently suppresses the activation and function of Th1 and Th17 cells. Th1 cells block the STAT4 signaling pathway, reducing the production of IFN- γ . Th17 cells inhibit the STAT3/ROR γ t pathway, decreasing the secretion of IL-17A and IL-12. Concurrently, IL-35 promotes proliferation and enhances the function of Foxp3⁺ Tregs, establishing a positive feedback regulatory loop. In B cells, IL-35 inhibits plasma cell differentiation and antibody production while also inducing the differentiation of Bregs. These Bregs further mediate immunosuppression by secreting anti-inflammatory cytokines such as IL-10. Additionally, IL-35 acts on dendritic cells, downregulating the expression of MHC class II molecules and costimulatory molecules, thereby attenuating their antigen-presenting capacity. STAT, signal transducer of activation; RORC, RAR-related orphan receptor C; IFN, interferon; Neu, neutrophil; MQ, macrophages; DC, dendritic cell; ROR γ t, transcription factors retinoic acid related orphan receptor gamma transcription; Mono, monocyte; Tregs, regulatory T cells; Bregs, Regulatory B cells; MHC-II, major histocompatibility complex class II; iTr35, inducible inhibitory T cells; TGF- β , transforming growth factor β ; FOXP3, factor forkhead box protein 3.

Table II. Immunomodulatory mechanisms of IL-35 in immune cells.

Authors, year	Target cell	Signaling pathway	Mechanism of action	Functional outcome	Disease relevance	(Refs.)
Dold <i>et al</i> , 2024	Tregs	•IL-35-STAT1/ STAT3 •EBI3/p35 dimer	Induces autocrine IL-35 production Enhances CTLA-4-mediated suppression	Suppresses Th1/Th17 responses	Autoimmunity (RA, MS)	(54)
Lee <i>et al</i> , 2024	Bregs	•IL-35-STAT1/ IL-10 crosstalk	Expands IL-10 ⁺ Breg population Upregulates PD-L1 expression	Inhibits plasma cell differentiation	SLE, Allo-transplant rejection	(55)
Zhang <i>et al</i> , 2025	Th17 cells	•IL-35-STAT1/ GATA3	Downregulates ROR γ t activity Reduces IL-17A/F secretion	Attenuates inflammatory tissue damage	IBP, Psoriasis	(56)
Liu <i>et al</i> , 2019	M2 Macrophages	•IL-35-STAT6/ PPAR γ	Promotes Arg1 ⁺ polarization Inhibits NLRP3 inflammasome activation	Enhances anti-inflammatory (IL-10 $\uparrow$ /TNF- α $\downarrow$)	Sepsis, Fibrosis	(57)
Liu <i>et al</i> , 2018	DCs	•IL-35-SOCS1	Suppresses CD80/86 co-stimulation Induces IDO expression	Induces T-cell anergy	Tumor immune evasion	(58)

Tregs, regulatory T cells; Bregs, regulatory B cells; DCs, dendritic cells; MS, multiple sclerosis; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; GATA3, GATA binding protein 3; IDO, indoleamine 2,3-dioxygenase; ROR γ t, retinoid-related orphan receptor gamma t; NLRP3, NLR family pyrin domain containing 3; RA, rheumatoid arthritis; TNF- α , necrosis factor α ; PPAR γ , proliferator-activated receptor gamma; STAT, signal transducer of activation; SLE, systemic lupus erythematosus; IDO, indoleamine 2,3-dioxygenase; SOCS, Suppressor of cytokine signaling, IL, interleukin; PD-L1, programmed cell death ligand-1; IBP, inflammatory demyelinating polyneuropathy.

are critical for inhibiting autoimmune diseases (64). Under noninflammatory conditions, a balance exists between Tregs and Th1 and Th17 cells (65), contributing to the regulation of autoimmune diseases and tumor progression. An imbalance between Th17 cells and Tregs can lead to increased Th1 and Th17 cell production in conditions such as IBD (66), driving uncontrolled inflammation. IL-35 may help maintain T-cell balance by influencing the genetic regulation of T-cell factor 1 (TCF) (47). IL-35 also reduces Th1 differentiation by lowering the level of ROR γ t (67) and contributes to the maintenance of Tregs by promoting Foxp3 expression (68). Through these mechanisms, IL-35 helps prevent inappropriate and excessive inflammatory responses.

Innate immunity is a complex and adaptive system, and both IL-35 and IL-37 play critical roles in regulating inflammation (Figs. 4 and 5). IL-37 markedly inhibits pathways that produce proinflammatory cytokines (Figs. 4 and 5) (69). Specifically, IL-37 suppresses the MAPK pathway by inhibiting the IL-18 pathway (70), whereas IL-35 prevents the

overexpression of proinflammatory cytokines such as IL-1 and TNF- α through inhibition of the p38 MAPK pathway (Fig. 5) (71). Additionally, IL-37 suppresses inflammation by enhancing TGF- β activity (71). Treatment with IL-35 and IL-37 results in an increased ratio of Tregs and a reduction in Th17 cells, key outcomes for managing autoimmune diseases and tumors. An imbalance in the Th17/Treg ratio is a significant factor in the development of autoimmune diseases or tumors (72). An excess of Th17 cells over Tregs increases susceptibility to autoimmune conditions such as IBD (73), whereas a greater number of Tregs than Th17 cells increases the risk of cancer (74). IL-35 and IL-37 also regulate Th1 activity and maintain the Th17/Treg balance by reducing T-bet and ROR γ levels while increasing FOXP3 expression. These actions help alleviate the inflammatory reactions associated with IBD (75,76), as illustrated in Fig. 5. Although studies have shown that IL-35 is abnormally expressed in patients with MG and may be involved in the regulation of immune imbalance, research on this pathway is still highly limited to

