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 (iTregs) and the recently reported regulatory B cells (Bregs). 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 iTregs; (iii) downregulation of type 17 helper T (Th17) cells; and (iv) conversion of Bregs into a Breg 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

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...
heterodimeric cytokines, which also comprises IL-12, IL-23 and IL-27 cytokines.\(^1,2\) IL-12 and IL-23 have the same \(\beta\) chain (P40) and different \(\alpha\) 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)-\(\beta\).\(^1,6\)

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-\(\beta\), IL-10 and Foxp3.\(^3,15\) Research identified a novel regulatory T cell type called the “iTr35 cell (IL-35+CD4+IL-10–TGF-\(\beta\)–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 iTreg cells have been described: iTreg-TGF-β, iTreg-IL-10 and iTreg35 cells. In addition, iTreg35 cells do not express Foxp3, which is different from iTreg-TGF-β. In addition, iTreg35 cells were more stable and retained greater immunosuppressive capacity than IL-10- or TGF-β-induced iTreg cells in vivo. In addition, CD8+ Treg, Breg and tolerogenic dendritic cells (DCs) are identified as new cellular sources of IL-35.

**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), 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).

The receptor for IL-35 (IL-35R) is composed of two subunits, IL-12Rβ2 and gp130. 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. IL-35R is composed of IL12Rβ2 and gp130, which subsequently activate signal transducers and activators of transcription (STAT) 1 and STAT4 signalling pathways. However, the signalling process of IL-35 is significantly different in B cells. 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. 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).

**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+ Treg cells have a significantly reduced ability to inhibit T cell proliferation. 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. In addition, IL-35 inhibits the function of Th1 and Th17 cells by promoting the proliferation of Treg cells and upregulating the expression of IL-10, while Treg 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 Th17 and Th1 cells. It is worth mentioning that IL-35 can significantly reduce Th2 effector cells. IL-35 inhibits proinflammatory cells and cytokines, increases the secreting of IL-10 and TGF-β and promotes the production of Treg and Breg cells, thus playing an immunomodulatory role (Figure 2).

In addition, one study identified IL-35-producing B cells as critical regulators of immunity. 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. This IL-35+ Breg-mediated protection was dependent on increased induction and expansion of endogenous Breg cells and Foxp3+ Treg cells and inhibition of the expansion of proinflammatory Th1 and Th17 cells (Figure 2).
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...
with IL-35 were decreased and anti-inflammatory cytokines (IL-10 and IL-2) were increased. Research using EBI3–/– mice has shown that EBI3 deficiency reinforces Th17 and Th1 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. In conclusion, IL-35 plays an immunosuppressive role in autoimmune diseases mainly by increasing Treg cells and inhibiting the differentiation of Th17 and Th1 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. Primary ITP is defined as isolated thrombocytopenia in the absence of other causes or disorders that may be associated with thrombocytopenia; and secondary ITP is defined as a secondary disorder, this might include thrombocytopenia secondary to systemic lupus erythematosus, hepatitis C infection or lymphoproliferative disorders. 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. 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. Several studies demonstrated that the frequency of circulating B cells secreting anti-glycoprotein (GP) IIb/IIIa antibody was significantly increased in ITP patients. 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. Significantly, with CD4+ T cell help, B cells are able to differentiate into platelet-reactive PCs that can secrete autoantibodies. Furthermore, GPIIb heterodimer and GPIb/IX complex were also expressed on megakaryocytes during the early stages of differentiation. The autoantibodies, specifically bound to platelet antigens, can fix complement on platelet and megakaryocyte (MK) membranes, triggering cell destruction through the complement system. Autoantibodies accelerate platelet clearance by removal via splenic macrophages and DCs, complement deposition and platelet apoptosis or by inhibiting megakaryocytic platelet production. 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. IL-35 suppressed the production of IgE by B cells stimulated with IL-4, IL-6 and IL-13 production by T_{h}2 and converted T_{h}2 cells into suppressive iTr35 cells. 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. 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. 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_{h}17 ratio, suggesting that the ability of these cells to regulate functional T cell subsets might be impaired in ITP patients (Figure 3).

