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Interleukin-35 in immune-related diseases: protection or destruction

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Summary

Interleukin-35 (IL-35) is a recently identified heterodimeric cytokine in the IL-12 family. It consists of an IL-12 subunit α chain (P35) and IL-27 subunit Epstein–Barr virus-induced gene 3 (EBI3) β chain. Unlike the other IL-12 family members, it signals through four unconventional receptors: IL-12Rβ2–IL-27Rα, IL-12Rβ2–IL-12Rβ2, IL-12Rβ2–GP130, and GP130–GP130. Interleukin-35 signaling is mainly carried out through the signal transducer and activator of transcription family of proteins. It is secreted not only by regulatory T (Treg) cells, but also by $CD8^+$ Treg cells, activated dendritic cells and regulatory B cells. It exhibits immunosuppressive functions distinct from those of other members of the IL-12 family; these are mediated primarily by the inhibition of T helper type 17 cell differentiation and promotion of Treg cell proliferation. Interleukin-35 plays a critical role in several immune-associated diseases, such as autoimmune diseases and viral and bacterial infections, as well as in tumors. In this review, we summarize the structure and function of IL-35, describe its role in immune-related disorders, and discuss the mechanisms by which it regulates the development and progression of diseases, including inflammatory bowel disease, collagen-induced arthritis, allergic airway disease, hepatitis, and tumors. The recent research on IL-35, combined with improved techniques of studying receptors and signal transduction pathways, allows for consideration of IL-35 as a novel immunotherapy target.

Keywords: immune-related diseases; immunosuppression; interleukin-35; regulatory T cells.

Introduction

Cytokines are a group of small proteins synthesized and secreted by a broad range of cells including immune cells such as monocytes, macrophages, T cells and B cells and certain non-immune cells such as endothelial cells, epidermal cells and fibroblasts.[1] Recent studies have uncovered a wealth of information on the role of cytokines in the regulation of immunomodulatory processes.[2,3] Endogenous and exogenous cytokines play a variety of roles in maintaining the delicate balance of the immune system, and disruption of this equilibrium may lead to debilitating immune-related diseases.[4] In 2007, Collison *et al.*[5] identified interleukin-35 (IL-35) as a novel inhibitory cytokine, consisting of Epstein–Barr virus-induced gene 3 (EBI3) and IL-12 subunit α chain (P35) subunits and belonging to the IL-12 cytokine family. Unlike other IL-12 family members, which are known to be primarily secreted by activated antigen-presenting cells,[6,7] IL-35 is primarily expressed in unstimulated regulatory T (Treg)

Abbreviations: AHR, airway hyperresponsiveness; CD, cluster of differentiation; CFA/I, colonization factor of enterotoxigenic *Escherichia coli*; CIA, collagen-induced arthritis; DC, dendritic cell; EBI3, Epstein–Barr virus-induced gene 3; FOXP3, forkhead box 3; GP130, glycoprotein 130; IBD, inflammatory bowel disease; IL-12Rβ1, interleukin 12 receptor, β1 subunit; IL-12Rβ2, interleukin 12 receptor, β2 subunit; IL-27Rα, interleukin 27 receptor, α (also known as WSX-1); IL, interleukin; JAK2, Janus-related kinase 2; STAT, signal transducer and activator of transcription; Teff, effector T; Th, T helper; Treg, regulatory T; TYK2, tyrosine kinase 2

cells in mice, but is not detected in the unstimulated human Treg cells.[5,8,9] Recently, it was shown to be secreted in a wide range of tissues and by different cell types, including regulatory B cells,[10,11] dendritic cells (DCs),[12] endothelial cells, smooth muscle cells, and monocytes.[13] Interleukin-35 has also been reported to play an important regulatory role in several autoimmune diseases, inflammatory diseases, bacterial and viral infectious diseases, and tumors.

Discovery and composition of IL-35

In 1997, Devergne *et al.* first reported that specific co-immune precipitates of P35 and EBI3 constitute a large portion of the nourishing component extract of the normal human full-term placenta, indicating that the two components heterodimerize *in vivo*.[14] In 2007, the heterodimer was formally named IL-35 at the 13th Immunology Conference. The same year, researchers demonstrated that the immunosuppressive cytokine IL-35 is a member of the IL-12 family of cytokines and that it regulates the function of Treg cells and inhibits the proliferation of $CD4^+$ $CD25^-$ effector T (Teff) cells.[5,15]

There are four members of the IL-12 family, namely, IL-12, IL-23, IL-27 and IL-35. All four are heterodimers: P35 and P40 subunits heterodimerize to form IL-12, P40 and P19 form IL-23, EBI3 and P28 form IL-27, and EBI3 and P35 form IL-35 (Fig. 1).[5,16–19] *EBI3* is widely expressed in B lymphocytes and tissues, including the spleen and tonsils transformed by the Epstein–Barr virus.[14] EBI3 plays a critical role in the immune response by down-regulating the expression of transcription factor RORγt in T helper type 17 (Th17) cells and inhibiting inflammatory response.[20] On the other hand, P35 can regulate inflammation, but has also been shown to lead to herpes simplex keratitis in mice.[21] Either subunit of IL-35 can independently regulate the immune response, and these functions are augmented in the IL-35 heterodimeric form.[22]

Studies suggest that except for IL-35, the other members of the IL-12 family are primarily secreted by antigen-presenting cells, including DCs, macrophages, and monocytes.[23,24] Initial studies reported that only $FOXP3^+$ Treg cells, but not Teff cells, can secrete active IL-35.[5,25] Moreover, Treg cells participate in a variety of inhibitory mechanisms. Wei *et al.*[26] reported that IL-35-producing Treg cells were distinct from the IL-10-producing subset. Furthermore, Seyerl *et al.* observed that human rhinovirus can activate DCs, and the activated DCs can secrete and release IL-35 into human peripheral blood.[27] In 2014, Shen *et al.*[10] further revealed that B cells can also secrete IL-35 and have an immunosuppressive function in autoimmune diseases and bacterial infectious diseases (Fig. 1). These results suggest that IL-35 production may be affected by multiple stimulants and immune microenvironments.