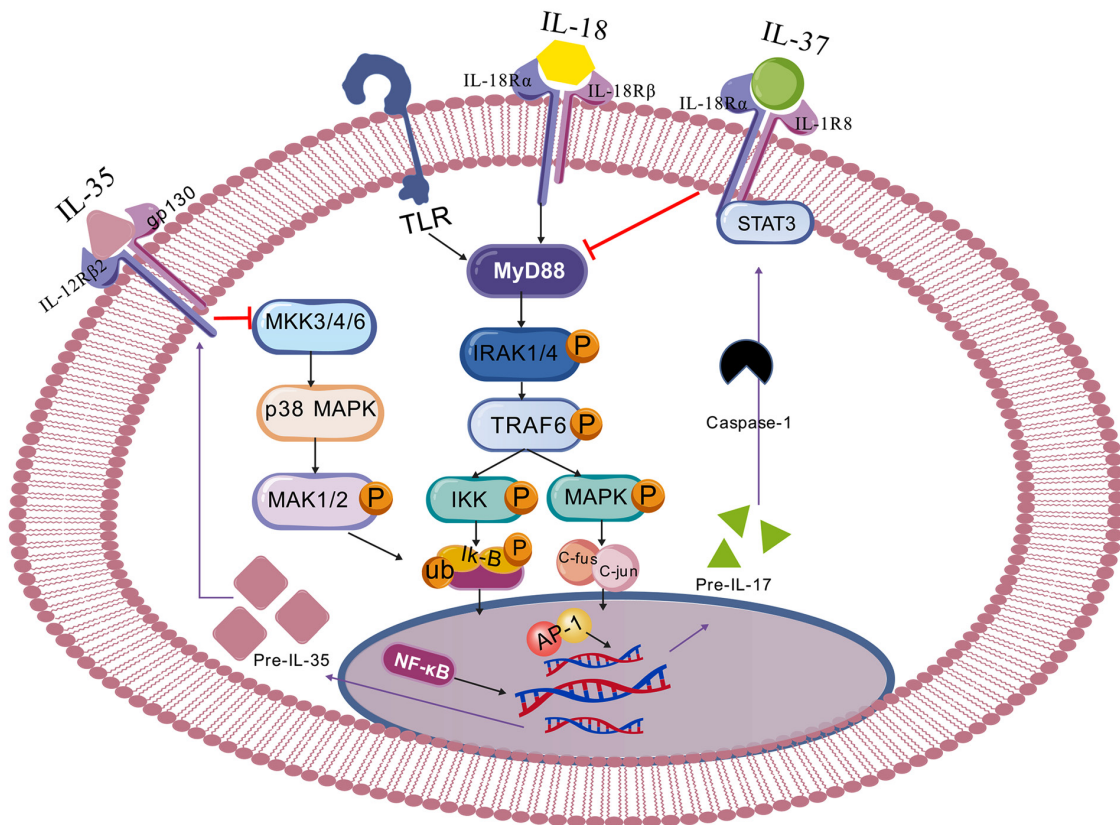


Figure 5. Regulation of inflammatory/anti-inflammatory signaling pathways by IL-35 and IL-37. Under inflammatory conditions, the MAPK and NF- κ B pathways are activated through inflammatory cytokine receptors (especially IL-18R), TLR ligands, and cellular environmental stress. The precursor IL-35 is subsequently generated. Mature IL-35 binds to the cell surface receptor complex gp130/IL-12R β 2, thereby inhibiting p38 MAPK. IL-35 reduces the production of proinflammatory cytokines by suppressing inflammatory signaling pathways. IL-37 inhibits the TLR/MyD88 pathway by blocking IL-18 signal transduction. The black and purple arrows indicate positive/activating effects, whereas the red lines and blunt ends indicate negative/inhibitory effects. IL, interleukin; IL-18R, IL-18 receptor; TLR, Toll-like receptor; MyD88, Myeloid differentiation primary response 88; IRAK, interleukin-1 receptor-associated kinase; TRAF, TNF receptor-associated factor 6; NF- κ B, Nuclear factor kappa-B; I κ B, Inhibitor of NF- κ B; IKK, I κ B kinase; MAPK, Mitogen-activated protein kinase; AP-1, Activator protein-1; MKK, MAP kinase kinase; P, phosphorylation; Ub, Ubiquitination.

the level of basic science and has not yet entered the stage of drug development or clinical intervention.

3. Overview of MG

The present section describes the pathogenesis of MG, immunomodulatory therapy for MG, the regulatory mechanism of IL-35 in MG and the regulatory mechanism of IL-35 in MG through the regulation of Bregs and Tregs, as follows:

Pathogenesis of MG. MG is an autoimmune disorder characterized by autoantibodies targeting AChRs or other associated proteins, such as muscle-specific kinase (MuSK) and low-density lipoprotein receptor related protein 4 (LRP4), on the postsynaptic membrane (77). The pathogenesis of MG is multifactorial and involves humoral and cellular immunity, thymic abnormalities, and genetic predispositions (77). The primary target organ is skeletal muscle, with patients predominantly presenting with muscle fatigability, which can be alleviated by anticholinergic drugs or rest. While various antibody types are present in patients with MG, the most common immunopathological subtype is the presence of autoantibodies against AChRs, accounting for ~85% of cases (78,79). The AChR is a pentameric transmembrane glycoprotein ion channel, and the autoantibodies targeting it are primarily of the IgG1 subclass, with a smaller proportion

of IgG3. The active or passive transfer of human AChR antibodies into animal models induces myasthenic symptoms and disease progression, demonstrating a direct pathogenic role for these autoantibodies in MG. Although the production of these antibodies is well understood in theory, the specific cellular immune mechanisms and characteristics underlying antibody production still require further investigation.

The core pathogenesis of MG centers on autoantibodies that target key proteins at the neuromuscular junction (NMJ), impairing synaptic transmission. Of AChR antibody-positive patients with MG, ~70% exhibit thymic abnormalities, such as thymic hyperplasia or thymoma, with the thymus considered the origin of the autoimmune response (80). Thymomas are typically observed in patients over 50 years of age, whereas younger or female patients are more likely to present with thymic lymphoid hyperplasia accompanied by B-cell infiltration (81). By contrast, MuSK-positive patients with MG do not exhibit thymic abnormalities (81). A small subset of LRP4-positive patients with MG also shows thymic hyperplasia resulting from lymphoid hyperplasia (82).

As a primary lymphoid organ, the thymus plays a critical role in T-cell differentiation. Although the frequency of CD8⁺ T cells exported to peripheral tissues remains unchanged in the thymuses of patients with MG, naturally differentiated Tregs exhibit impaired function, and partially dysfunctional

Table III. The advantages and disadvantages of various treatment methods for myasthenia gravis.

Authors, year	Treatment type	Representative drugs/methods	Advantage	Limitation	(Refs.)
Crisafulli <i>et al</i> , 2024	Traditional drugs	Bromocriptin, glucocorticoids	Fast onset of action (bromopyridine) and low price	Long-term use can easily lead to obesity and infections (hormones)	(96)
Gu <i>et al</i> , 2024	Immunosuppressant	Azathioprine, Tacrolimus	Suitable for refractory patients	Slow onset (takes more than 4 weeks); risk of liver and kidney function damage	(97)
Vu <i>et al</i> , 2025	Biological targeted agents	Rozalizumab monoclonal antibody	Covering AChR/MuSK antibody-positive patients with fast onset (several days)	High cost, requiring injection administration	(98)
Ng <i>et al</i> , 2021	Surgical treatment	Thymectomy	Suitable for patients with thymic hyperplasia/thymoma, with a high long-term remission rate	Long-term medication maintenance is required after surgery	(99)
de Meel <i>et al</i> , 2015	First aid measures	Plasma exchange, intravenous immunoglobulin injection	Quickly eliminate antibodies for crisis rescue	Short-term effect (several weeks), expensive cost	(100)

AChR, anti-acetylcholine receptor; MuSK, Muscle-specific kinase.

Tregs are also present in peripheral tissues (83). These alterations in T-cell immunoregulatory function have been linked to functional deficiencies in Tregs in patients with MG (83). Additionally, effector T cells (Teffs) in patients with MG can resist the immunosuppressive effects of Tregs (79), likely due to the inflammatory microenvironment within the thymus.

Beyond humoral immunity, T cells, particularly the imbalance among Th1, Th17 and Tregs, play a critical role in the pathogenesis of MG (84,85). These findings suggest that the inflammatory environment alters the functionality and plasticity of CD4+ T cells, leading to abnormal Treg and effector T-cell functions.

AChR antibodies directly damage the postsynaptic membrane by activating the complement cascade (such as C3 and the MAC complex). AChR-specific CD4+ helper T (Th) cells are essential for the progression of MG (85). Some studies suggest that the imbalance between Th1 and Th2 cells, as well as the cytokines they secrete, is closely associated with the pathogenesis of MG (86-88). In an experimental autoimmune MG model, Th17 cells influence autoantibody release by shifting the Th1/Th2 cytokine balance, reducing Treg numbers and downregulating Foxp3 expression (89). One study reported that serum IL-35 concentrations are markedly lower in patients with MG than in controls and are associated with anti-AChR antibody titers (90), indicating a regulatory role for IL-35 in the onset and progression of MG.

MGs are associated with specific HLA alleles, such as HLA-DR3 and HLA-B8, as well as non-HLA genes, such as PTPN22 and CTLA4 (91). Genetic studies have identified MHC types carrying risk alleles for MG (91). GWAS findings have confirmed that CTLA4 and TNFRSF11A are involved in MG pathogenesis (92-94). CTLA4 transmits signals to T cells, whereas TNFRSF11A regulates interactions between T cells and DCs (93,94). Additionally, factors such as infections (such as EBV), medications (such as D-penicillamine) and vitamin D deficiency may trigger MG (95). A deeper understanding of these mechanisms will help pave the way for precision therapies for MG and provide insights into how novel biologics may target these pathological processes, improving the prediction of the durability of their therapeutic effects.

Immunomodulatory therapy for MG. Various treatment options are available for MG, including symptomatic therapy, thymectomy, immunomodulatory therapy and long-term immunosuppressive therapy (96). For severe, widespread MG, prompt initiation of immunosuppressive therapy is critical (97). Commonly used immunomodulatory treatments for patients with MG include corticosteroids, immunosuppressants such as azathioprine, mycophenolate mofetil and methotrexate and newer therapies such as calcineurin inhibitors. As outlined in Table III (96-100), each treatment method offers distinct advantages and limitations. Recently, treatment strategies have

Table IV. The mechanism of action, advantages and applicable population of precision targeted therapy for myasthenia gravis.