**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 thrombocytopenia. Apoptosis and perforin/granzyme-mediated cytotoxicity constitute the main pathway used by CTLs to destruct autologous platelets. It was previously shown that CTLs stimulated with anti-CD3 were capable of inducing autologous platelet lysis and desialylation. In addition, the interaction of upregulated FasL and TNF-a with their respective receptors on the surface of
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\(_{\text{reg}}\) cells.\(^{25}\) The elevated IL-35 levels in chronic hepatitis B enhanced the suppressive function of CD4\(^+\)CD25\(^+\) T\(_{\text{reg}}\) 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\(_{\text{reg}}\) cells to IL-35.\(^{44}\) Research shows that IL-35 suppresses the expansion of CTLs and inhibits antigen-specific IFN-\(\gamma\) 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

Figure 3. The effect of interleukin (IL)-35 on the pathogenesis of primary immune thrombocytopenia (ITP) is mediated by T and B cells. B cells require the help of helper T (T\(_{\text{h}}\)) 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\(_{\text{h}}\) cells. Regulatory T (T\(_{\text{reg}}\)) and B cells (B\(_{\text{reg}}\)) 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 (iT\(_{\text{reg}}\)) cells; a new regulatory T cell) can inhibit the proliferation and function of T\(_{\text{h}}\), T\(_{\text{reg}}\), Th2 and Th17 cells, suppress cytotoxic activity and promote T\(_{\text{reg}}\) and B\(_{\text{reg}}\) 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.
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 Th cells probably also affects the CTL response. The experimental results showed that the Treg/Th17 cell balance changed in favour of Th17 cells in patients with ITP, implying an association between elevated Th17 cell numbers and the development of ITP.46 Th17 cells, like Treg 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 Treg-deficient mice developed thrombocytopenia and thrombocytopenic mice produced IgG anti-platelet antibodies that mainly targeted GPIb/IX.10 Several mechanisms have been implicated in the suppressive activity of Treg 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, Treg cells are an important regulator of infectious tolerance via the ability to convert Tconv cells into iTreg cells directly by producing suppressive cytokines, such as IL-10, TGF-β, or the newly identified IL-35.44 iTreg cells showed stronger and more durable immunosuppression than Treg cells.49 Thus, while Treg cells play a fundamental role in the maintenance of immune tolerance to prevent autoimmune disease, Th17 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 Th17 cells.36

The responses of Th1 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, Th2 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 Th1 cells produce IFN-γ and are mainly involved in macrophage activation.52 In contrast, ITP patients have a Th1 profile and Th1 dominance that not only promotes antibody production, but it also facilitates cell-mediated cytotoxicity.42,54 Th2 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 Th2 development by repressing GATA3 and IL-4 expression and limiting Th2 proliferation.56 IL-35 can also mediate the conversion of Th2 cells to Treg cells, although this can be blocked by IFN-γ.56 Furthermore, IL-35, via thymic stromal lymphopoietin, also inhibited DC priming of Th2 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 Th1 and Th17 cells.3 IL-23 can promote a Th17 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{12} The first-line treatment of ITP includes corticosteroids and intravenous immunoglobulins.\textsuperscript{60} The second-line therapies include rituximab, thrombopoietin and splenectomy.\textsuperscript{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.\textsuperscript{61} Conversely, the levels of IL-4, IL-10 and IL-35 in patients with ITP were considerably higher than those before treatment.\textsuperscript{61} Similarly, intravenous immunoglobulin treatment corrects the peripheral deficiency of T\textsubscript{reg} cells, and increases IL-35 levels and platelet counts.\textsuperscript{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\textsubscript{h}1 and T\textsubscript{h}17 cells, promoting the transformation of naive T cells into T\textsubscript{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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**References**

1. Collison LW, Workman CJ, Kuo TT, et al. The inhibitory cytokine IL-35 contributes to
regulatory T-cell function. *Nature* 2007; 450: 566–569. DOI: 10.1038/nature06306.

2. Zufferey A, Kapur R and Semple JW. Pathogenesis and Therapeutic Mechanisms in Immune Thrombocytopenia (ITP). *J Clin Med* 2017; 6: 16. DOI: 10.3390/jcm6020016.

3. Vignali DA and Kuchroo VK. IL-12 family cytokines: immunological playmakers. *Nat Immunol* 2012; 13: 722–728. DOI: 10.1038/ni.2366.

4. Beadling C and Slifka MK. Regulation of innate and adaptive immune responses by the related cytokines IL-12, IL-23, and IL-27. *Arch Immunol Ther Exp (Warsz)* 2006; 54: 15–24. DOI: 10.1007/s00005-006-0002-6.

5. Collison LW, Delgoffe GM, Guy CS, et al. The composition and signaling of the IL-35 receptor are unconventional. *Nat Immunol* 2012; 13: 290–299. DOI: 10.1038/ni.2227.

6. Collison LW, Chaturvedi V, Henderson AL, et al. IL-35-mediated induction of a potent regulatory T cell population. *Nat Immunol* 2010; 11: 1093–1101. DOI: 10.1038/ni.1952.

