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Immunomodulatory cytokine interleukin-35 and immune thrombocytopaenia

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Abstract

Considerable attention has been paid to interleukin (IL)-35 because of its immunosuppressive effects in a variety of autoimmune diseases. IL-35, a recently identified cytokine of the IL-12 family, is a negative regulatory factor secreted by IL-35-inducible regulatory T cells (iTr35 cells) and the recently reported regulatory B cells (B_{reg} cells). Four biological effects of IL-35 have been discovered *in vitro* and *in vivo*: (i) suppression of T cell proliferation; (ii) conversion of naive T cells into iTr35 cells; (iii) downregulation of type 17 helper T (T_h17) cells; and (iv) conversion of B_{reg} cells into a B_{reg} subset that produces IL-35 and IL-10. IL-35 plays an important role in a variety of autoimmune diseases, such as rheumatoid arthritis, allergic asthma and systemic lupus erythematosus. Primary immune thrombocytopaenia (ITP), which is characterized by isolated thrombocytopaenia and mild mucocutaneous to life-threatening bleeding, is an autoimmune disease with complex dysregulation of the immune system. Both antibody-mediated and/or T cell-mediated platelet destruction are key processes. In addition, impairment of T cells and cytokine imbalances have now been recognized to be important. This review summarizes the immunomodulatory effects of IL-35 and its role in the pathogenesis of ITP as mediated by T and B cells.

Keywords

Interleukin-35, IL-35-inducible regulatory T cells, primary immune thrombocytopaenia

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Introduction

Since it was first identified in 2007, interleukin (IL)-35 has become the focus of considerable attention as it is an important immunosuppressive cytokine. IL-35 is a member of the IL-12 family of

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heterodimeric cytokines, which also comprises IL-12, IL-23 and IL-27 cytokines.[1,2] IL-12 and IL-23 have the same β chain (P40) and different α chains: P35 and P19, respectively.[3] IL-12 and IL-23 are proinflammatory cytokines that induce type 1 helper T (T_h1) cells and T_h17 cells, respectively.[3] IL-27 consists of a heterodimer of P28 and Epstein–Barr virus-induced gene 3 (EBI3); and some research has demonstrated that IL-27 is an immunoregulatory cytokine, although it was initially thought to be a proinflammatory cytokine.[4] The biological function of IL-35 has been directly shown to include the following: suppression of the proliferation of conventional T cells (T_{conv} cells) and conversion of naive T_{conv} cells into a strongly suppressive induced T_{reg} cell population known as iTr35 cells.[3,5,6] These iTr35 cells mediate immunosuppressive function via IL-35 and IL-10 but not via the inhibitory cytokine transforming growth factor (TGF)-β.[1,6]

Primary immune thrombocytopaenia (ITP) is an acquired immune disorder characterized by both platelet destruction and impaired megakaryocyte and platelet production.[7] The pathogenesis of the immune dysregulation resulting in ITP is not completely understood and likely quite complex. Currently, ITP is known to be primarily due to immunoglobulin G (IgG) autoantibodies opsonizing the individual's platelets, resulting in markedly enhanced Fc receptor (FcR)-mediated phagocytosis and destruction by macrophages.[8] Research has shown the presence of activated platelet-specific autoreactive T cells that recognize and respond to autologous platelet antigens and drive the generation of platelet reactive autoantibodies by B cells.[9] A proinflammatory T_h1, T_h17, and T_h22 cytokine milieu predominates in many ITP patients that together promote macrophage function, autoreactive B cell development and T cell cytotoxicity.[10]

Previous research has shown that the negative regulator IL-35, as an immunosuppressive cytokine, could inhibit the effect of T_{conv}/B_{conv} cells and convert them into T_{reg}/B_{reg} cells that induce immunoregulatory factors such as IL-35 and IL-10, inhibit T_h1 and T_h17 cells and inhibit the secretion and function of IL-17 and other inflammatory factors.[11] Studies in mice have demonstrated that IL-35 plays key roles in autoimmune diseases,[1,12] allergic diseases[13] and other diseases such as infections.[14,15] As such, IL-35 offers a unique target for therapies aimed at treating autoimmune diseases. This current review focuses on the relationship between IL-35 and ITP by describing the structure, pathways and biological function of IL-35 and summarizes the research status of ITP pathogenesis. Based on the function of IL-35 in regulating the immune system, the review describes the direct and circumstantial effects of IL-35 in the pathogenesis of ITP.