Authors, year	Drug category	Representative drugs	Mechanism of action	Main advantages	Applicable population	(Refs.)
Zhu <i>et al</i> , 2023	FcRn inhibitor	Rozalizumab, Aigamimod	Blocking neonatal Fc receptors to accelerate the clearance of pathogenic IgG antibodies	Quick onset (superior to traditional drugs), convenient administration (subcutaneous injection), and good safety	Fast onset of action (superior to unified medication for generalized myasthenia gravis patients with positive AChR antibody and MuSK antibody), convenient administration (subcutaneous injection), and good safety.	(101)
Stascheit <i>et al</i> , 2025	Complement inhibitor	ravulizumab, zilucoplan	Inhibit complement C5 and block immune inflammatory response	Rapid improvement of symptoms, long-lasting efficacy, and good safety	Generalized myasthenia gravis patients with positive AChR antibodies	(102)
Piehl <i>et al</i> , 2025	B-cell depletion agent	Rituximab	Eliminate CD20+B cells and reduce antibody production	Effective for some refractory patients	Generalized myasthenia gravis patients with multiple antibody subtypes	(103)

FcRn, neonatal Fc receptor; AChR, anti-acetylcholine receptor; MuSK, muscle-specific kinase.

evolved from traditional immunosuppressive approaches to precision-targeted therapies aimed at achieving disease remission or improved symptom control, minimizing treatment side effects. For example, oral corticosteroid doses are often reduced to ≤ 5 mg/day, with side effects maintained at minimal levels (≤ 1). As shown in Table IV (101-103), precision-targeted therapeutic drugs such as FcRn inhibitors, complement inhibitors and B-cell depletion agents offer diverse regulatory mechanisms, therapeutic benefits, and target populations for MG treatment. These therapies have led to rapid improvements in MG-Activities of Living (ADL) and Quantitative Myasthenia Gravis (QMG) scores, as well as reductions in corticosteroid use (104). Notably, rozalizumab is the first and only approved biological agent in China for patients with AChR and MuSK antibody positivity. Plasma IL-35 levels markedly increase with improvements in MG following immunomodulatory therapy (105). Therefore, plasma IL-35 levels can serve as a valuable biomarker for evaluating the therapeutic efficacy of MG treatments. Further studies revealed that in an experimental autoimmune MG (EAMG) model, treatment with IL-35 combined with low-dose tacrolimus (30% of the conventional dose) resulted in greater clinical improvement (40% increase in muscle strength scores) than did full-dose tacrolimus alone, with a more significant reduction in serum anti-AChR antibody levels (65% decrease in the combination group vs. 45% decrease in the tacrolimus-alone group) (106-108). In anti-AChR antibody-positive MG mice, IL-35 (10 μ g per dose; twice weekly) combined with eculizumab (dose reduced by

half) was administered for 4 weeks. Postsynaptic membrane complement deposition was decreased by 80% (vs. 50-60% in the single agent group) and the muscle strength recovery time was shortened by 40% compared with that in the single agent group (109). In the anti-MuSK antibody-positive MG mouse model, comparisons were made between IVIG alone (1 g/kg), IL-35 alone (20 μ g/kg) and combination therapy (IVIG 0.5 g/kg + IL-35 10 μ g/kg). The combination group showed a 32% improvement in the muscle strength recovery rate (compared with IVIG alone); anti-MuSK IgG4 levels decreased by 58% (only a 35% reduction in the IVIG-alone group); and the postsynaptic membrane AChR cluster density recovered to 85% of normal levels (compared with 60-70% in the single-agent groups) (110). Although current immunomodulatory therapy can effectively control the symptoms of most MG patients, there are still key bottlenecks, such as delayed onset, significant toxic side effects, difficulty in treating some patients, and high treatment costs. The future direction lies in developing more precise, safe, and durable targeted immune intervention strategies combined with personalized assessment to optimize treatment pathways.

IL-35 in MG. IL-35 plays a pivotal role in the onset and progression of MG. Through IL-35, Tregs and Bregs modulate various pathways and proteins, influencing the development and progression of this disease. As shown in Table V (9,111-116), the complex interactions between various cytokines and inflammatory factors markedly influence the pathogenesis

Table V. Key cytokine networks involved in MG pathogenesis.

Authors, year	Cytokine/ inflammatory factor	Source cells	Target cells/ pathways	Proposed mechanisms in MG	Clinical/ therapeutic implications	(Refs.)
Arslan <i>et al</i> , 2024	IL-17A	Th17 cells, $\gamma\delta$ T cells	• Muscle cells • B cells	• Promotes AChR antibody production (B-cell activation) • Induces complement activation at NMJ	Anti-IL-17 biologics (such as secukinumab) in trials	(111)
Cao <i>et al</i> , 2016	IFN- γ	Th1 cells, NK cells	• Macrophages • Follicular helper T cells (Tfh)	• Upregulates MHC class II on APCs • Enhances germinal center reactions (autoantibody production)	Correlates with disease severity (serum levels)	(112)
Dalakas <i>et al</i> , 2019	BAFF/BLyS	Macrophages, DCs	• B cells	• Survival signal for autoreactive B cells • Sustains plasma cell longevity	Belimumab (BAFF inhibitor) shows partial efficacy	(113)
Huda <i>et al</i> , 2023	IL-6	Macrophages, Th2 cells	• B cells • Tfh cells	• Drives plasma cell differentiation • Promotes Th17 polarization	Tocilizumab (anti-IL-6R) reduces MG exacerbations	(114)
Yilmaz <i>et al</i> , 2015	TNF- α	Macrophages, Mast cells	• Muscle cells • Endothelial cells	• Disrupts NMJ integrity (via MMP-9$\uparrow$) • Enhances leukocyte infiltration	Etanercept (TNF blocker) may worsen MG (controversial)	(115)
Wu <i>et al</i> , 2025	IL-10	Tregs, Bregs	• Th1/Th17 cells • B cells	• Suppresses autoantibody production • Limits pro-inflammatory cytokine release	Low IL-10 in patients with MG correlates with thymic hyperplasia	(116)
Martinez Salazar <i>et al</i> , 2025	Complement (C3a/C5a)	Antibody-activated cascade	• NMJ postsynaptic membrane	• Membrane attack complex (MAC) formation $\rightarrow$ AChR degradation	Eculizumab (anti-C5) approved for refractory MG	(9)

NMJ, neuromuscular junction; MMP-9, matrix metalloproteinase-9; MG, myasthenia gravis; APCs, antigen-presenting cells; BLyS, B lymphocyte stimulator; MHC, major histocompatibility complex; TNF- α , Tumor Necrosis Factor alpha; BAFF, B-cell activating factor; IL, Interleukin; MG, myasthenia gravis; MAC, membrane attack complex; AChR, acetylcholine receptors.

of MG. Previous studies have shown that the ratio of Tregs is decreased in patients with MG and is negatively associated with disease activity, suggesting that Treg deficiency is linked to the progression of MG (117,118). Tregs are the primary source of IL-35 (119), and their deficiency may contribute to reduced IL-35 expression in patients with MG. Furthermore, IL-35 levels are markedly inversely associated with neurological function scores (QMGs) and ADL scores in these patients (119), indicating that IL-35 is a reliable marker for assessing the severity of MG (120). IL-35 can inhibit

Th cell proliferation and promote the generation of Tregs, which, in turn, release IL-35. A reduction in IL-35 levels may disrupt the balance of T-cell subsets, leading to the release of cytokines that exacerbate the condition of MG (121). The imbalance between T and B-cell subsets plays a critical role in the progression and outcome of MG, particularly within the T-cell compartment (122). IL-35 levels are lower in untreated patients with MG, particularly those with GMG or comorbid thymoma (116), but they increase following treatment and are negatively associated with functional disability scores (123),

highlighting the importance of IL-35 in MG disease outcomes. The reduction in IL-35 during the acute phase may be related to a decrease in Treg and Breg proportions. Compared with those in healthy controls (HCs), both the ratio and function of Bregs are diminished in patients with MG (124). Additionally, IL-35 is negatively associated with Th17 cells and their secreted factor, IL-17 (125), suggesting that IL-35 may exert its immunosuppressive effects by regulating Th17 cells in MG. These studies suggest that IL-35 is an important anti-inflammatory factor that regulates MG and has the potential to be used for the treatment of MG. However, some studies suggest that it may promote disease progression in certain chronic inflammatory or tumor environments, exhibiting a 'double-edged sword' characteristic. In MG, a highly heterogeneous autoimmune disease, further research is needed to investigate whether IL-35 accidentally activates other immune pathways or leads to immune escape.