IL-35 receptors and signaling pathways

The receptors of IL-12 family cytokines are dimers that signal through the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway.[11,28–31] The IL-12 receptor is composed of IL-12Rβ1 and IL-12Rβ2, both of which are members of the GP130 cytokine receptor family. Interaction between IL-12 and its receptor causes the mutual phosphorylation of Janus-related kinase 2 (JAK2) and tyrosine kinase 2 (TYK2), which further induces tyrosine phosphorylation of the receptors, and recruits STAT1, STAT3, STAT4, and STAT5.[32,33] Together, IL-23R and IL-12Rβ1 comprise the IL-23 receptor complex on IL-23-responsive cells.[29] After binding to the receptor, IL-23 too induces the phosphorylation of JAK2 and TYK2, which, in turn, phosphorylate STAT1, STAT3, STAT4, and STAT5.[29,34] The IL-27 receptor is a heterodimer formed by IL-27Rα and GP130.[35] After binding to the receptor, STAT1, STAT3, STAT4, and STAT5 are activated through phosphorylation by JAK1 and JAK2.[36,37] Unlike other IL-12 family members, the receptors of IL-35 are: GP130–GP130, IL-12Rβ2–IL-12Rβ2, IL-12Rβ2–GP130, and IL-12Rβ2–IL-27Rα (Fig. 1).[31] Following the binding of IL-35 to its receptors, its downstream signal transduction occurs through a unique heterodimer formed by STAT1 and STAT4, leading to the expression of target genes, including *EBI3* and *P35*. These target genes lead to a feedback loop promoting the expression of IL-35.[31] The IL-35 signaling pathway consists of STAT1, STAT3, STAT4, JAK1, and JAK2 molecules (Fig. 1).[38,39] However, the binding of IL-35 to different receptor subunits depends on the cell-type. In T cells, IL-35 signaling uses three receptor subunits, namely, GP130–GP130, IL-12Rβ2–IL-12Rβ2, and IL-12Rβ2–GP130, which activate STAT1 and STAT4.[40,41] Regulatory B cells have four receptor subunits: GP130, IL-27Rα, IL-12Rβ1, and IL-12Rβ2. Interestingly, a study employing small interfering RNA to silence each subunit individually showed that IL-35 signaling in B cells occurs through IL-27Rα and IL-12Rβ2, which activate STAT1 and STAT3, rather than through GP130 and IL-12Rβ1.[11,42]

IL-35 and immune-related diseases

The last decade has seen remarkable progress in IL-35 research. Studies have shown that it has two major biological effects in a variety of disease models: inhibition of T-cell proliferation[43,44] and inhibition of the development and differentiation of Th17 cells.[45,46] It has been shown to play an important role in the development and progression of inflammatory and autoimmune diseases. Recent results suggest that the plasma concentration of IL-35 is lower in psoriasis patients than in healthy individuals.[47] Furthermore, it was found that IL-35 could alleviate the pathological characteristics of severe psoriatic

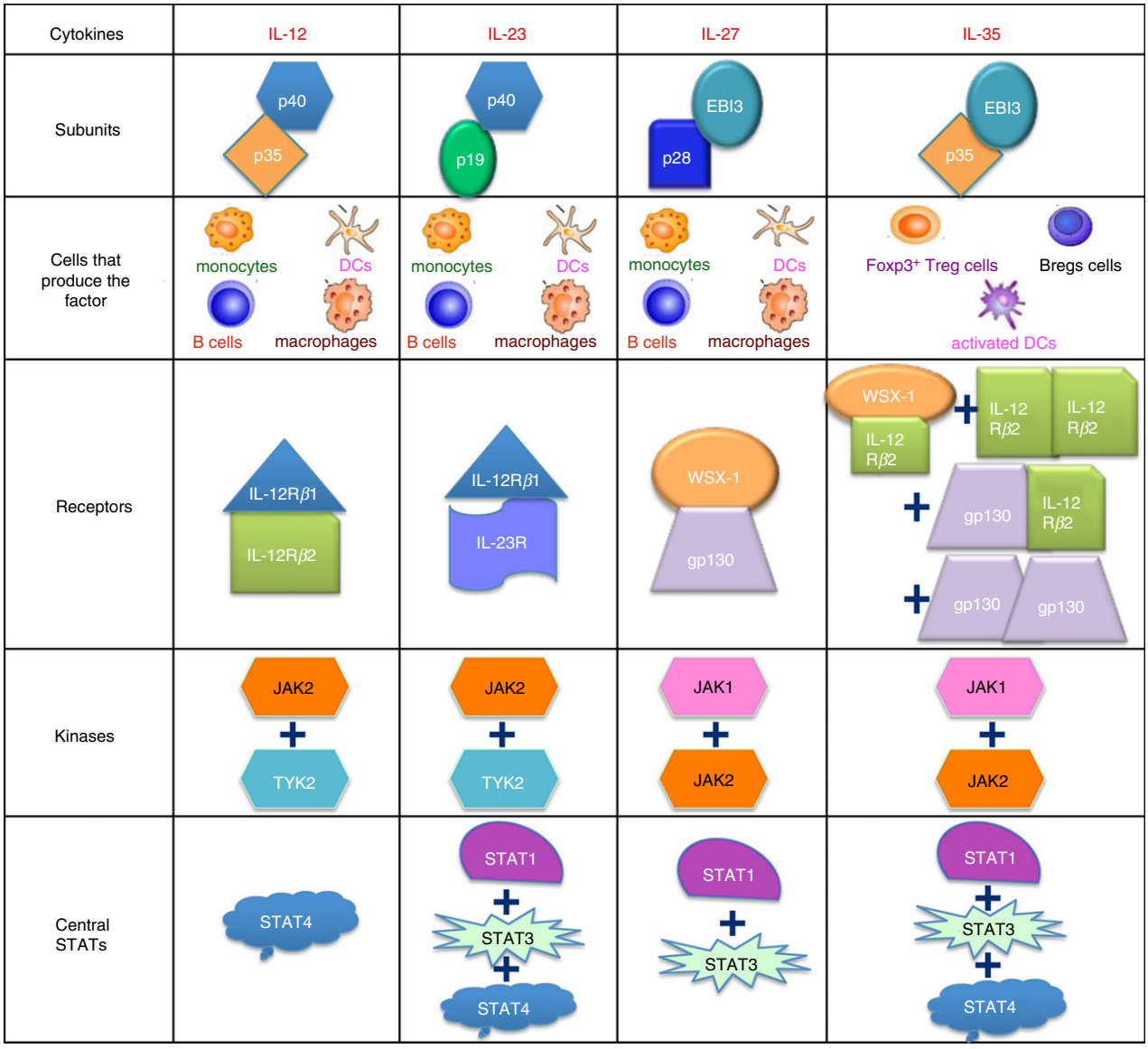

Figure 1. Four members of the interleukin-12 (IL-12) cytokine family and their downstream signaling pathways: IL-12 (p35/p40) signals through IL-12Rβ1 and IL-12Rβ2, IL-23 (p19/p40) signals through IL-23R and IL-12Rβ1, IL-27 (p28/Ebi3) signals through gp130 and WSX-1, while IL-35 (p35/Ebi3) uses IL-12Rβ2 and WSX-1 heterodimers, IL-12Rβ2 and IL-12Rβ2 homodimers, IL-12Rβ2 and gp130 heterodimers, and gp130 and gp130 homodimers.