The immunosuppressive cytokine IL-35

iTr35 cells: the main source of IL-35

Research has demonstrated that EBI3 and p35 are highly expressed by mouse Foxp3+ T_{reg} cells but not by resting or activated effector CD4+ T cells.[16] Therefore, it was initially believed that IL-35 was mainly secreted directly by $CD4+CD25+Foxp3+T_{reg}$ cells.[1] As research progressed, a new type of IL-35-induced T_{reg} cell, known as iTr35 cells,[6] was found to be the primary type of cell that secretes IL-35, independent of TGF-β, IL-10 and Foxp3.[15] Research identified a novel regulatory T cell type called the "iTr35 cell (IL-35+CD4+IL-10–TGF-β–Foxp3–)", which may be a new member of the existing regulatory T cell family.[1,6,17] T_{reg} cells, a unique subset of CD4+ T cells, normally comprises only about 4% of CD4+ T cells in adult peripheral blood.[18]

According to the cytokines that induce them, three types of iT_{reg} cells have been described: iTr-TGF-β, iTr-IL-10 and iTr35 cells.[15] iTr35 cells do not express Foxp3, which is different from iTr-TGF-β.[15] In addition, iTr35 cells were more stable and retained greater immunosuppressive capacity than IL-10- or TGF-β-induced iT_{reg} cells *in vivo*.[6,13] In addition, CD8+ T_{reg}, B_{reg} and tolerogenic dendritic cells (DCs) are identified as new cellular sources of IL-35.[19]

Composition and signalling pathway

Similar to other members of the IL-12 family, IL-35 also consists of two subunits, an α chain (p35) and a β chain (EBI3),[3] but the expression of the two subunits is different. In contrast to the subunits of IL-12 and IL-23, which are secreted as covalently linked heterodimers, IL-12p35 and EBI3 are secreted as independent subunits and research indicates that the two subunits associate during inflammatory conditions to form the bioactive heterodimer (Figure 1).[20]

The receptor for IL-35 (IL-35R) is composed of two subunits, IL-12Rβ2 and gp130.[3] A previous study found that IL-35 signalled through a unique heterodimer of the receptor chains IL-12Rβ2 and gp130 or homodimers of each chain; and the complete IL-12Rβ2-gp130 receptor was required for maximal suppression.[1] IL-35R is composed of IL12Rβ2 and gp130, which subsequently activate signal transducers and activators of transcription (STAT) 1 and STAT4 signalling pathways.[21] However, the signalling process of IL-35 is significantly different in B cells.[22] A previous study demonstrated that in B cells, interference of gp130 expression or neutralization of gp130 with antibodies did not affect the IL-35-induced inhibition of B cell proliferation and IL-10 induction.[22] A study employing small interfering RNA to silence each subunit individually showed that IL-35 signalling in B cells occurs through IL-27Rα and IL-12Rβ2, which activate STAT1 and STAT3 (Figure 1).[12]

Biological function

The predominant mechanism of suppression associated with the activity of IL-35 is its ability to suppress T cell proliferation and effector functions. For example, experiments in IL-12a–/– and EBI3–/– mice have shown that CD4+ T_{reg} cells have a significantly reduced ability to inhibit T cell proliferation.[1,6] Further studies showed that IL-35 can inhibit the secretion of proinflammatory cytokines by T cells, such as interferon (IFN)-γ, IL-12 and IL-17.[23] In addition, IL-35 inhibits the function of T_h1 and T_h17 cells by promoting the proliferation of T_{reg} cells and upregulating the expression of IL-10, while T_{reg} cells can promote the expression of IL-35 via a positive feedback loop to participate in immunoregulation and inhibit the differentiation of CD4+ T cells into T_h17 and T_h1 cells.[24,25] It is worth mentioning that IL-35 can significantly reduce T_h2 effector cells.[26] IL-35 inhibits proinflammatory cells and cytokines, increases the secreting of IL-10 and TGF-β and promotes the production of T_{reg} and B_{reg} cells, thus playing an immunomodulatory role (Figure 2).[15]

In addition, one study identified IL-35-producing B cells as critical regulators of immunity.[22] Humoral immunity has also been shown to be suppressed by IL-35, which induces the conversion of human B cells into regulatory B cells that produce IL-35 as well as IL-10.[14] This IL-35+ B_{reg}-mediated protection was dependent on increased induction and expansion of endogenous B_{reg} cells and Foxp3+ T_{reg} cells and inhibition of the expansion of proinflammatory T_h1 and T_h17 cells (Figure 2).[22,27]