Bregs and IL-35. B cells, as precursors to plasma cells, play a key role in promoting humoral immune responses through T-cell activation (126). Studies have highlighted the specific protective functions of B cells in regulating immune responses (126-128). In a study involving 41 patients with MG who did not receive any medication treatment and 30 HCs, the proportions of CD19+ IL-10+ cells and CD19+CD24hiCD38hi cell subsets in patients with MG were markedly lower than those in HCs; in thymus tissues, the percentage of CD19+ IL-10+ cells was highest in healthy children (~8%), followed by healthy adults (~3%) and was lowest in patients with MG (~0.5%) (129). In another study involving 112 patients with MG, Breg infiltration in the TME decreased with MG aggravation, whereas the opposite trend was observed for Tfh cells. The Breg/Tfh ratios in the peripheral blood and TME were broadly consistent and the levels of both types of cells were markedly lower in patients with aggravated MG. Therefore, Breg cells have been confirmed to have an immunosuppressive function and play an important role in MG (130). These B-cell subsets, known as Bregs, downregulate immune reactions by secreting cytokines such as IL-10 and TGF- β (131). As shown in Fig. 6, IL-10 secreted by Bregs inhibits Th17 cell differentiation, enhances Th2 polarization, and suppresses DC activation; IL-35 secreted by Bregs inhibits Th1/Th17 activation and promotes Treg expansion; and TGF- β secretion by Bregs inhibits Th1 activation and further promotes Treg expansion (131).

IL-35 regulates the immune response of the body by modulating B lymphocytes. IL-35 is secreted primarily by Tregs and Bregs (28). Under conditions such as TLR activation, an inflammatory microenvironment (such as in autoimmune diseases or tumors) and Treg-Breg interactions, Bregs secrete IL-35 (28,132,133). Naïve B cells can differentiate into immunosuppressive Breg subsets, termed IL-35-induced Bregs (i35-Bregs) upon IL-35 stimulation. These i35-Bregs have been shown to prevent immune responses by blocking Th1/Th17 cells (134), representing a promising new avenue for future autoimmune disease therapies. IL-35 promotes the differentiation of naïve B cells into Bregs through the STAT1/STAT3 signaling pathway (135) and enhances Breg function. IL-35-stimulated i35-Bregs exhibit stronger immunosuppressive capabilities, producing and releasing anti-inflammatory

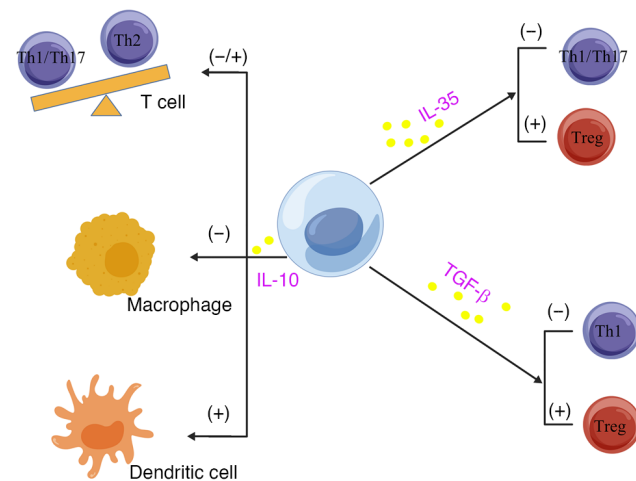


Figure 6. Bregs mediate immune responses through IL-10, TGF- β , and/or IL-35. IL-10 secreted by Bregs inhibits the differentiation of Th1/Th17 cells and enhances Th2 polarization. IL-10 from Bregs also suppresses the activation of macrophages and dendritic cells. IL-35 secreted by Bregs inhibits Th1/Th17 activation and promotes the expansion of Tregs. TGF- β secreted by Bregs inhibits Th1 activation and facilitates the expansion of Tregs. TGF- β , transforming growth factor- β ; IL, interleukin; Tregs, regulatory T cells; Bregs, regulatory B cells; Th, T-helper type.

factors such as TGF- β (15). Additionally, IL-35 suppresses Th1/Th17 responses, reduces proinflammatory cytokines such as IL-17, promotes Treg expansion, inhibits CD8+ T cells, facilitates tumor immune escape, and enhances the suppressive function of Bregs (136). These findings suggest that IL-35 may serve as a therapeutic agent for treating MG.

IL-35 regulates the immune response in autoimmune disease models by modulating Bregs. In models of autoimmune diseases, such as rheumatoid arthritis, IL-35-producing Breg (IL-35+ Breg) cells markedly ameliorate inflammatory damage. In EAE and IBD, IL-35+ Bregs alleviate disease symptoms by reducing effector T-cell proliferation (137,138). Further studies have shown that IL-35 treatment increases the proportion of Bregs (such as CD19+CD24hiCD38hi or IL-10+ Bregs) and suppresses the production of pathogenic antibodies, such as anti-AChR antibodies (15,139,140). IL-35 likely promotes Breg differentiation via the STAT3 pathway while preventing the transformation of B cells into plasma cells (141). IL-35+ Bregs block the proliferation of proinflammatory T cells (such as Th1 and Th17) by releasing IL-10, which in turn reduces the expression of IFN- γ and IL-17 (142). These findings suggest that IL-35 can improve the prognosis of patients with autoimmune diseases by regulating Bregs.

IL-35 regulates the immune response in MG models by modulating Bregs. In MG, T follicular helper (Tfh) cells induce germinal center reactions and autoantibody production, whereas IL-35 may indirectly inhibit Tfh differentiation through Bregs (143). In an EAMG mouse model, injection of recombinant IL-35 alleviated muscle weakness symptoms, reduced serum AChR antibody titers, and increased the proportion of Bregs (144). Combination therapy using IL-35 and adoptive transfer of Bregs (such as CD19+CD24hiCD38hi Bregs) has synergistic therapeutic effects (144). Delivery of the IL-35 gene via adenoviral vectors, such as AAV-IL-35, can maintain long-term Breg function and delay EAMG progression (145). Bregs in MG exhibit functional defects (such as reduced IL-10

Table VI. Differences in the effects of IL-35 on B and T cells.

Feature	B lymphocytes	T lymphocytes
Primary receptor	IL-12R β 2/gp130 (low expression)	IL-12R β 2/gp130 (high expression, especially on Tregs)
Core signaling	STAT3 $\rightarrow$ IL-10 secretion	STAT1 $\rightarrow$ inhibition of Teffs; STAT3 $\rightarrow$ expansion of Tregs
Functional output	Promotes Bregs differentiation, inhibits antibody production	Inhibits Teffs, enhances Tregs function
Pathological association (Refs.)	Autoimmune diseases (such as RA) (147)	Tumor immune escape, transplant tolerance (148)
Authors, year	Choi <i>et al</i> , 2021	Ito <i>et al</i> , 2020

IL, interleukin; Tregs, regulatory T cells; Bregs, regulatory B cells; Teffs, effector T cells; STAT, signal transducer and activator of transcription.

Table VII. Mechanisms of Treg-mediated immunosuppression.

Authors, year	Mechanism	Key molecules/ pathways	Target cells/ effects	Relevance to MG	Therapeutic potential	(Refs.)
Tekguc <i>et al</i> , 2021	Cell-contact inhibition	CTLA-4, LAG-3, PD-1	Blocks CD80/CD86 on DCs $\rightarrow$ inhibits T-cell activation	$\downarrow$ CTLA-4 in thymoma-associated MG	Abatacept (CTLA-4-Ig fusion)	(149)
Zong <i>et al</i> , 2024	Cytokine secretion	IL-10, TGF- β , IL-35	Suppresses Th1/Th17; induces iTr35	IL-35 $\downarrow$ correlates with severe MG (QMG score)	Recombinant IL-35 trials (Phase I)	(150)
Yang <i>et al</i> , 2022	Metabolic disruption	CD25 (IL-2R α), IDO, CD39/CD73	Depletes IL-2 $\rightarrow$ Teff apoptosis; adenosine production	Altered CD25 expression in refractory MG	Low-dose IL-2 therapy	(151)
Sun <i>et al</i> , 2020	Cytotoxicity	Granzyme B, Perforin	Direct killing of Teff/APCs	$\uparrow$ Granzyme B ⁺ Treg post-thymectomy	CAR-Treg engineering	(152)
Wang <i>et al</i> , 2015	Epigenetic regulation	Foxp3-dependent chromatin remodeling	Silences IFN- γ /IL-17 loci via HDACs	Foxp3 methylation defects in MG Tregs	HDAC inhibitors (such as Vorinostat)	(153)