lesions in K14-VEGF-A transgenic mice by reducing the ratio of the total number of macrophages to M1/M2 macrophages.[48,49]

IL-35 and inflammatory bowel disease

Currently, the pathogenesis of inflammatory bowel disease (IBD) is not clear. It is widely believed that intestinal flora and environmental factors play an important role in the etiology of IBD.[50] In 2007, Collison *et al.* first demonstrated in an IBD model that the inhibitory effect of Treg cells on Teff cells, and thereby the therapeutic effect on the IBD model, is greatly reduced by the deletion of the two subunits EBI3 and P35. The researchers also observed that $CD4^+$ $CD25^-$ iTR35 cells stimulated by exogenous IL-35 could have an immunosuppressive effect in IBD model mice.[51,52] Wirtz *et al.* reported that enteritis symptoms in the IBD mouse model were significantly alleviated by vector-mediated IL-35 overexpression. At the same time, the authors showed that, after the establishment of the IBD model in *EBI3*$^{-/-}$ and *P28*$^{-/-}$ mice, the *EBI3*$^{-/-}$ animals suffered more severe inflammation, indicating that IL-35, rather than IL-27 regulates IBD inflammation.[43] Wang *et al.* demonstrated that IL-35

recombinant protein could regulate IBD inflammation by promoting the secretion of IL-10 and inhibiting the expression of pro-inflammatory cytokines such as IL-6, tumor necrosis factor-α, and IL-17 in an acute model of colitis.[49] When IL-35 was integrated into mesenchymal stem cells, the cells could ameliorate ulcerative colitis by down-regulating the expression of pro-inflammatory cytokines.[53] The above studies suggest that IL-35 plays a significant biological role in inhibiting IBD.

IL-35 and collagen-induced arthritis

The collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis is induced by type II collagen immunization.[54] IL-35 was first reported to have a therapeutic effect on the collagen-induced arthritis model in 2007. This study showed that IL-35 could promote the proliferation of $CD4^+$ $CD25^-$ and $CD4^+$ $CD25^+$ T cells *in vitro* and increase the secretion of interferon-γ. Simultaneously, IL-35 inhibited the aggravation of the CIA model by inhibiting the differentiation of $CD4^+$ T cells into Th17 cells.[15] In previous studies, the colonization factor of enterotoxigenic *Escherichia coli* (CFA/I) could improve the CIA model by inducing the proliferation of IL-10-secreting $CD4^+$ $CD39^+$ $Foxp3^+$ T cells.[55,56] Kochetkova *et al.* reported that, in the CIA model, $CD4^+$ $CD39^+$ $Foxp3^+$ T cells could secrete IL-35 after CFA/I treatment. The therapeutic effect of CFA/I on the model was greatly reduced and IL-35 was blocked *in vivo*.[57] Other studies have shown that exogenous IL-35 could inhibit the proliferation of Th1 and Th17 cells and promote $CD4^+$ T cells to express CD39 in the CIA model. Meanwhile, the authors have also shown that $CD4^+$ $CD39^+$ $CD25^-$ T cells isolated from mice in the IL-35-treated group could express IL-10 strongly, but not induce $CD4^+$ $CD39^+$ T cells when IL-35 was used to treat $Il10^{-/-}$ mice.[44] The study reveals that when IL-35 treatment is applied in the CIA model, it can induce the production of IL-10 in $CD4^+$ $CD39^+$ Treg cells.

IL-35 and allergic airway disease

Clinically defined allergic asthma is characterized by reversible airway obstruction and airway hyper-responsiveness. The main feature of allergic airway disease is that the hyperproliferative Th2 cells promote the secretion of IL-4, IL-5, and IL-13, which increases IgE levels in the respiratory tract, leading to increased numbers of eosinophils and the secretion of mucus into the airway.[58,59] In recent years, studies have shown that IL-17 produced by macrophages or Th17 cells plays an important role in allergic airway disease.[60,61] Whitehead *et al.* observed that the allergen-induced allergic airway disease is more severe when EBI3 or P35 are absent in $CD278^+$ Treg cells. However, in IL-10- or transforming growth factor-β-deficient mice, the disease model did not show any sign of exacerbation. The p28 subunit was expressed at relatively low levels in $CD278^+$ Treg cells; however, EBI3 can pair with p28 as well to form IL-27. Thus IL-35, but not IL-27, is selectively expressed by murine $CD278^+$ Treg cells. This study also showed that endogenous IL-35 expressed by $CD278^+$ Treg cells had a protective effect in the allergic airway disease model.[62] In ovalbumin-sensitized allergic airway disease mouse models, IL-35 alleviated the symptoms of the disease by inhibiting the recruitment of inflammatory DCs at the inflammatory sites and draining lymph nodes.[63] Interleukin-35 vectors can effectively treat allergic airway inflammation induced by memory/effector Th2 cells reactive to allergen mites. Furthermore, this therapeutic effect is mainly achieved by regulating the inflammatory factors IL-4, IL-5, and IL-13 and the chemokines CCL2, CXCL1, and CXCL5.[64]

Increased eosinophils could be pathogenic in various diseases, such as eosinophilic pneumonias, allergic asthma, and eosinophil-associated gastrointestinal disorders.[65,66] Recent findings suggest that IL-35 decreases airway eosinophilia by reducing the production of the eosinophil-attracting chemokines CCL11 and CCL24, which demonstrates that IL-35 may provide a new therapeutic strategy to reduce tissue recruitment of eosinophils in such diseases.[67] In addition, studies have shown that the expression of IL-35 is down-regulated in chronic obstructive pulmonary disease, a chronic bronchitis and emphysema characterized by airflow obstruction.[68] Moreover, decreased IL-35 levels were negatively correlated with the smoking status, indicating that IL-35 can serve as a biomarker to estimate progression of chronic obstructive pulmonary disease.[69] The above results suggest that IL-35 may be a good indicator of allergic inflammation and can be used as a biomarker.