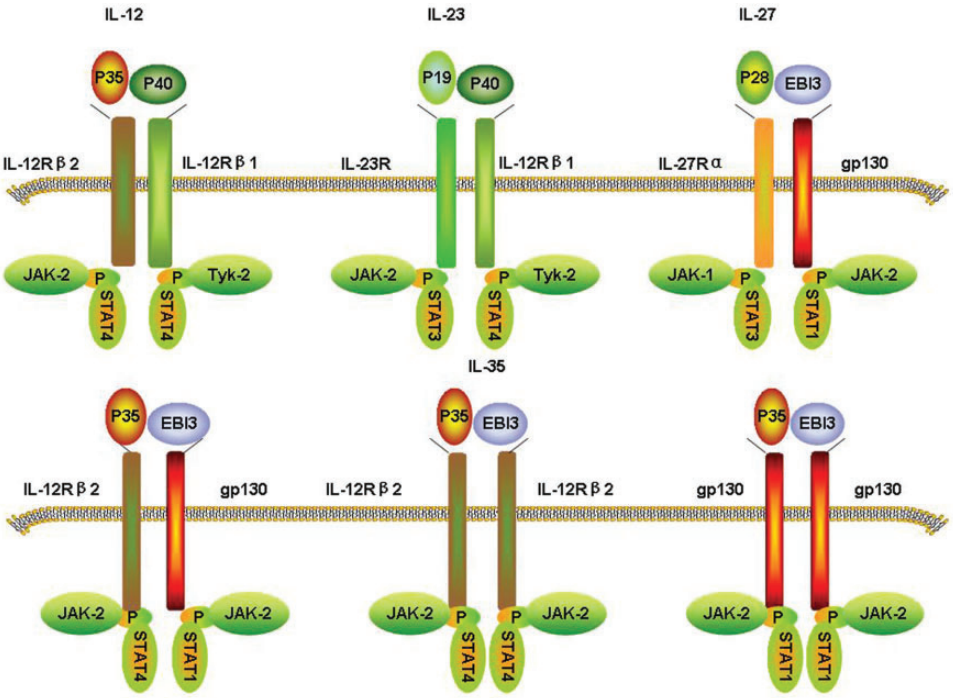

Figure 1. Interleukin (IL)-12 family members, their corresponding receptors and regulation of downstream signalling pathways. If both IL-12Rβ2 and gp130 in T cells are absent, the inhibition of IL-35 would be completely eliminated; if only one of the two is missing, then the inhibition of IL-35 would be partially suppressed. As members of the IL-12 family, IL-12, IL-23, IL-27 and IL-35 are heterodimeric, sharing subunits and their corresponding receptors. Each member is composed of an α chain (p19, p28 or p35) and a β chain (p40 or Epstein–Barr virus-induced gene 3 [EBI3]) whose expression is regulated independently. Receptors for the IL-12 family cytokines also share subunits. The IL-35 receptor consists of IL-12Rβ2/gp130 heterodimer or homodimer. IL-12Rβ2 is a component of the IL-12 receptor, whereas gp130 is a component of the IL-27 receptor. Upon receptor binding, cytokines transmit their signals through activation of members of the Janus activating kinase (JAK) family and activation and nuclear translocation of signal transducer and activator of transcription (STAT) family members. The colour version of this figure is available at: http://imr.sagepub.com.

IL-35 and autoimmune disease

As an inhibitory cytokine, IL-35 plays an important regulatory role in autoimmune diseases. For example, in allergic asthma murine models, IL-35 infusion can effectively reduce the severity of airway sensitivity, reduce the number of inflammatory cells in bronchoalveolar lavage fluid and the levels of IL-4, IL-5, IL-13 and IL-17, increase T_{reg} cells in lung tissues and inhibit excessive airway mucus secretion.[13] A previous study found that the serum IL-35 level and the percentage of CD4+ EBI3+ T cells were dramatically decreased and negatively correlated with the systemic lupus erythematosus (SLE) disease activity index score in patients with SLE, suggesting that IL-35 and CD4+ EBI3+ T cells play protective roles in patients with active SLE.[28] A study using a mouse model of SLE found that plasma proinflammatory factors (e.g. IFN, TNF, IL-6 and IL-17) in mice treated