APC, antigen-presenting cells; Teff, effector T cells; IFN- γ , interferon- γ ; FOXP3, forkhead box protein 3; LAG-3, lymphocyte activation gene 3; PD-1, programmed death 1; CAR, chimeric antigen receptor; Th, T helper; TGF- β , transforming growth factor β ; IL, interleukin; QMG, quantitative myasthenia gravis scale; HDACs, histone deacetylases; IDO, indoleamine 2,3-dioxygenase; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; MG, myasthenia gravis; Treg, regulatory T cells.

secretion) and supplementation with IL-35 alone may need to be combined with other immunomodulatory strategies, such as low-dose IL-2 (129,146). As shown in Table VI (147,148), the regulatory pathways and mechanisms of action of IL-35 vary between T and B lymphocytes: In B lymphocytes, IL-35 primarily modulates STAT3 to promote Breg differentiation and inhibit antibody production, whereas in T lymphocytes, it modulates both STAT3 and STAT1 to inhibit Teffs and enhance Treg function. These findings suggest that IL-35 can improve the prognosis of MG models by regulating Bregs. However, the current research on IL-35 regulation by Bregs

in MG is still at the basic association level and lacks mechanistic analysis and clinical translation support. In the future, larger prospective studies, *in vitro* functional experiments, and animal model validation are needed to clarify whether IL-35 can be used as a new target for MG therapy.

Tregs and IL-35. As shown in Table VII (149-153), Treg-induced immunosuppression involves multiple mechanisms, including CTLA4-mediated suppression of APCs and the production of immunosuppressive metabolites. In a study involving 39 patients with MG, the percentages of CD4⁺CD25⁺ Treg

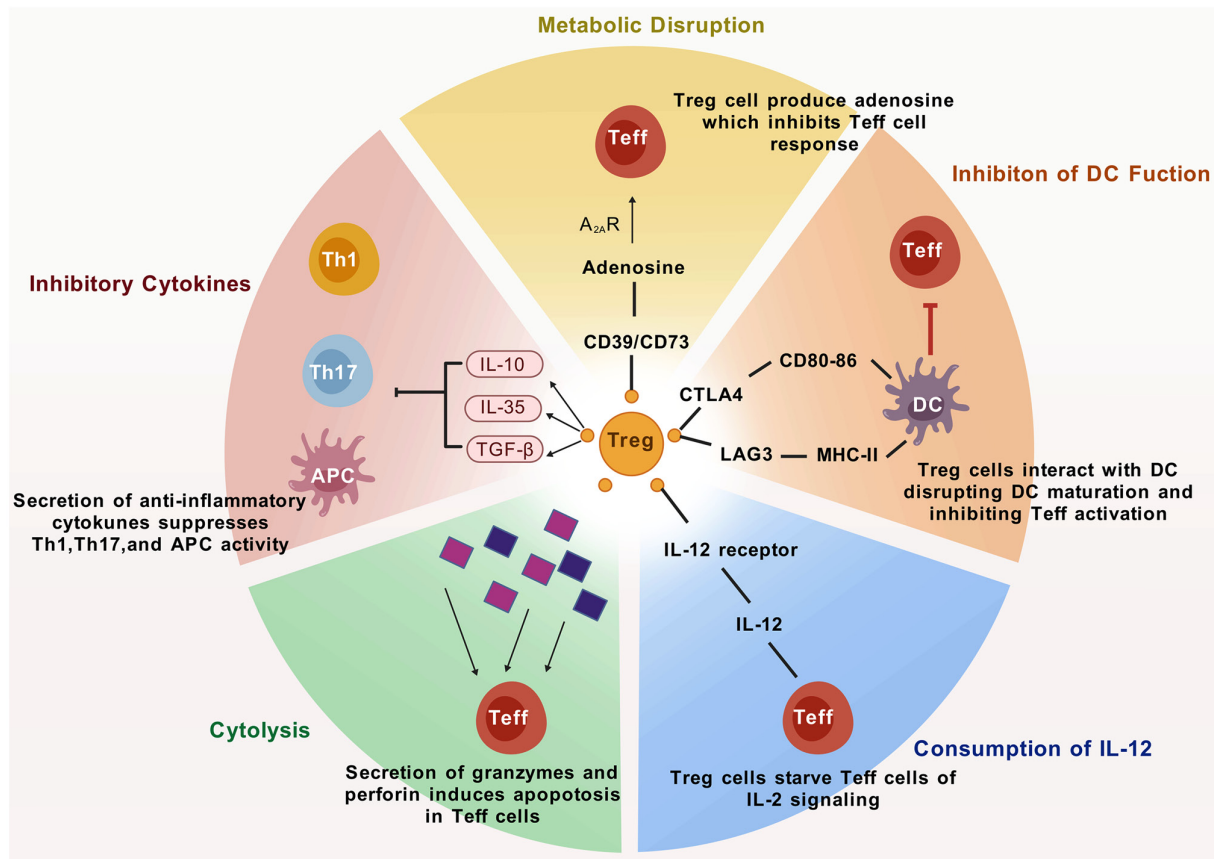


Figure 7. Immunosuppressive mechanisms mediated by Tregs. Tregs modulate immune responses by suppressing the functions of Teffs and APCs through multiple mechanisms, including i) interactions between CTLA-4 and LAG-3 expressed by Tregs and the CD80/86 costimulatory molecules and MHC class II molecules expressed by DCs, respectively, to inhibit DC function and maturation, thereby inducing IDO generation and suppressing Teff cell activation. ii) Metabolic disruption: Tregs can disrupt metabolic processes by expressing the ectoenzymes CD39/CD73, leading to the generation of adenosine, which binds to the A2AR exposed on effector T cells, or by IL-2 deprivation. iii) Production and secretion of the anti-inflammatory cytokines IL-10, IL-35, and TGF- β , which inhibit Th1 and Th17 immune responses and the production of IFN- γ and IL-17, respectively. iv) Direct cytotoxicity: Tregs can also directly kill effector cells by releasing granzyme A, granzyme B, and perforin, thereby inducing target apoptosis. APC, antigen-presenting cell; TGF- β , transforming growth factor- β ; A2AR, adenosine A2A receptor; IL, interleukin; IFN, interferon; Teff, effector T cell; Treg, regulatory T cell; CD, cluster of differentiation; IDO, indoleamine 2,3-dioxygenase; DC, dendritic cell; CTLA-4, cytotoxic T-lymphocyte antigen 4; LAG3, lymphocyte-activation gene 3; MHC, major histocompatibility complex.

cells in the peripheral blood of patients with OMG and GMG were both lower than those of healthy individuals ($P < 0.05$); however, the percentage of patients with OMG was not distinctly different from that of patients with GMG ($P = 0.475$). Additionally, the percentages of CD3+CD4+CD25+Foxp3+ Treg cells in the OMG and GMG patient groups were lower than those in the healthy group (154). Another study included 13 children with serum AChR antibody-positive ocular-type MG and 18 age-matched controls. The percentages of Tregs among peripheral blood CD4(+) T cells in the active stage, remission stage, and control groups were 3.3 ± 1.3 , 4.8 ± 1.7 , and $5.0 \pm 0.6\%$, respectively. The Treg population was markedly lower in the active stage than in the remission stage and in the control group. Furthermore, the Treg percentage is markedly lower during the relapse of myasthenia symptoms (155). Tregs employ both direct and indirect pathways to suppress various immune cells, with indirect effects often involving one cell type affecting another, ultimately leading to immune cell suppression (156). The release of cytokines such as TGF- β and the generation of lytic enzymes such as granzymes often induce immune apoptosis (157). Additionally, Tregs directly inhibit target cells by releasing CD39/CD73, which lowers extracellular ATP levels by generating adenosine and

AMP (158). Tregs suppress the ability of autoreactive B cells to produce harmful autoantibodies, a process that also disrupts the autoreactive B cells themselves (159). Moreover, Tregs utilize granzyme B and perforin to induce pore formation and lyse effector B cells, thereby reducing autoantibody production (160). Tregs also act specifically on monocytes, inhibiting their differentiation and cytokine production (161). When cocultured with Tregs, monocytes acquire characteristics of M2 macrophages, such as increased expression of CD206 and CD163, along with reduced responsiveness to proinflammatory stimuli. This is evidenced by decreased IL-6 production and the inhibition of NF- κ B activation (162). By contrast, monocytes cocultured with Tregs secreted 2.3 times more IL-17 (as measured by ELISA, $P < 0.01$), but this difference was not statistically significant (163). This effect may be related to the high expression of OX40L on expanded Tregs, which activates a proinflammatory monocyte phenotype via the NF- κ B pathway, as validated by scRNA-seq (163). The mechanisms of Treg-mediated immunosuppression are summarized in Fig. 7.