7. Nugent D, McMillan R, Nichol JL, et al. Pathogenesis of chronic immune thrombocytopenia: increased platelet destruction and/or decreased platelet production. *Br J Haematol* 2009; 146: 585–596. DOI: 10.1111/j.1365-2457.2009.07717.x.

8. Kistangari G and McCrae KR. Immune thrombocytopenia. *Hematol Oncol Clin North Am* 2013; 27: 495–520. DOI: 10.1016/j.hoc.2013.03.001.

9. Zhang J, Zhang Q, Li Y, et al. Immune dysregulation in primary immune thrombocytopenia patients. *Hematology* 2018; 23: 510–516. DOI: 10.1080/10245332.2018.1435021.

10. Nishimoto T, Satoh T, Simpson EK, et al. Predominant autoantibody response to GPIb/IX in a regulatory T-cell-deficient mouse model for immune thrombocytopenia. *J Thromb Haemost* 2013; 11: 369–372. DOI: 10.1111/jth.12079.

11. Sun RJ, Yuan D, Liu SY, et al. Reduced IL-35 in patients with immune thrombocytopenia. *Blood Coagul Fibrinolysis* 2020; 31: 543–550. DOI: 10.1097/MBC.0000000000000961.

12. Yang Y, Xuan M, Zhang X, et al. Decreased IL-35 levels in patients with immune thrombocytopenia. *Hum Immunol* 2014; 75: 909–913. DOI: 10.1016/j.humimm.2014.06.019.

13. Li Y, Pan X, Peng X, et al. Adenovirus-mediated interleukin-35 gene transfer suppresses allergic airway inflammation in a murine model of asthma. *Inflamm Res* 2015; 64: 767–774. DOI: 10.1007/s00011-015-0858-1.

14. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 2014; 507: 366–370. DOI: 10.1038/nature12979.

15. Teymouri M, Pirro M, Fallarino F, et al. IL-35, a hallmark of immune-regulation in cancer progression, chronic infections and inflammatory diseases. *Int J Cancer* 2018; 143: 2105–2115. DOI: 10.1002/ijc.31382.

16. Li X, Mai J, Virtue A, et al. IL-35 is a novel responsive anti-inflammatory cytokine—a new system of categorizing anti-inflammatory cytokines. *PLoS One* 2012; 7: e33628. DOI: 10.1371/journal.pone.0033628.

17. Bettini M and Vignali DA. Regulatory T cells and inhibitory cytokines in autoimmunity. *Curr Opin Immunol* 2009; 21: 612–618. DOI: 10.1016/j.coi.2009.09.011.

18. Danikowski KM, Jayaraman S and Prabhakar BS. Regulatory T cells in multiple sclerosis and myasthenia gravis. *J Neuroinflammation* 2017; 14: 117. DOI: 10.1186/s12974-017-0892-8.

19. Friedman A and Liao KL. The role of the cytokines IL-27 and IL-35 in cancer. *Math Biosci Eng* 2015; 12: 1203–1217. DOI: 10.3934/mbe.2015.12.1203.

20. Dambuza IM, He C, Choi JK, et al. IL-12p35 induces expansion of IL-10 and IL-35-expressing regulatory B cells and ameliorates autoimmune disease. *Nat Commun* 2017; 8: 719. DOI: 10.1038/s41467-017-00838-4.

21. Guo Y, Cao W and Zhu Y. Immunoregulatory Functions of the IL-12 Family of Cytokines in Antiviral Systems. *Viruses* 2019; 11:772. DOI: 10.3390/v11090772.
22. Wang RX, Yu CR, Dambuza IM, et al. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat Med* 2014; 20: 633–641. DOI: 10.1038/nm.3554.

23. Hou C, Wu Q, Ouyang C, et al. Effects of an intravitreal injection of interleukin-35-expressing plasmid on pro-inflammatory and anti-inflammatory cytokines. *Int J Mol Med* 2016; 38: 713–720. DOI: 10.3892/ijmm.2016.2688.

24. Niedbala W, Wei XQ, Cai B, et al. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. *Eur J Immunol* 2007; 37: 3021–3029. DOI: 10.1002/eji.200737810.

25. Dong Y, Li X, Yu Y, et al. JAK/STAT signaling is involved in IL-35-induced inhibition of hepatitis B virus antigen-specific cytotoxic T cell exhaustion in chronic hepatitis B. *Life Sci* 2020; 252: 117663. DOI: 10.1016/j.lfs.2020.117663.