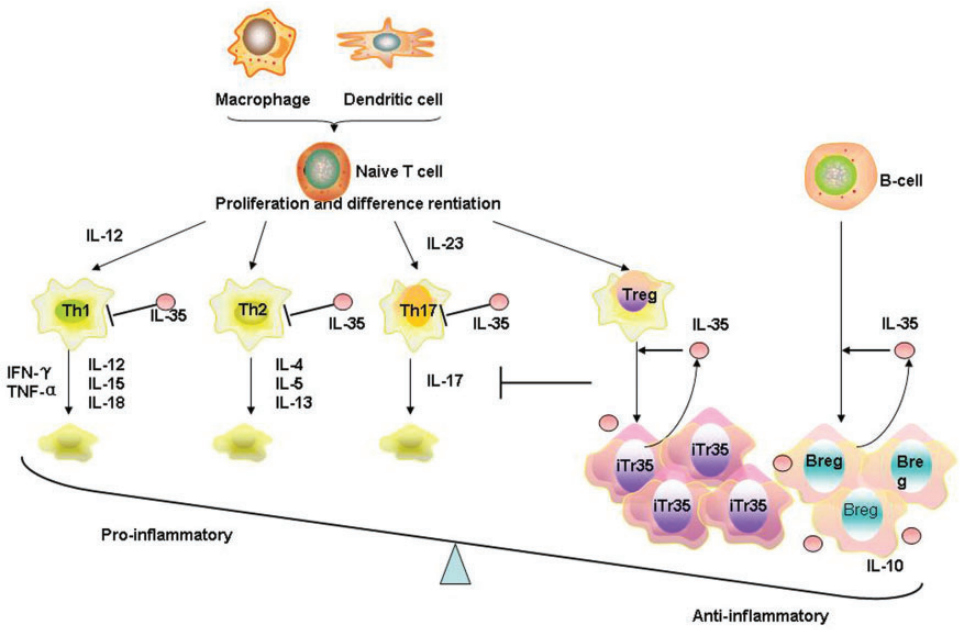

Figure 2. Interleukin (IL)-35 regulates T and B cell-mediated pro-inflammatory and anti-inflammatory immune responses. IL-35 suppresses conventional T cells and promotes their conversion to IL-35-inducible regulatory T cells (iTr35 cells), which play a vital role in immune regulation as they inhibit various immune responses due to the suppressive effects of IL-35. IL-35 can contribute to the induction of iTr35 cells and regulatory B (B_{reg}) cell populations and induces anti-inflammatory effects via the inhibition of type 1 helper T (T_h1) cell, T_h2 cell and T_h17 cell responses. The black arrows indicate positive effects and the T-shaped end indicates negative effects. IFN, interferon; TNF, tumour necrosis factor. The colour version of this figure is available at: http://imr.sagepub.com.

with IL-35 were decreased and anti-inflammatory cytokines (IL-10 and IL-2) were increased.[29] Research using EBI3–/– mice has shown that EBI3 deficiency reinforces T_h17 and T_h1 cell responses in the central nervous system, increases T cell production of IL-12 and IL-17 in peripheral lymphoid organs and enhances the development of experimental autoimmune encephalitis.[30] In conclusion, IL-35 plays an immunosuppressive role in autoimmune diseases mainly by increasing T_{reg} cells and inhibiting the differentiation of T_h17 and T_h1 cells, providing new ideas and targets for the treatment of autoimmune diseases.

Potential relationship between IL-35 and ITP

Pathogenesis of ITP

According to the International Working Group, ITP is divided into two types: primary ITP and secondary ITP.[8] Primary ITP is defined as isolated thrombocytopaenia in the absence of other causes or disorders that may be associated with thrombocytopaenia; and secondary ITP is defined as a secondary disorder, this might include thrombocytopaenia secondary to systemic lupus erythematosus, hepatitis C infection or lymphoproliferative disorders.[21] Drugs,

autoimmune diseases and chronic infections can lead to ITP through different mechanisms, so this review mainly will focus on the pathogenesis of primary ITP. Determination of the concentrations of IL-35 in serum samples collected from patients with ITP showed decreased plasma IL-35 levels compared with the levels in patients with ITP in remission and control individuals.[12] The remainder of the review will briefly describe the potential role of IL-35 in the development and treatment of primary ITP.