Several studies have reported that T1DM, MG, and similar autoimmune diseases are associated with Treg deficiency (Table VIII) (84,164-167). IL-35 promotes Treg proliferation and function, such as through the inhibition of Th1/Th17

Table VIII. Treg functional defects in major autoimmune diseases.

Authors, year	Disease	Key Treg defect	Molecular mechanism	Validated therapeutic targets	(Refs.)
Zhang <i>et al</i> , 2023	Type 1 diabetes	•Quantitative deficiency (↓40%) •Impaired suppression (20% efficacy)	•Foxp3 epigenetic instability •IL-2Rα hypomethylation	•Low-dose IL-2 •Teplizumab (CD3 mAb)	(164)
Hosseini <i>et al</i> , 2020	Multiple sclerosis	•Migration failure (↓CCR6) •Th17 imbalance (↑3-fold)	•STAT3 hyperactivation •AHR signaling defect	•Siponimod (S1P modulator) •Treg adoptive transfer	(165)
Uzawa <i>et al</i> , 2021	Myasthenia gravis	•IL-35⁺ subset loss (↓70%) •Thymic escape of autoreactive T-cells	•Ebi3 transcriptional silencing •OX40L overexpression	•Recombinant IL-35 •Thymectomy + Treg expansion	(84)
Rezaei <i>et al</i> , 2022	Rheumatoid arthritis	•Pro-inflammatory skewing (IL-17⁺) •Synovial accumulation defects	•Foxp3 hypermethylation •RORγt co-expression	•JAK inhibitors (tofacitinib) •HDAC inhibitors	(166)
Huang <i>et al</i> , 2024	Systemic lupus	•Mitochondrial dysfunction •CD4+CTLA-4^{lo} phenotype	•mTOR overactivation •PD-1/PD-L1 axis disruption	•Rapamycin analogs •CAR-Treg therapy	(167)

FOXP3, forkhead box protein 3; JAK, Janus kinase; AHR, aryl hydrocarbon receptor; CCR6, CC chemokine receptor 6; PD-1/PD-L1, programmed death protein 1 (PD-1) and its ligand programmed death-ligand 1; mTOR, mammalian target of rapamycin; OX40L, receptor ligand; OX40L, transmembrane glycoprotein OX40 ligand; FOXP3, forkhead box protein 3; CAR, chimeric antigen receptor; CTLA-4, cytotoxic-T-lymphocyte-antigen-4; S1P, sphingosine-1-phosphate; STAT, signal transducer of activation; HDAC, histone deacetylase.

responses, by activating the STAT1/STAT3 signaling pathway in Tregs (168). In the EAMG model, exogenous IL-35 restores the suppressive function of Tregs and reduces the levels of AChR autoantibodies (169). IL-35 also converts conventional T cells into novel iTR35 cells, which, although independent of Foxp3, possess potent immunosuppressive functions (170). In MG, iTR35 cells may compensate for the functional impairment of conventional Tregs. In AChR-induced EAMG rats, recombinant IL-35 injection markedly alleviates clinical symptoms, such as reducing myasthenia scores and decreasing immune complex deposition at the NMJ (171). IL-35 suppresses the differentiation of pathogenic B cells and the secretion of autoantibodies through Tregs while also downregulating proinflammatory cytokines such as IFN- γ (172). Compared with conventional Tregs, IL-35-pretreated Tregs (IL-35+ Tregs) show enhanced therapeutic efficacy (50). IL-35 may augment immunosuppression through the Treg-Breg axis, with Treg-derived IL-35 promoting the expansion of Bregs, thus forming a negative feedback loop (50). In the serum of patients with GMG, IL-35 expression in Tregs inversely correlates with disease severity (such as MGFA classification). Treg functional deficiency is more pronounced in patients with MG with thymomas, potentially because of insufficient IL-35 secretion (173). Combining IL-35 with low-dose IL-2 (which can expand Tregs) may help restore immune balance in patients with MG (174). Adenoviral vectors, such as AAV-IL-35, have

demonstrated long-term efficacy in animal models, although safety concerns must be addressed for clinical application (175). IL-35 nanoparticles targeting Tregs may improve local efficacy, particularly by targeting the thymus or lymph nodes (176). CRISPR gene editing can also increase the IL-35 secretion capacity of Tregs (177). Thus, by regulating the function and expansion of Tregs, IL-35 has significant immunosuppressive and therapeutic potential in MG models. At present, research on the regulatory effects of IL-35 on Tregs in MG is still in its early stages and is limited mainly by the use of animal models, a lack of human data, incomplete elucidation of the mechanism of action, and a lack of specific therapies targeting this pathway for clinical application. Although new therapies such as CAR-T cells have begun to target B cells or rebuild immune tolerance, research on direct intervention in MG via the IL-35/Treg axis is still at the basic level.

Differential roles of IL-35 in various MG subtypes. Among patients with MG, the AChR antibody-positive (AChR+) subtype represents the majority, accounting for 80-85% of cases (178). In an EAMG model induced by AChR, exogenous IL-35 markedly reduced serum anti-AChR antibody titers and decreased complement deposition at the NMJ (144). This effect likely results from the ability of IL-35 to inhibit B-cell differentiation into plasma cells, thereby reducing the production of autoantibodies. Several studies have shown that

Table IX. Research on IL-35 subtypes or isoforms.

Authors, year	Research dimensions	Current key content	Does it involve 'subtypes/isomers'	(Refs.)
Wang <i>et al.</i> , 2023 Gao <i>et al.</i> , 2017	Molecular structure	P35 + EB13 heterodimer, structurally stable	No	(183)
	Splicing variation	No reports have been found on the generation of new isoforms of p35 or EB13 in the context of IL-35	No	(184)
	Functional diversity	Functional differences caused by source cells, receptor usage, and microenvironmental influences	Yes (non structural level)	
	Detection method	ELISA, Flow cytometry and quantitative PCR detection of p35/EB13 mRNA	Not distinguishing between 'subtypes'	
	Development direction of treatment	Recombinant IL-35 for anti-inflammatory treatment; blocking IL-35 to enhance anti-tumor immunity	Targeting a single molecular form	

IL-35, interleukin 35; EB13, Epstein-Barr virus-induced gene 3; ELISA, enzyme-linked immunosorbent; P35, interleukin-12 subunit p35.

serum IL-35 levels are markedly greater in AChR+ patients with MG than in HCs and are associated with disease severity (such as MGFA classification) (116,179,180). By contrast, the anti-muscle-specific kinase antibody-positive (MuSK+) subtype accounts for 5-10% of MG cases (178), with an immunopathological mechanism distinct from that of AChR+ MG. Although IL-35 in MuSK+ MG has received increasing attention, research in this area remains limited. In the MuSK-induced EAMG model, the efficacy of IL-35 may be weaker than that in the AChR EAMG model, potentially due to the IgG4 nature of MuSK antibodies, which have lower complement activation capabilities. MuSK+ MG is more strongly associated with B-cell tolerance defects than with T-cell-driven inflammation. Th17 cells may play a lesser role in MuSK+ MG, indicating that the promotion of Tregs by IL-35 may not be as significant as that in AChR+ MG. Research has shown that IL-35 levels in MuSK+ patients with MG may not be markedly associated with disease severity (181,182), indicating that the mechanisms underlying the role of IL-35 in these subtypes may differ.

IL-35 subtypes or isoforms. At present, according to Table IX (183,184), no classical subtypes or isoforms of IL-35 have been found; however, according to Table X (185-187), its functional diversity may stem from different cellular sources, receptor combinations, and signaling pathway activation patterns.