IL-35 and type I diabetes

Type I diabetes is a chronic autoimmune disease, which is primarily caused by the destruction of the β-cells that secrete insulin, resulting in significantly elevated blood glucose levels.[25,70] Currently, intramuscular injection of insulin is the primary treatment for type I diabetes, which only temporarily relieves the symptoms and does not cure the disease; cell therapy involving islet or insulin-secreting cell transplantation presents a more effective strategy.[71] In recent years, researchers have established IL-35 transgenic mice, in which IL-35 is only expressed on islet β-cells, and found that IL-35 can inhibit the occurrence of primary and secondary type I diabetes. The inhibitory effect of IL-35 occurs primarily through inhibition of the proliferation and infiltration of $CD4^+$ and $CD8^+$ T cells.[72] Similarly, transgenic IL-35 expression selectively targeted against β-cells in NOD mice can reduce the number of

islet-resident conventional $CD4^+$ and $CD8^+$ T cells, DCs, and islet $Foxp3^+$ Treg cells.[73] Although IL-35 administration did not increase the number of Treg cells, it decreased the number of Th1 and Th17 cells, as well as interferon-γ- or IL-17A-expressing $CD8^+$ T cells, and reduced the infiltration of mononuclear cells in the islets.[74] At the same time, clinical research studies showed that the expression of IL-35 in serum was markedly lower in C-peptide-negative patients, and this was associated with a simultaneous decrease in the proportion of $IL\text{-}35^+$ Treg cells, $IL\text{-}35^+$ regulatory B cells, and IL-35-producing $CD8^+$ $FOXP3^+$ cells.[45] These results indicate that high expression of IL-35 can affect autoimmune diabetes. Moreover, the expression of IL-35 in local islet β-cells can control the immune response of $CD4^+$ and $CD8^+$ T cells, which suggests that IL-35 plays an important role in the regulation of the immune response in type I diabetes.

IL-35 and hepatitis

Hepatitis virus causes destruction of liver parenchymal cells, leading to severe liver disorders, such as cirrhosis and hepatocellular carcinoma.[75] Hepatitis is a common and frequently occurring disease in the clinic, its incidence being highest in Asia.[76] The T-cell-mediated immune response plays an important role in the pathogenesis of the disease.[77] Studies suggest that unstimulated human Treg cells do not express IL-35;[8] however, substantial up-regulation of EBI3 and P35, not IL-10 and transforming growth factor-β, was observed in activated human Treg cells compared with conventional T cells.[9] On the other hand, IL-35 expression has been detected in peripheral blood $CD4^+$ T lymphocytic cells of chronic hepatitis B patients.[78] However, whether IL-35 is involved in the development of hepatitis has not yet been confirmed. Researchers have found that IL-35 is highly expressed in the peripheral blood of patients with hepatitis B, and the immunomodulatory function of Teff cells activated by hepatitis virus antigens is significantly inhibited after IL-35 treatment, with a concomitant decrease in the ability to secrete interferon-γ.[79] Conversely, a study showed that the level of IL-35 was significantly decreased in patients with chronic HBV compared with healthy control individuals.[80] These studies further suggest that IL-35 plays an important immunomodulatory role in the occurrence and development of hepatitis.

IL-35 and tumors

Inflammation is a well-established factor in the regulation of cancer progression, and recent studies have suggested that IL-35 might influence tumor-related inflammation.[81] In advanced gastric cancer, the frequency of IL-35-producing B cells is significantly increased, suggesting that these cells may participate in the progression of the disease.[82] Elevated expression of IL-35 can be detected in the peripheral blood of adult patients with acute myeloid leukemia, and the expression levels depend on the severity of the disease. In leukemia patients, IL-35 can aggravate the disease condition by promoting the proliferation of Treg cells and by inhibiting $CD4^+$ $CD25^-$ Teff cells.[83] On the other hand, IL-35 was not detected in the peripheral blood of patients with non-small cell lung cancer, but its expression was significantly increased at the tumor site.[84] Similarly, high expression of IL-35 can be detected in tumor tissues of human nasopharyngeal carcinoma, melanoma, and malignant B-cell lymphoma. The IL-35 produced by these tumors can activate $CD11b^+$ $GR1^+$ bone-marrow-derived immunosuppressive cells in the tumor microenvironment to help the immune escape of tumor cells.[85] Turnis *et al.* suggested that the presence of IL-35 in the tumor microenvironment leads to reduced lymphocytic infiltration, decreases effector cell proliferation, increases tumor burden, and decreases survival of the immunocompetent host. Furthermore, Treg-derived IL-35 promotes the expression of multiple inhibitory receptors (PD1, TIM3, and LAG3), thereby facilitating intratumoral T-cell exhaustion.[86] Another study showed that IL-35 promoted the growth of pancreatic cancer cells *in vitro* by inhibiting their apoptosis through increasing the expression of CDK2, CDK4, cyclin B, and cyclin D.[87] Overexpression of IL-35 in hepatoma cells (HepG2) enhanced apoptotic sensitivity and induced cell cycle arrest of hepatocellular carcinoma through the regulation of genes related to the cell cycle and apoptosis, including an increase in *FAS* expression and down-regulation of the expression of cyclin D1, survivin, and Bcl-2.[88] Similar clinical test results indicate that the mean serum concentrations of IL-35 were significantly higher in patients with prostate cancer than in the healthy control group. These findings indicate that IL-35 is possibly involved in tumor progression.[89] Recent results suggest that IL-35 can promote tumor progression by functioning as an upstream cytokine to promote cancer-associated inflammation and control neutrophil polarization.[90] Research results suggest that IL-35 can promote tumor progression by functioning as an upstream cytokine for promoting cancer-associated inflammation and controlling neutrophil polarization.[90] Studies also demonstrated that IL-35 expression was elevated in both serum and tumors in patients with colorectal cancer. These elevated IL-35 can suppress T-cell proliferation and may participate in tumor immunotolerance.[91] However, Zhang *et al.* demonstrated that IL-35 levels are decreased in human colon cancer and that IL-35 is capable of exerting anti-tumor activity by suppressing β-catenin expression. Interleukin-35 inhibits cell migration, invasion, proliferation, and colony formation when it is highly expressed in colon cancer cells.[92] A study has found that IL-35 could inhibit angiogenesis and inflammation in rheumatoid arthritis by down-regulating the expression of vascular