IL-35 and B cells: participating in the pathogenesis of primary ITP

Classically, ITP is primarily due to IgG autoantibodies opsonizing the individual's platelets, resulting in markedly enhanced FcR-mediated phagocytosis and destruction by macrophages in the reticuloendothelial system within the spleen.[31] Several studies demonstrated that the frequency of circulating B cells secreting anti-glycoprotein (GP) IIb/IIIa antibody was significantly increased in ITP patients.[32] B cells secreting anti-GP IIb/IIIa antibody in the peripheral blood of ITP patients are mostly platelet-reactive plasma cells (PCs) that are released from the spleen after activation through antigen-specific interaction.[33] Significantly, with CD4+T cell help, B cells are able to differentiate into platelet-reactive PCs that can secrete autoantibodies.[34] Furthermore, GPIIb heterodimer and GPIb/IX complex were also expressed on megakaryocytes during the early stages of differentiation.[7] The autoantibodies, specifically bound to platelet antigens, can fix complement on platelet and megakaryocyte (MK) membranes, triggering cell destruction through the complement system.[35] Autoantibodies accelerate platelet clearance by removal via splenic macrophages and DCs, complement deposition and platelet apoptosis or by inhibiting megakaryocytic platelet production.[33]

Interleukin-35 suppressed the proliferation of primary mouse B cells and induced the expansion of IL-10-producing CD19+B220hiCD5–B cells (IL10-B_{reg} cells) and IL-35-producing B cells.[36] IL-35 suppressed the production of IgE by B cells stimulated with IL-4, IL-6 and IL-13 production by T_h2 and converted T_h2 cells into suppressive iTr35 cells.[37] In addition, B_{reg} cells can inhibit the production of CD4+ T cells at least in part via IL-10, TGF-β and IL-35 secretion and play a regulatory role through direct interactions with effector T cells and monocytes.[38,39] Meanwhile, IL-35 exerts an immunosuppressive effect by reducing infiltration of local macrophages and the ratio of inflammatory M1 macrophages to anti-inflammatory M2 macrophages.[40] A previous study demonstrated that the number of B_{reg} cells was positively correlated with both the T_{reg} count and the T_{reg}/T_h17 ratio, suggesting that the ability of these cells to regulate functional T cell subsets might be impaired in ITP patients (Figure 3).[14]

IL-35 and T cells: participating in the pathogenesis of primary ITP

In patients with ITP with no identifiable anti-platelet autoantibodies, cytotoxic T lymphocytes (CTLs) have been shown to increase both platelet and MK lysis, suggesting the role of these cells in mediating thrombocytopaenia.[34] Apoptosis and perforin/granzyme-mediated cytotoxicity constitute the main pathway used by CTLs to destruct autologous platelets.[41] It was previously shown that CTLs stimulated with anti-CD3 were capable of inducing autologous platelet lysis and desialylation.[42] In addition, the interaction of upregulated FasL and TNF-a with their respective receptors on the surface of