4. Studies on IL-35 in patients with MG

A study involving 43 patients with MG with positive anti-AChR antibodies and 25 HCs reported that the serum levels of 24 inflammatory cytokines were measured. Elevated serum

concentrations of a proliferation-inducing ligand (APRIL), IL-28A and IL-35 were detected in patients with MG, and the IL-20 and IL-35 levels decreased markedly after treatment. Among these cytokines, APRIL, IL-19 and IL-35 concentrations are markedly greater in AChR-positive patients with MG. According to clinical subtype analyses, APRIL and IL-20 are increased in patients with late-onset MG, and IL-35 levels are increased in patients with thymoma-associated MG compared with healthy controls (188). This increase in IL-35 may represent a compensatory regulatory response to the autoimmune reaction, helping to alleviate symptoms by inhibiting Th17 cells, which led to a 40% reduction in the number of IL-17+ cells (189). Li *et al.* (190) reported that the proportions of Th1 (IFN- γ +) and Th17 cells in the blood of thymoma-associated MG (TMG) patients were increased by 1.8-fold compared with those in HCs (flow cytometry, $P < 0.001$). Patients with comorbid thymoma presented an even greater increase in Th17 cell proportions (3.2-fold), which were positively associated with IL-6 levels ($r = 0.62$) (191). The underlying mechanism may involve Th17 cells directly damaging the postsynaptic membrane of the NMJ through IL-17A, as electrophysiological experiments confirmed a 40% reduction in the compound muscle action potential amplitude (163). Further studies in 25 treatment-naïve AChR-positive patients with MG and 28 controls revealed decreased levels of cytokines promoting Th2 polarization and a reduction in Th1-related factors, such as IL-4 and IL-22 (184). However, the serum concentrations of IL-10, IL-12p40, IL-12p70, IL-20, IL-22, IL-26, IL-28A, IL-29 and IL-35 are elevated in AChR-positive patients with MG (192). These altered cytokine profiles contribute to promoting B-cell proliferation, increasing Th1/Th2 ratios and enhancing Th17 cell proliferation (193). Immunosuppressive

Table X. Research on the functional diversity of IL-35.

Authors, year	Dimension	Description	Relevant research insights	(Refs.)
Li <i>et al</i> , 2025	Cell source specificity	The IL-35 produced by different types of regulatory immune cells (such as Foxp3+Treg vs. iTr35 vs. Breg) may have different regulatory effects	Similar to the recognition of different molecular subtypes in tumors (such as KRASG12C enriched immune regulatory subtypes), it suggests that functional heterogeneity may stem from differences in cellular background	(185)
Smyth <i>et al</i> , 2025	Differences in receptor usage	IL-35 can transmit signals through multiple receptor combinations, leading to varying degrees of activation of downstream pathways such as STAT1/STAT3 phosphorylation.	The mechanism of multiple receptor utilization is similar to the specific binding of FGFR2b to its ligand, reflecting functional plasticity	(186)
Haley <i>et al</i> , 2025	Microenvironment dependent effects	In inflammation, tumors, or autoimmune diseases, the role of IL-35 may shift from immunosuppression to pro-inflammatory or tissue repair	Just as the classification of polycystic ovary syndrome shows that different subtypes have unique metabolic and reproductive outcomes, the function of IL-35 may also vary depending on the 'microenvironment subtype'	(187)

IL-35, interleukin 35; FOXP3, factor forkhead box protein 3; Treg, regulatory T cells; iTr35, inducible regulatory T cells 35; STAT, signal transducer of activation; KRASG12C, Kirsten rat sarcoma viral oncogene homolog, glycine to cysteine mutation at codon 12; FGFR2b, fibroblast growth factor receptor 2b.

treatment markedly reduces the plasma concentrations of IL-20 and IL-35 (188). In the context of MG, inflammation mediated by Th1 and Th17 cells seems to increase Treg activity, leading to increased IL-35 production, which in turn results in reduced Th2 polarization (194). IL-35 can mitigate established inflammation in severe patients with MG, with its serum concentration increasing during the acute stage but gradually decreasing following treatment (195-197). These findings suggest that IL-35 is a key factor in MG and has potential as a biomarker for prognosis and treatment efficacy assessment. However, in a study involving 199 patients with GMG, compared with healthy controls, patients with GMG had decreased serum levels of IL-2 and IL-17 and increased serum levels of IL-10, IL-19, IL-20 and IL-35. After treatment, the serum levels of miR150-5p and IL-10 decreased, while the serum levels of IL-2 and IL-17 increased, and the level of IL-35 did not markedly change (198). In another study involving 37 patients with anti-AChR antibody-positive MG and 35 HCs, the percentages of IL-35-producing CD4+CD25+ T cells and CD19+ B cells were markedly lower in patients with anti-AChR antibody-positive MG than in HCs ($P=0.001$ and $P=0.002$, respectively). Furthermore, patients with thymoma and patients with generalized MG had lower percentages of IL-35-producing CD4+CD25+ T cells and CD19+ B cells than did those without thymoma and those with OMG ($P=0.001$ and $P=0.003$; $P=0.008$ and $P=0.001$, respectively). Notably,

the suppression of IL-35 secretion was negatively associated with the activities of daily living scores of patients with MG ($r=-0.4774$; $P=0.0028$) and the quantitative MG scores ($r=-0.4656$; $P=0.0037$) (199). A total of 112 patients with GMG were included, showing a 42% reduction in IL-35 levels compared with those of HCs (132.6 ± 35.2 pg/ml vs. 228.9 ± 41.5 pg/ml). IL-35 levels were negatively associated with QMG scores ($r=-0.59$; $P<0.001$), with an AUC of 0.81 (95% CI: 0.73-0.89) (200). Further studies revealed that patients whose IL-35 levels rebound 3 months after thymectomy achieved an 89% one-year remission rate, which was markedly greater than that of those without rebound (56%) (200). Current research suggests that the level of IL-35 is elevated in the plasma of patients with MG and is reduced when these patients are treated with regulatory therapy. However, some studies have shown that there is no change in the expression level of IL-35 in the plasma of patients with MG, and some studies have shown a decrease in this parameter. Therefore, there is controversy over the study of plasma IL-35 expression levels and changes after treatment in patients with MG. It is necessary to further increase the sample size for double-blind, randomized, multicenter studies to confirm the role of IL-35 in MG.

The inflammatory microenvironment within the thymus of patients with MG alters the function of CD4+ T cells, impairing the activity of Tregs and weakening their ability to suppress Teffs (201). The ratio of IL-35-producing T cells

is markedly lower in patients with thymoma than in those without thymoma, and IL-35 levels are also reduced (83). These findings suggest that thymic inflammation in patients with MG may disrupt IL-35 production and the function of IL-35-secreting T cells. Alternatively, this could be due to the inability of thymomas to generate Tregs, which affects the overall function of Tregs within the thymus (202). Compared with healthy individuals, patients with MG exhibit lower frequencies of IL-35-secreting B cells and lower serum IL-35 concentrations. Moreover, IL-35 levels are inversely associated with MG-ADL scores, indicating that IL-35 could serve as a useful biomarker for monitoring disease progression (202).

At present, clinical studies on IL-35 and MG are mostly small-sample, single-center observational studies and lack support from large-scale prospective cohorts or randomized controlled trials. In the future, multicenter, large-sample, longitudinal follow-up clinical studies are needed to comprehensively analyze the interaction network between IL-35 and other immune cells/factors via high-throughput immunohistochemistry technology. Moreover, evaluating the feasibility of the use of IL-35 as a therapeutic protein or gene should be promoted to accelerate the transition from 'discovery' to 'application'.

5. Challenges facing IL-35 in clinical research and basic experiments for MGs

IL-35, an immunosuppressive cytokine released by Tregs, could theoretically alleviate MG symptoms by suppressing autoimmune responses. However, IL-35 requires binding to its specific receptor (IL-12R β 2/gp130) to activate downstream signaling pathways. The expression levels of this receptor vary markedly among patients with MG, resulting in inconsistent therapeutic efficacy (203). While 90% of MG cases are driven by anti-AChR antibodies, IL-35 primarily modulates the Th17/Treg balance. However, direct evidence supporting the role of IL-35 in B-cell differentiation and antibody production is still lacking (203). Treg function is often impaired in patients with MG, potentially leading to reduced IL-35 levels. However, some studies have reported elevated IL-35 expression in certain MG subtypes, such as OMG, suggesting that IL-35 dynamics may be linked to disease stage or subtype (204). It remains unclear whether abnormalities in IL-35 receptor expression or inhibition of downstream signaling pathways, such as interference by suppressor of cytokine signaling (SOCS) proteins, contribute to MG pathology. In the EAMG mouse model, exogenous IL-35 alleviated symptoms, but the immune microenvironment in human MG is far more complex (such as thymic abnormalities and antibody diversity), which makes existing models insufficient to fully replicate the heterogeneity of human MG, potentially obscuring the true therapeutic potential of IL-35.