26. Choi J, Leung PS, Bowlus C, et al. IL-35 and Autoimmunity: a Comprehensive Perspective. *Clin Rev Allergy Immunol* 2015; 49: 327–332. DOI: 10.1007/s12016-015-8468-9.

27. Choi JK and Egwuagu CE. Interleukin 35 Regulatory B Cells. *J Mol Biol* 2020. S0022-2836(20)30474-5. DOI: 10.1016/j.jmb.2020.07.019.

28. Ouyang H, Shi YB, Liu ZC, et al. Decreased interleukin 35 and CD4+EBI3+ T cells in patients with active systemic lupus erythematosus. *Am J Med Sci* 2014; 348: 156–161. DOI: 10.1097/MAJ.0000000000000215.

29. Cai Z, Wong CK, Dong J, et al. Remission of systemic lupus erythematous disease activity with regulatory cytokine interleukin (IL)-35 in Murphy Roths Large (MRL)/lpr mice. *Clin Exp Immunol* 2015; 181: 253–266. DOI: 10.1111/cei.12639.

30. Liu JQ, Liu Z, Zhang X, et al. Increased Th17 and regulatory T cell responses in EBV-induced gene 3-deficient mice lead to marginally enhanced development of autoimmune encephalomyelitis. *J Immunol* 2012; 188: 3099–3106. DOI: 10.4049/jimmunol.1100106.

31. LeVine DN and Brooks MB. Immune thrombocytopenia (ITP): Pathophysiology update and diagnostic dilemmas. *Vet Clin Pathol* 2019; 48 Suppl 1: 17–28. DOI: 10.1111/vcp.12774.

32. Chen JF, Yang LH, Chang LX, et al. The clinical significance of circulating B cells secreting anti-glycoprotein IIb/IIIa antibody and platelet glycoprotein IIb/IIIa in patients with primary immune thrombocytopenia. *Hematology* 2012; 17: 283–290. DOI: 10.1179/1607845412Y.0000000014.

33. Audia S, Mahévas M, Samson M, et al. Pathogenesis of immune thrombocytopenia. *Autoimmun Rev* 2017; 16: 620–632. DOI: 10.1016/j.autrev.2017.04.012.

34. Cooper N and Ghanima W. Immune Thrombocytopenia. *N Engl J Med* 2019; 381: 945–955. DOI: 10.1056/NEJMcp1810479.

35. Najaoui A, Bakchoul T, Stoy J, et al. Autoantibody-mediated complement activation on platelets is a common finding in patients with immune thrombocytopenic purpura (ITP). *Eur J Haematol* 2012; 88: 167–174. DOI: 10.1111/j.1600-0609.2011.01718.x.

36. Huang A, Cheng L, He M, et al. Interleukin-35 on B cell and T cell induction and regulation. *J Inflamm (Lond)* 2017; 14: 16. DOI: 10.1186/s12950-017-0164-5.

37. Su LC, Liu XY, Huang AF, et al. Emerging role of IL-35 in inflammatory autoimmune diseases. *Autoimmun Rev* 2018; 17: 665–673. DOI: 10.1016/j.autrev.2018.01.017.

38. Li X, Zhong H, Bao W, et al. Defective regulatory B-cell compartment in patients with immune thrombocytopenia. *Blood* 2012; 120: 3318–3325. DOI: 10.1182/blood-2012-05-432575.

39. Boldison J, Da Rosa LC, Davies J, et al. Dendritic cells license regulatory B cells to produce IL-10 and mediate suppression of antigen-specific CD8+ T cells. *Cell Mol Immunol* 2020; 17: 843–855. DOI: 10.1038/s41423-019-0324-z.

40. Zhang J, Lin Y, Li C, et al. IL-35 Decelerates the Inflammatory Process by Regulating Inflammatory Cytokine Secretion and M1/M2 Macrophage Ratio in Psoriasis. *J Immunol* 2016;
41. Zhang F, Chu X, Wang L, et al. Cell-mediated lysis of autologous platelets in chronic idiopathic thrombocytopenic purpura. *Eur J Haematol* 2006; 76: 427–431. DOI: 10.1111/j.1600-0609.2005.00622.x.

42. Olsson B, Andersson PO, Jernas M, et al. T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. *Nat Med* 2003; 9: 1123–1124. DOI: 10.1038/nm921.

43. Li S, Wang L, Zhao C, et al. CD8⁺ T cells suppress autologous megakaryocyte apoptosis in idiopathic thrombocytopenic purpura. *Br J Haematol* 2007; 139: 605–611. DOI: 10.1111/j.1365-2141.2007.06737.x.