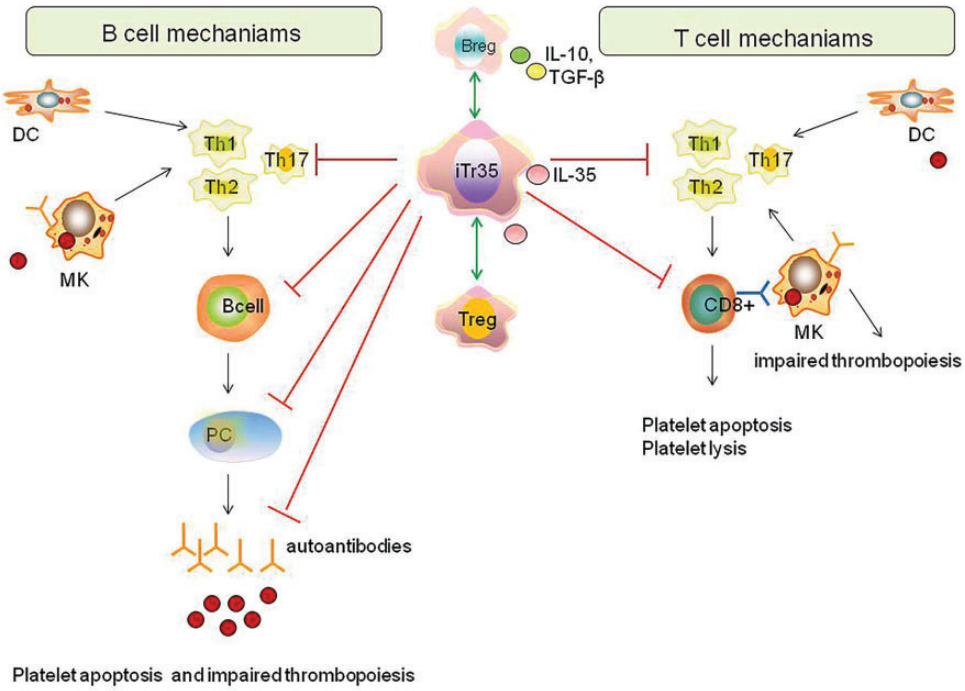

Figure 3. The effect of interleukin (IL)-35 on the pathogenesis of primary immune thrombocytopaenia (ITP) is mediated by T and B cells. B cells require the help of helper T (T_h) cells to efficiently develop into antibody-secreting plasma cells. CD8+ T cells have been shown to directly lyse platelets, induce platelet apoptosis and inhibit thrombopoiesis by megakaryocytes (MK). These responses are based on macrophages and dendritic cells (DC) phagocytizing platelet fragments and presenting them to T_h cells. Regulatory T (T_{reg}) and B cells (B_{reg}) are important regulators that keep both B- and T cell-mediated autoimmunity in check. IL-35 (an immunosuppressive cytokine) and IL-35-inducible regulatory T cells (iTr35 cells; a new regulatory T cell) can inhibit the proliferation and function of T_h1, T_h2 and T_h17 cells, suppress cytotoxic activity and promote T_{reg} and B_{reg} cells. The arrows indicate positive effects and the T-shaped ends indicate negative effects. PC, platelet-reactive plasma cells. The colour version of this figure is available at: http://imr.sagepub.com.

target cells may result in apoptosis of autologous platelets.[41] Furthermore, CTLs can further inhibit platelet production by inhibiting MK apoptosis.[43] Many factors can cause CTL dysfunction or exhaustion and one of these factors is the inhibitory function of T_{reg} cells.[25] The elevated IL-35 levels in chronic hepatitis B enhanced the suppressive function of CD4+CD25+ T_{reg} cells, while reduced cytolytic and noncytolytic activity of hepatitis B antigen-specific CD8+ T cells, which might be due to the direct response and positive feedback mechanisms of T_{reg} cells to IL-35.[44] Research shows that IL-35 suppresses the expansion of CTLs and inhibits antigen-specific IFN-γ secretion by CTLs.[45] In addition, IL-35 may inhibit the proliferation of effector T cells by blocking the cell cycle, causing G1 phase arrest and inhibiting the proliferation of immature T cells.[36] A series of studies have found that CD4+ T cells with the IL-35 gene modified can achieve immunosuppressive effects by

inhibiting the proliferation of other T cells, including CD4+ T cells and CD8+ T cells.[1,24]

As CTLs are also dependent on the help of T cells to efficiently perform effector functions, the polarization of T_h cells probably also affects the CTL response. The experimental results showed that the T_{reg}/T_h17 cell balance changed in favour of T_h17 cells in patients with ITP, implying an association between elevated T_h17 cell numbers and the development of ITP.[46] T_h17 cells, like T_{reg} cells, differentiate from CD4+ T cells in the presence of IL-6 and TGF-β;[23] and the secretion of IL-17 stimulates the production of pro-inflammatory cytokines such as IL-1, IL-6 and IFN-γ leading to anti-platelet antibody production in patients with ITP.[47] In animal models, approximately 40% of T_{reg}-deficient mice developed thrombocytopaenia and thrombocytopaenic mice produced IgG anti-platelet antibodies that mainly targeted GPIb/IX.[10] Several mechanisms have been implicated in the suppressive activity of T_{reg} cells, including secretion of immunosuppressive cytokines IL-10 and TGF-β,[16] as well as the down-regulation of surface cytotoxic T lymphocyte antigen-4.[48] Significantly, T_{reg} cells are an important regulator of infectious tolerance via the ability to convert T_{conv} cells into iT_{reg} cells directly by producing suppressive cytokines, such as IL-10, TGF-β, or the newly identified IL-35.[44] iTr35 cells showed stronger and more durable immunosuppression than T_{reg} cells.[49] Thus, while T_{reg} cells play a fundamental role in the maintenance of immune tolerance to prevent autoimmune disease, T_h17 cells play the opposite role.[50,51] IL-35 intervention in CD4+ T cells inhibits the expression of STAT3 protein, RORgt mRNA and IL-17A mRNA and up-regulates STAT5b levels, supporting the conclusion that IL-35 inhibits the function of T_h17 cells.[36]