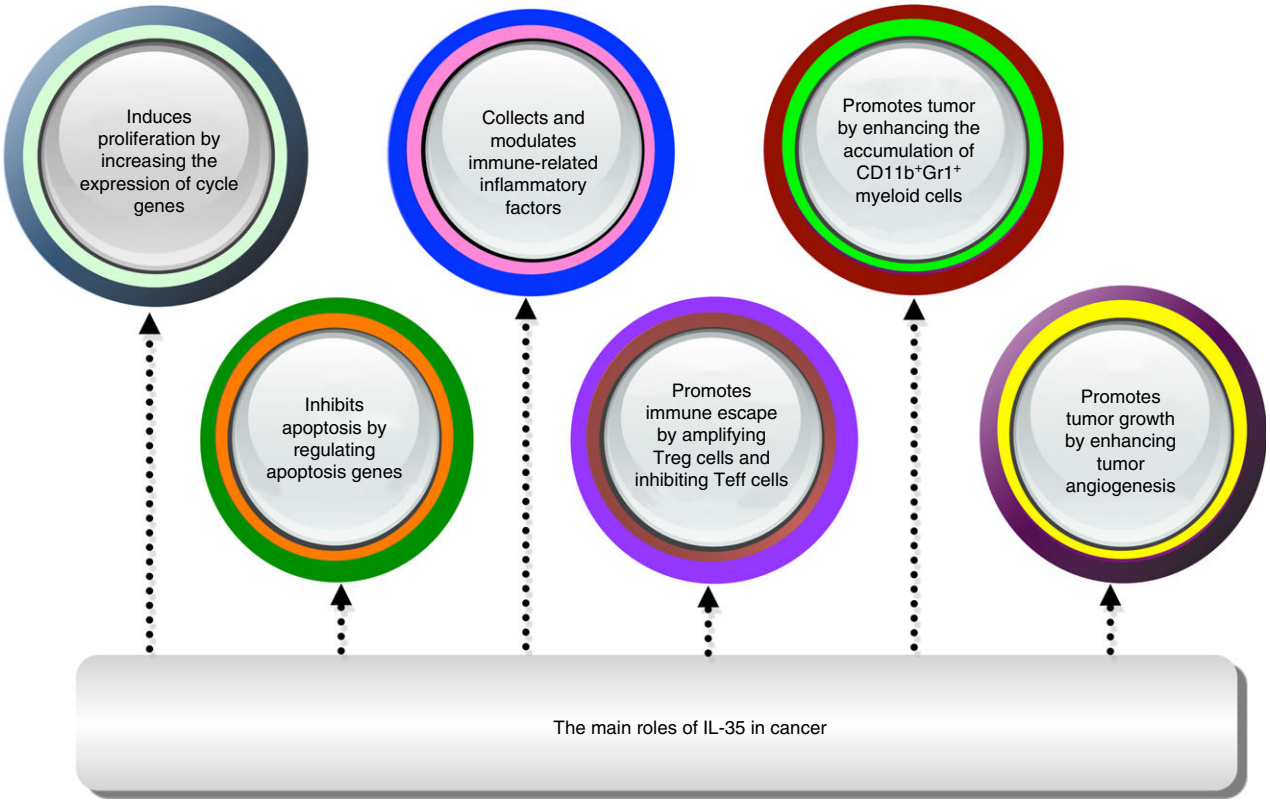

Figure 2. The main roles of interleukin-35 (IL-35) in tumors are shown.

endothelial growth factor-induced ANG2 and disturbing ANG2/TIE2 signaling.[93] Conversely, IL-35 produced by tumor cells can promote tumor growth by promoting the recruitment of $CD11b^+$ $GR1^+$ myeloid-derived suppressor cells and increase angiogenesis in the tumor microenvironment.[85] In conclusion, IL-35 has significant immunomodulatory effects in the process of tumor formation and development, and the molecular mechanism of IL-35-mediated immune regulation varies based on tumor type. It has been confirmed that IL-35 can not only promote tumor proliferation and progression by a variety of mechanisms such as angiogenesis promotion, but also inhibit tumor cell apoptosis by regulating apoptosis genes (Fig. 2). Furthermore, IL-35 can also promote tumor growth through a variety of mechanisms, such as by inhibiting Teff cells and promoting the proliferation of Treg cells and the accumulation of bone-marrow-derived immunosuppressive $CD11b^+$ $GR1^+$ cells (Fig. 2). Immunoregulation by IL-35 is also achieved by collecting and modulating the secretion of multiple immune-related inflammatory factors (Fig. 2).

Concluding remarks

In summary, IL-35 shares numerous similarities in structure and composition with other members of the IL-12 cytokine family. However, it differs considerably in function, to the extent of having effects opposite to those of the other members. In this review, we have discussed data that suggest that targeting IL-35-producing Treg cells has significant therapeutic potential. Furthermore, IL-35 plays an important role in the immune system as an inhibitory cytokine and can modulate dysfunctional T cells in immune dysfunctions, activate bone-marrow-derived immunosuppressive cells, and regulate multiple immune-related inflammatory factors. Therefore, the regulation of IL-35 is of great significance in immune disorders. Advances in research on IL-35, combined with improved techniques of studying receptors and signal-transduction pathways, allow for consideration of IL-35 as a novel therapeutic target for immune regulation. The possibility of incorporating IL-35 in novel therapeutic strategies to treat deadly immune diseases promise a greater clinical significance of IL-35 in the near future. According to the different immunomodulatory effects of IL-35 in different diseases, IL-35 can be considered as a treatment strategy for immune-related diseases. For example, if the expression of IL-35 is reduced in a certain disease, then treat with IL-35 recombinant protein. If the expression of IL-35 is significantly elevated in a certain disease, then IL-35 can be blocked.

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Author contributions

HBX and CPS conceptualized the review and finalized the manuscript preparation. YSZ, QPW, CLL, and HXD modified the grammar of this review. JFZ performed the literature search and drafted the manuscript.

Disclosures

The authors have no financial conflicts of interest.

References

1 Gajiyev JN, Tagiyev EG, Gadjiyev NJ. Impact of roncoleukin on balance of cytokins in complex treatment of obturation jaundice of nontumoral genesis. *Klin Khir* 2016; **2**:24–7.