44. Cheng ST, Yuan D, Liu Y, et al. Interleukin-35 Level Is Elevated in Patients with Chronic Hepatitis B Virus Infection. *Int J Med Sci* 2018; 15: 188–194. DOI: 10.7150/ijms.21957.

45. Li X, Tian L, Dong Y, et al. IL-35 inhibits HBV antigen-specific IFN-gamma-producing CTLs in vitro. *Clin Sci (Lond)* 2015; 129: 395–404. DOI: 10.1042/CS20140511.

46. Ji L, Zhan Y, Hua F, et al. The ratio of Treg/Th17 cells correlates with the disease activity of primary immune thrombocytopenia. *PLoS One* 2012; 7: e50909. DOI: 10.1371/journal.pone.0050909.

47. Yoh K, Morito N, Ojima M, et al. Overexpression of RORγt under control of the CD2 promoter induces polyclonal plasmacytosis and autoantibody production in transgenic mice. *Eur J Immunol* 2012; 42: 1999–2009. DOI: 10.1002/eji.201142250.

48. Chambers CA, Kuhns MS, Egen JG, et al. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Annu Rev Immunol* 2001; 19: 565–594. DOI: 10.1146/annurev.immunol.19.1.565.

49. Shamji MH, Layhadi JA, Achkova D, et al. Role of IL-35 in sublingual allergen immunotherapy. *J Allergy Clin Immunol* 2019; 143: 1131–1142 e1134. DOI: 10.1016/j.jaci.2018.06.041.

50. Noack M and Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev* 2014; 13: 668–677. DOI: 10.1016/j.autrev.2013.12.004.

51. Wang X, Zheng XY, Ma C, et al. Mitigated Tregs and augmented Th17 cells and cytokines are associated with severity of experimental autoimmune neuritis. *Scand J Immunol* 2014; 80: 180–190. DOI: 10.1111/sji.12201.

52. Muhammad Yusoff F, Wong KK and Mohd Redzwan N. Th1, Th2, and Th17 cytokines in systemic lupus erythematosus. *Autoimmunity* 2020; 53: 8–20. DOI: 10.1080/08916934.2019.1693545.

53. Hua F, Ji L, Zhan Y, et al. Aberrant frequency of IL-10-producing B cells and its association with Treg/Th17 in adult primary immune thrombocytopenia patients. *Biomed Res Int* 2014; 2014: 571302. DOI: 10.1155/2014/571302.

54. Panitsas FP, Theodoropoulou M, Kouraklis A, et al. Adult chronic idiopathic thrombocytopenic purpura (ITP) is the manifestation of a type-1 polarized immune response. *Blood* 2004; 103: 2645–2647. DOI: 10.1182/blood-2003-07-2268.

55. Crane IJ and Forrester JV. Th1 and Th2 lymphocytes in autoimmune disease. *Crit Rev Immunol* 2005; 25: 75–102. DOI: 10.1615/critrevimmunol.v25.i2.10.

56. Zhang X, Zhang Z, He Z, et al. Interleukin 35 induced Th2 and Tregs bias under normal conditions in mice. *PeerJ* 2018; 6: e5638. DOI: 10.7717/peerj.5638.

57. Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nat Rev Immunol* 2005; 5: 521–531. DOI: 10.1038/nri1648.

58. Zhou H, Qiu JH, Wang T, et al. Interleukin 27 inhibits cytotoxic T-lymphocyte-mediated platelet destruction in primary immune thrombocytopenia. *Blood* 2014; 124: 3316–3319. DOI: 10.1182/blood-2014-06-580084.

59. Li Q, Yang M, Xia R, et al. Elevated expression of IL-12 and IL-23 in patients with primary immune thrombocytopenia. *Platelets* 2015; 26: 453–458. DOI: 10.3109/095373104.2014.934217.

60. Samson M, Fraser W and Lebowitz D. Treatments for Primary Immune
61. Li Q, Zhang L, Xia R, et al. Plasma Levels of Interleukin 12 Family Cytokines and Their Relevant Cytokines in Adult Patients with Chronic Immune Thrombocytopenia before and after High-Dose Dexamethasone Treatment. Med Princ Pract 2015; 24: 458–464. DOI: 10.1159/000433472.

62. Wang SC, Yang KD, Lin CY, et al. Intravenous immunoglobulin therapy enhances suppressive regulatory T cells and decreases innate lymphoid cells in children with immune thrombocytopenia. Pediatr Blood Cancer 2020; 67: e28075. DOI: 10.1002/pbc.28075.