The responses of T_h1 cells, characterized primarily by IL-2, IFN-γ, TNF-α and TNF-β production, are involved in response to intracellular pathogens and generally promote pro-inflammatory, complement fixing phenotypes.[52] In contrast, T_h2 cells, characterized by IL-4, IL-5, IL-6 and IL-13 cytokine production, function in the fight against extracellular pathogens; and typically elicit an immediate-type hypersensitivity response.[53] T_h1 cells produce IFN-γ and are mainly involved in macrophage activation.[52] In contrast, ITP patients have a T_h1 profile and T_h1 dominance that not only promotes antibody production, but it also facilitates cell-mediated cytotoxicity.[42,54] T_h2 cells produce conventional cytokines IL-4, IL-5 and IL-13 that are essentially responsible for the induction of effector cells and the induction of B cells to produce allergen specific IgE antibodies.[55] Previous research showed that IL-35 inhibited the allergic T cell response and had the ability to modulate the production of IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, IL-23, IL-27 and TNF-α in mice with autoimmune disease.[23,28] Moreover, IL-35 blocks T_h2 development by repressing GATA3 and IL-4 expression and limiting T_h2 proliferation.[56] IL-35 can also mediate the conversion of T_h2 cells to T_{reg} cells, although this can be blocked by IFN-γ.[56] Furthermore, IL-35, via thymic stromal lymphopoetin, also inhibited DC priming of T_h2 responses.[37]

Imbalance between IL-35 and other cytokines

Interleukin-12, IL-23 and IL-27 are also members of the same cytokine family as IL-35; and each has been demonstrated to be involved in the development of T cells.[16] IL-12 and IL-23 can induce and promote the generation of proinflammatory T_h1 and T_h17 cells.[3] IL-23 can promote a T_h17 response and macrophage apoptosis; and IL-27 can inhibit macrophage activation.[57]

Recent data suggested that IL-27 could inhibit platelet destruction by negatively regulating CTL cytotoxicity toward autologous platelets in ITP.[58] The plasma levels of IL-12, IL-23, IL-27, IFN-γ and IL-17 were significantly increased in patients with ITP with compared with the levels in normal controls, while the levels of IL-4, IL-10 and IL-35 were considerably lower than the levels in controls.[59] It may be that IL-12 competitively inhibits the IL-35 receptor pathway, thereby participating in the immunosuppressive effect of IL-35 and facilitating the development of ITP.

IL-35: could it be a marker of ITP

In particular, the plasma IL-35 concentration was positively correlated with platelet count, suggesting that IL-35 may be a biomarker that reflects the activity of ITP.[12] The first-line treatment of ITP includes corticosteroids and intravenous immunoglobulins.[60] The second-line therapies include rituximab, thrombopoietin and splenectomy.[60] After high-dose dexamethasone, the plasma levels of IL-12p70, IL-23, IL-27, IFN-γ and IL-17A were significantly decreased in patients with ITP compared with patients before high-dose dexamethasone treatment.[61] Conversely, the levels of IL-4, IL-10 and IL-35 in patients with ITP were considerably higher than those before treatment.[61] Similarly, intravenous immunoglobulin treatment corrects the peripheral deficiency of T_{reg} cells, and increases IL-35 levels and platelet counts.[62] These findings suggest that IL-35 levels might be used to evaluate the effectiveness of clinical treatment and predict disease severity.

Conclusion

Interleukin-35 is a negative regulator that functions mainly by inhibiting T_h1 and T_h17 cells, promoting the transformation of naive T cells into T_{reg} cells, inhibiting the secretion and function of IL-17 and other inflammatory factors; and promoting and cooperating with the immunosuppressive factor IL-10. Data from murine models and human studies have established that IL-35 has an immunosuppressive effect on autoimmune diseases. Although the fundamental mechanisms of IL-35 function remain unclear, it has been confirmed that serum IL-35 levels are elevated in normal individuals and correlate with increased platelet count. Further studies are needed to assess the importance of the IL-35 signalling pathway in human disease. Nevertheless, IL-35 plays a role in controlling effector immunity and may therefore constitute a treatment target in primary ITP.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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