2 Banchereau J, Pascual V, O'Garra A. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. *Nat Immunol* 2012; **13**:925–31.

3 Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. *Nature* 2017; **542**:177–85.

4 Oyesola OO, Fruh SP, Webb LM, Tait Wojno ED. Cytokines and beyond: regulation of innate immune responses during helminth infection. *Cytokine* 2018; **18**:30359–9.

5 Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM *et al.* The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 2007; **450**:566–9.

6 Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* 2007; **25**:221–42.

7 Ma X, Trinchieri G. Regulation of interleukin-12 production in antigen-presenting cells. *Adv Immunol* 2001; **79**:55–92.

8 Bardel E, Larousserie F, Charlot-Rabiega P, Coulomb-L'Hermine A, Devergne O. Human $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells do not constitutively express IL-35. *J Immunol* 2008; **181**:6898–905.

9 Chaturvedi V, Collison LW, Guy CS, Workman CJ, Vignali DA. Cutting edge: human regulatory T cells require IL-35 to mediate suppression and infectious tolerance. *J Immunol* 2011; **186**:6661–6.

10 Shen P, Roch T, Lampropoulou V, O'Connor RA, Stervbo U, Hilgenberg E *et al.* IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 2014; **507**:366–70.

11 Wang RX, Yu CR, Dambuza IM, Mahdi RM, Dolinska MB, Sergeev YV *et al.* Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat Med* 2014; **20**:633–41.

12 Dixon KO, van der Kooij SW, Vignali DA, van Kooten C. Human tolerogenic dendritic cells produce IL-35 in the absence of other IL-12 family members. *Eur J Immunol* 2015; **45**:1736–47.

13 Sha X, Meng S, Li X, Xi H, Maddaloni M, Pascual DW *et al.* Interleukin-35 inhibits endothelial cell activation by suppressing MAPK-AP-1 pathway. *J Biol Chem* 2015; **290**:19307–18.

14 Devergne O, Birkenbach M, Kieff E. Epstein–Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. *Proc Natl Acad Sci U S A* 1997; **94**:12041–6.

15 Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB *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**:3021–9.

16 Ringkowski S, Thomas PS, Herbert C. Interleukin-12 family cytokines and sarcoidosis. *Front Pharmacol* 2014; **5**:233.

17 Gately MK, Desai BB, Wolitzky AG, Quinn PM, Dwyer CM, Podlaski FJ *et al.* Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte maturation factor). *J Immunol* 1991; **147**:874–82.

18 Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B *et al.* Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 2000; **13**:715–25.

19 Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J *et al.* IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive $CD4^+$ T cells. *Immunity* 2002; **16**:779–90.

20 Yang J, Yang M, Htut TM, Ouyang X, Hanidu A, Li X *et al.* Epstein–Barr virus-induced gene 3 negatively regulates IL-17, IL-22 and RORγt. *Eur J Immunol* 2008; **38**:1204–14.

21 Frank GM, Divito SJ, Maker DM, Xu M, Hendricks RL. A novel p40-independent function of IL-12p35 is required for progression and maintenance of herpes stromal keratitis. *Invest Ophthalmol Vis Sci* 2010; **51**:3591–8.

22 Li X, Fang P, Yang WY, Wang H, Yang X. IL-35, as a newly proposed homeostasis-associated molecular pattern, plays three major functions including anti-inflammatory initiator, effector, and blocker in cardiovascular diseases. *Cytokine* 2017; **17**:30169–2.

23 Hibbert L, Pflanz S, De Waal Malefyt R, Kastelein RA. IL-27 and IFN-α signal via Stat1 and Stat3 and induce T-Bet and IL-12Rβ2 in naive T cells. *J Interferon Cytokine Res* 2003; **23**:513–22.

24 Collison LW, Vignali DA. Interleukin-35: odd one out or part of the family? *Immunol Rev* 2008; **226**:248–62.

25 Bettini M, Vignali DA. Regulatory T cells and inhibitory cytokines in autoimmunity. *Curr Opin Immunol* 2009; **21**:612–18.

26 Wei X, Zhang J, Gu Q, Huang M, Zhang W, Guo J *et al.* Reciprocal expression of IL-35 and IL-10 defines two distinct effector Treg subsets that are required for maintenance of immune tolerance. *Cell Rep* 2017; **21**:1853–69.

27 Seyerl M, Kirchberger S, Majdic O, Seipelt J, Jindra C, Schrauf C *et al.* Human rhinoviruses induce IL-35-producing Treg via induction of B7–H1 (CD274) and sialoadhesin (CD169) on DC. *Eur J Immunol* 2010; **40**:321–9.

28 Presky DH, Yang H, Minetti LJ, Chua AO, Nabavi N, Wu CY *et al.* A functional interleukin 12 receptor complex is composed of two β-type cytokine receptor subunits. *Proc Natl Acad Sci USA* 1996; **93**:14002–7.

29 Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J *et al.* A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. *J Immunol* 2002; **168**:5699–708.

30 Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF *et al.* WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. *J Immunol* 2004; **172**:2225–31.

31 Collison LW, Delgoffe GM, Guy CS, Vignali KM, Chaturvedi V, Fairweather D *et al.* The composition and signaling of the IL-35 receptor are unconventional. *Nat Immunol* 2012; **13**:290–9.

32 Mikus LD, Rosenthal LA, Sorkness RL, Lemanske RF Jr. Reduced interferon-γ secretion by natural killer cells from rats susceptible to postviral chronic airway dysfunction. *Am J Respir Cell Mol Biol* 2001; **24**:74–82.

33 Gollob JA, Murphy EA, Mahajan S, Schnipper CP, Ritz J, Frank DA. Altered interleukin-12 responsiveness in Th1 and Th2 cells is associated with the differential activation of STAT5 and STAT1. *Blood* 1998; **91**:1341–54.

34 Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS *et al.* STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem* 2007; **282**:9358–63.

35 Liu S, Han J, Gong H, Li Y, Bao X, Qi J *et al.* Soluble interleukin-27 receptor α is a valuable prognostic biomarker for acute graft-versus-host disease after allogeneic haematopoietic stem cell transplantation. *Sci Rep* 2018; **8**:10328.

36 Holscher C, Holscher A, Ruckerl D, Yoshimoto T, Yoshida H, Mak T *et al.* The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. *J Immunol* 2005; **174**:3534–44.