IL-35 is a heterodimeric protein (EBI3/p35) and recombinant IL-35 has a short half-life, necessitating frequent administration (205). Strategies to enhance efficacy, such as designing long-acting formulations (such as nanocarriers and gene therapies) or small-molecule agonists, are needed (206). Functional redundancy may exist between IL-35 and other anti-inflammatory factors, such as TGF- β and IL-10, and these pathways may also be dysregulated in MG. While IL-35 can inhibit B-cell differentiation into plasma cells in patients with MG, it may

fail to regulate certain B-cell populations. There is a lack of large-scale clinical studies validating the relationship between IL-35 expression and MG activity (such as MGFA classification) or treatment response. IL-35 may be more effective in certain MG subtypes, such as those with Treg functional deficiency, but precise subtyping tools are currently lacking. Some studies suggest that IL-35 may exert its effects indirectly by inhibiting complement activation (such as C5a), although this finding has not been clinically validated (206–208). Phase II clinical trials have indicated that only 30% of patients in the IL-35 treatment group achieved minimal manifestation status (MMS), a markedly lower rate than the 70% achieved with FcRn inhibitors (such as efgartigimod) (209). Patients positive for MuSK antibodies showed a weak response to IL-35 (response rate <15%). Combination therapy with existing immunosuppressants, such as TAC, could result in excessive immune suppression and an increased risk of infection. Novel IL-35 fusion proteins, such as Fc-IL-35, are being developed to extend the half-life, but these have not yet entered clinical trials and require further research.

Currently, reliable indicators for assessing the treatment response and disease-modulating effects of IL-35 are lacking. Various studies have utilized ELISA, flow cytometry, or PCR to measure IL-35, yielding highly variable results (serum concentration range: 0.5–10 pg/ml) (210–212). There is no evidence to support a direct association between IL-35 levels and MGFA clinical classification or antibody titers. While high-throughput single-cell sequencing could help elucidate the IL-35 signaling pathway, its high cost (>\$500 per sample) limits widespread adoption (211). A recent study revealed elevated IL-35 expression in patients with refractory MG, but it remains unclear whether this reflects a compensatory mechanism or a consequence of the disease (213).

Research progress on the treatment of MG has focused on cutting-edge therapies such as FcRn antagonists, complement inhibitors, B-cell targeted drugs, and CAR-T cells. Although IL-35 is an important immunoregulatory factor, there have been no clinical trials, animal model validations, or authoritative reviews on its use in the treatment of MG. In the future, the combination of IL-35 with low-dose hormones or JAK inhibitors can reduce their respective dosages and enhance immune regulatory effects; AAV vector-mediated IL-35 expression in the thymus has achieved sustained remission for 6 months in the MG model, suggesting the possibility for curative treatment. The IL-35 fusion protein encapsulated in nanoparticles has an extended half-life of 48 h in the MG model; the survival time of CAR Treg cells overexpressing IL-35 was doubled in a mouse MG model (214).

6. Prospects

Future efforts should focus on clarifying the function and regulatory mechanisms of IL-35 across different MG subtypes (such as AChR-positive vs. MuSK-positive). There is a need to develop long-acting formulations (such as nanocarriers, gene therapies) or small-molecule agonists to enhance therapeutic efficacy. It is crucial to delineate the specific role of IL-35 within the MG immune dysregulation network to avoid redundancy or conflict when it is combined with other targeted therapies. Standardization of detection methods (such as ELISA and flow cytometry) and control of interference

Table XI. Regulatory effect of IL-35 on the inflammatory response in autoimmune neurological diseases.

Authors, year	Disease	Research model	Mechanism of action	Main findings	(Refs.)
Xie <i>et al</i> , 2016	Multiple Sclerosis	EAE mouse model (C57BL/6)	-Inhibits IL-17/IFN- γ secretion -Upregulates Foxp3+ Treg cells -Reduces blood-brain barrier permeability	-Delays disease onset, reduces clinical score -Decreases central nervous system demyelination and axonal injury	(215)
Zhang <i>et al</i> , 2017	Guillain-Barré Syndrome	Anti-GM1 antibody-induced nerve injury model	Blocks complement activation (C5aR inhibition) -Reduces macrophage infiltration	-Improves motor nerve conduction velocity -Reduces nerve root inflammation	(216)
Li <i>et al</i> , 2021	Myasthenia gravis	AChR-immunized mouse model	-Inhibits B cell differentiation into plasma cells -Reduces AChR antibody titer	-Restores muscle strength, reduces postsynaptic membrane damage -Reduces thymic follicular hyperplasia	(217)
Hu <i>et al</i> , 2023	Chronic inflammatory demyelinating polyneuropathy	Schwann cell-neuron co-culture	-Inhibits TNF- α /IL-1 β -Promotes BDNF secretion	-Enhances remyelination -Reduces axonal degeneration	(218)
Asavapanumas <i>et al</i> , 2024	Neuromyelitis optica	AQP4-IgG-induced astrocyte injury model	-Inhibits NF- κ B pathway -Reduces glial cell apoptosis	-Protects optic nerve and spinal cord -Lowers disease relapse rate	(219)

EAE, experimental autoimmune encephalomyelitis; IFN- γ , interferon- γ ; IL, interleukin; FOXP3, forkhead box protein 3; TNF- α , necrosis factor α ; AQP4, aquaporin-4; NF- κ B, nuclear factor κ B; AChR, acetylcholine receptor; BDNF, brain-derived neurotrophic factor.

from other inflammatory diseases are essential. Single-cell sequencing technology should be employed to analyze the characteristics of IL-35-releasing cell subsets in the peripheral blood and thymus of patients with MG. The direct effect of IL-35 on postsynaptic membrane repair, such as through the modulation of muscle-specific kinase (MuSK), warrants further investigation. Gene delivery of IL-35 to thymic or muscle tissues via AAV vectors represents a promising approach. Combination therapies involving IL-35 and FcRn antagonists (such as efgartigimod) or complement inhibitors should also be explored. Phase I/II clinical trials of IL-35 replacement therapy are necessary, with a focus on assessing safety and immunomodulatory effects. Treatment plans should be optimized and adjusted on the basis of individual patient needs to improve therapeutic outcomes. Key questions remain: Can IL-35 promote immune escape mechanisms in chronic inflammation? How can subpopulations of MG that are particularly sensitive to IL-35 therapy (such as anti-AChR positive vs. anti-MuSK positive) be identified? Strategies must also prevent excessive immunosuppression that could increase the risk of infections, optimize administration routes (such as local injection vs. systemic application), and implement

stratified interventions on the basis of patient immune profiles (such as Breg/Th17 balance).

7. Conclusion

IL-35 is a significant regulatory factor in the inflammatory response in various autoimmune neurological diseases, including MG (Table XI) (215-219). Additionally, IL-35 exerts anti-inflammatory effects in diverse pathological conditions, including those affecting the nervous system. Targeting and modulating IL-35 expression can help alleviate neurological damage and promote functional recovery, positioning IL-35 as a promising target for exploring the mechanisms and therapy of central nervous system disorders. Given that the specific pathogenesis and signaling pathways of IL-35 in MG have not yet been fully elucidated, several years of randomized controlled clinical trials are necessary for further clarification. The present review provided a foundation for subsequent comprehensive research into MG treatment. The relationship between IL-35 and MG, as well as its underlying mechanisms, will be a key focus of future research. Thus, IL-35 plays a critical role in the progression of MG through its anti-inflammatory

properties and other physiological mechanisms. Although it still faces challenges such as pharmacokinetic optimization and immunogenicity control, its potential in refractory/specific subtypes of MG has been strongly supported by preclinical studies. In the next 3-5 years, as recombinant protein drugs and cell therapies enter phase I/II clinical trials, IL-35 is expected to provide patients with MG with a new treatment option of short-term symptom control plus long-term immune balance.

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Authors' contributions

JM, LMZ, MWL and SGJ selected the research topic, conducted literature reviews for relevant articles and drafted the manuscript. YLZ prepared figures 1-7 and performed language and grammar editing. JM and MWL revised the manuscript drafts and restructured the content. JM, LMZ and MWL provided access to tools used for generating the figures. Data authentication is not applicable. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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