37 Yoshimura T, Takeda A, Hamano S, Miyazaki Y, Kinjyo I, Ishibashi T *et al.* Two-sided roles of IL-27: induction of Th1 differentiation on naive $CD4^+$ T cells versus suppression of proinflammatory cytokine production including IL-23-induced IL-17 on activated $CD4^+$ T cells partially through STAT3-dependent mechanism. *J Immunol* 2006; **177**:5377–85.

38 Floss DM, Schonberg M, Franke M, Horstmeier FC, Engelowski E, Schneider A *et al.* IL-6/IL-12 cytokine receptor shuffling of extra- and intracellular domains reveals canonical STAT activation via synthetic IL-35 and IL-39 signaling. *Sci Rep* 2017; **7**:15172.

39 Wu S, Li Y, Yao L, Li Y, Jiang S, Gu W *et al.* Interleukin-35 inhibits angiogenesis through STAT1 signalling in rheumatoid synoviocytes. *Clin Exp Rheumatol* 2017; **36**:223–7.

40 Liu H, Zhang W, Tian FF, Kun A, Zhou WB, Xiao B *et al.* IL-35 is involved in the pathogenesis of guillain-barre syndrome through its influence on the function of $CD4^+$ T cells. *Immunol Invest* 2015; **44**:566–77.

41 Garbers C, Aparicio-Siegmund S, Rose-John S. The IL-6/gp130/STAT3 signaling axis: recent advances towards specific inhibition. *Curr Opin Immunol* 2015; **34**:75–82.

42 Olson BM, Sullivan JA, Burlingham WJ. Interleukin 35: a key mediator of suppression and the propagation of infectious tolerance. *Front Immunol* 2013; **4**:315.

43 Wirtz S, Billmeier U, Mchedlidze T, Blumberg RS, Neurath MF. Interleukin-35 mediates mucosal immune responses that protect against T-cell-dependent colitis. *Gastroenterology* 2011; **141**:1875–86.

44 Kochetkova I, Golden S, Holderness K, Callis G, Pascual DW. IL-35 stimulation of $CD39^+$ regulatory T cells confers protection against collagen II-induced arthritis via the production of IL-10. *J Immunol* 2010; **184**:7144–53.

45 Espes D, Singh K, Sandler S, Carlsson PO. Increased interleukin-35 levels in patients with type 1 diabetes with remaining C-peptide. *Diabetes Care* 2017; **40**:1090–5.

46 Collison LW, Pillai MR, Chaturvedi V, Vignali DA. Regulatory T cell suppression is potentiated by target T cells in a cell contact, IL-35- and IL-10-dependent manner. *J Immunol* 2009; **182**:6121–8.

47 Li T, Gu M, Liu P, Liu Y, Guo J, Zhang W *et al.* Clinical significance of decreased interleukin-35 expression in patients with psoriasis. *Microbiol Immunol* 2018; **62**:454–61.

48 Zhang J, Lin Y, Li C, Zhang X, Cheng L, Dai L *et al.* IL-35 decelerates the inflammatory process by regulating inflammatory cytokine secretion and M1/M2 macrophage ratio in psoriasis. *J Immunol* 2016; **197**:2131–44.

49 Wang Y, Mao Y, Zhang J, Shi G, Cheng L, Lin Y *et al.* IL-35 recombinant protein reverses inflammatory bowel disease and psoriasis through regulation of inflammatory cytokines and immune cells. *J Cell Mol Med* 2018; **22**:1014–25.

50 Barnett MP, Dommels YE, Butts CA, Zhu S, McNabb WC, Roy NC. Inoculation with enterococci does not affect colon inflammation in the multi-drug resistance 1a-deficient mouse model of IBD. *BMC Gastroenterol* 2016; **16**:31.

51 Collison LW, Chaturvedi V, Henderson AL, Giacomin PR, Guy C, Bankoti J *et al.* IL-35-mediated induction of a potent regulatory T cell population. *Nat Immunol* 2010; **11**:1093–101.

52 McLean MH, Neurath MF, Durum SK. Targeting interleukins for the treatment of inflammatory bowel disease-what lies beyond anti-TNF therapy? *Inflamm Bowel Dis* 2014; **20**:389–97.

53 Yan Y, Zhao N, He X, Guo H, Zhang Z, Liu T. Mesenchymal stem cell expression of interleukin-35 protects against ulcerative colitis by suppressing mucosal immune responses. *Cytotherapy* 2018; **20**:911–18.

54 Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. *Nature* 1980; **283**:666–8.

55 Kochetkova I, Thornburg T, Callis G, Pascual DW. Segregated regulatory $CD39^+CD4^+$ T cell function: TGF-β-producing Foxp3- and IL-10-producing $Foxp3^+$ cells are interdependent for protection against collagen-induced arthritis. *J Immunol* 2011; **187**:4654–66.

56 Kochetkova I, Trunkle T, Callis G, Pascual DW. Vaccination without autoantigen protects against collagen II-induced arthritis via immune deviation and regulatory T cells. *J Immunol* 2008; **181**:2741–52.

57 Kochetkova I, Thornburg T, Callis G, Holderness K, Maddaloni M, Pascual DW. Oral *Escherichia coli* colonization factor antigen I fimbriae ameliorate arthritis via IL-35, not IL-27. *J Immunol* 2014; **192**:804–16.

58 Herrick CA, Bottomly K. To respond or not to respond: T cells in allergic asthma. *Nat Rev Immunol* 2003; **3**:405–12.

59 Robinson DS. The role of regulatory T lymphocytes in asthma pathogenesis. *Curr Allergy Asthma Rep* 2005; **5**:136–41.

60 Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; **199**:1567–75.

61 Hansen JJ. Immune responses to intestinal microbes in inflammatory bowel diseases. *Curr Allergy Asthma Rep* 2015; **15**:61.

62 Whitehead GS, Wilson RH, Nakano K, Burch LH, Nakano H, Cook DN. IL-35 production by inducible costimulator (ICOS)-positive regulatory T cells reverses established IL-17-dependent allergic airways disease. *J Allergy Clin Immunol* 2012; **129**:207–15.e1-5.

63 Dong J, Wong CK, Cai Z, Jiao D, Chu M, Lam CW. Amelioration of allergic airway inflammation in mice by regulatory IL-35 through dampening inflammatory dendritic cells. *Allergy* 2015; **70**:921–32.

64 Huang CH, Loo EX, Kuo IC, Soh GH, Goh DL, Lee BW *et al.* Airway inflammation and IgE production induced by dust mite allergen-specific memory/effector Th2 cell line can be effectively attenuated by IL-35. *J Immunol* 2011; **187**:462–71.

65 Giovannini-Chami L, Blanc S, Hadchouel A, Baruchel A, Boukari R, Dubus JC *et al.* Eosinophilic pneumonias in children: a review of the epidemiology, diagnosis, and treatment. *Pediatr Pulmonol* 2016; **51**:203–16.

66 George L, Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. *Ther Adv Chronic Dis* 2016; **7**:34–51.

67 Kanai K, Park AM, Yoshida H, Tsunoda I, Yoshie O. IL-35 suppresses lipopolysaccharide-induced airway eosinophilia in EBI3-deficient mice. *J Immunol* 2017; **198**:119–27.

68 Himani G, Badini A, Nanji K. Depression and its associated factors among patients with chronic obstructive pulmonary disease in Karachi, Pakistan. *Cureus* 2018; **10**: e2930.

69 Jiang S, Shan F, Zhang Y, Jiang L, Cheng Z. Increased serum IL-17 and decreased serum IL-10 and IL-35 levels correlate with the progression of COPD. *Int J Chron Obstruct Pulmon Dis* 2018; **13**:2483–94.

70 Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 2001; **358**:221–9.

71 Luo X, Herold KC, Miller SD. Immunotherapy of type 1 diabetes: where are we and where should we be going? *Immunity* 2010; **32**:488–99.

72 Bettini M, Castellaw AH, Lennon GP, Burton AR, Vignali DA. Prevention of autoimmune diabetes by ectopic pancreatic β-cell expression of interleukin-35. *Diabetes* 2012; **61**:1519–26.

73 Manzoor F, Johnson MC, Li C, Samulski RJ, Wang B, Tisch R. β-cell-specific IL-35 therapy suppresses ongoing autoimmune diabetes in NOD mice. *Eur J Immunol* 2017; **47**:144–54.

74 Singh K, Kadesjo E, Lindroos J, Hjort M, Lundberg M, Espes D *et al.* Interleukin-35 administration counteracts established murine type 1 diabetes – possible involvement of regulatory T cells. *Sci Rep* 2015; **5**:12633.

75 Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**:582–92.

76 Yarygin KN, Lupatov AY, Kholodenko IV. Cell-based therapies of liver diseases: age-related challenges. *Clin Interv Aging* 2015; **10**:1909–24.

77 Yoshio S, Kanto T. Immunopathogenesis of hepatitis B virus infection. *Nihon Rinsho* 2015; **73**(Suppl 9):361–5.

78 Liu F, Tong F, He Y, Liu H. Detectable expression of IL-35 in $CD4^+$ T cells from peripheral blood of chronic hepatitis B patients. *Clin Immunol* 2011; **139**:1–5.

79 Li X, Tian L, Dong Y, Zhu Q, Wang Y, Han W *et al.* IL-35 inhibits HBV antigen-specific IFN-γ-producing CTLs *in vitro*. *Clin Sci (Lond)* 2015; **129**:395–404.

80 Cheng ST, Yuan D, Liu Y, Huang Y, Chen X, Yu HB *et al.* Interleukin-35 level is elevated in patients with chronic hepatitis B virus infection. *Int J Med Sci* 2018; **15**:188–94.

81 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**:646–74.

82 Wang K, Liu J, Li J. IL-35-producing B cells in gastric cancer patients. *Medicine (Baltimore)* 2018; **97**:e0710.

83 Tao Q, Pan Y, Wang Y, Wang H, Xiong S, Li Q *et al.* Regulatory T cells-derived IL-35 promotes the growth of adult acute myeloid leukemia blasts. *Int J Cancer* 2015; **137**:2384–93.

84 Wang HM, Zhang XH, Feng MM, Qiao YJ, Ye LQ, Chen J *et al.* Interleukin-35 suppresses the antitumor activity of T cells in patients with non-small cell lung cancer. *Cell Physiol Biochem* 2018; **47**:2407–19.

85 Wang Z, Liu JQ, Liu Z, Shen R, Zhang G, Xu J *et al.* Tumor-derived IL-35 promotes tumor growth by enhancing myeloid cell accumulation and angiogenesis. *J Immunol* 2013; **190**:2415–23.

86 Turnis ME, Sawant DV, Szymczak-Workman AL, Andrews LP, Delgoffe GM, Yano H *et al.* Interleukin-35 limits anti-tumor immunity. *Immunity* 2016; **44**:316–29.

87 Nicholl MB, Ledgewood CL, Chen X, Bai Q, Qin C, Cook KM *et al.* IL-35 promotes pancreas cancer growth through enhancement of proliferation and inhibition of apoptosis: evidence for a role as an autocrine growth factor. *Cytokine* 2014; **70**:126–33.

88 Long J, Zhang X, Wen M, Kong Q, Lv Z, An Y *et al.* IL-35 over-expression increases apoptosis sensitivity and suppresses cell growth in human cancer cells. *Biochem Biophys Res Commun* 2013; **430**:364–9.

89 Chatrabnous N, Ghaderi A, Ariafar A, Razeghinia MS, Nemati M, Jafarzadeh A. Serum concentration of interleukin-35 and its association with tumor stages and FOXP3 gene polymorphism in patients with prostate cancer. *Cytokine* 2018; **13**:221–27.

90 Zou JM, Qin J, Li YC, Wang Y, Li D, Shu Y *et al.* IL-35 induces N2 phenotype of neutrophils to promote tumor growth. *Oncotarget* 2017; **8**:33501–14.

91 Ma Y, Chen L, Xie G, Zhou Y, Yue C, Yuan X *et al.* Elevated level of interleukin-35 in colorectal cancer induces conversion of T cells into iTr35 by activating STAT1/STAT3. *Oncotarget* 2016; **7**:73003–15.

92 Zhang J, Mao T, Wang S, Wang D, Niu Z, Sun Z *et al.* Interleukin-35 expression is associated with colon cancer progression. *Oncotarget* 2017; **8**:71563–73.

93 Jiang S, Li Y, Lin T, Yuan L, Li Y, Wu S *et al.* IL-35 inhibits angiogenesis through VEGF/Ang2/Tie2 pathway in rheumatoid arthritis. *Cell Physiol Biochem* 2016; **40**:1105–16.