Cytokine 122 (2019) 154076

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

IL-35, as a newly proposed homeostasis-associated molecular pattern, plays three major functions including anti-inflammatory initiator, effector, and blocker in cardiovascular diseases

Xinyuan Li, Pu Fang, William Y. Yang, Hong Wang, Xiaofeng Yang*

Centers for Metabolic Disease Research, Cardiovascular Research, and Thrombosis Research, Departments of Pharmacology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140, USA

ARTICLE INFO

Keywords:
IL-35
Cardiovascular disease
Vascular inflammation
Atherosclerosis
HAMP

ABSTRACT

IL-35 is a new anti-inflammatory cytokine identified in 2007, which inhibits inflammation and immune responses by inducing regulatory T cells and regulatory B cells and suppressing effector T cells and macrophages. The unique initiator and effector anti-inflammatory properties of IL-35 bring tremendous interest in investigating its role during cardiovascular disease (CVD) development, in which inflammatory processes are firmly established as central to its development and complications. In this review, we update recent understanding of how IL-35 is produced and regulated in the cells. In addition, we outline the signaling pathways affected by IL-35 in different cell types. Furthermore, we summarize the roles of IL-35 in atherosclerosis, diabetes, and sepsis. We propose a new working model that IL-35 and its receptors are novel homeostasis-associated molecular pattern (HAMP) and HAMP receptors, respectively, which explains the complex nature of IL-35 signaling as an anti-inflammatory initiator, effector and blocker. Thorough understanding of this topic is significant towards development of new anti-inflammatory therapies against CVDs and other diseases. (total words: 163)

1. Introduction

Interleukin 35 (IL-35) is an anti-inflammatory cytokine that belongs to the IL-12 cytokine family. Besides IL-35, the IL-12 family cytokines also include IL-12, IL-23, and IL-27. IL-35 is a dimeric cytokine with two subunits, IL-12A and Epstein-Barr virus induced 3 (EBI3) [1,2]. Of note, the IL-12A subunit of IL-35 is shared by IL-12 (composed of IL-12A and IL-12B), whereas its EBI3 subunit is also shared by IL-27 (composed of EBI3 and IL-27p28) [3]. Secreted by regulatory T cells (Treg) and regulatory B cells (Breg), IL-35 suppresses the activities of T helper 1 (Th1) cells and T helper 17 (Th1) cells while promoting the development of Treg and Breg [4,5]. In T cells and endothelial cells, IL-35 signals through either heterodimer or homodimer of interleukin 12 receptor subunit beta 2 (IL12RB2)/interleukin 6 signal transducer (IL6ST), which subsequently activates heterodimer of signal transducer and activator of transcription 1 (STAT1)/STAT4 [6,7]. In B cells, IL-35 engages with heterodimer of IL12RB2/interleukin 27 receptor subunit alpha (IL27RA), which then induces the activation of STAT1/STAT3 [8].

Cardiovascular diseases (CVDs) are the leading causes of morbidity and mortality in the western worlds. Nearly three quarters of the CVD-related death is the result of atherosclerosis, which is a form of chronic autoimmune inflammatory condition predominantly occurred in middle/large sizes of arteries associated with specific CVD risk factors including hyperlipidemia, hyperglycemia, hyperhomocysteinemia, classical obesity, cigarette smoke, hypertension, infections, etc. [9,10]. Development of atherosclerosis are fueled by aberrant immune-mediated responses, which contribute to its adverse clinical outcome including myocardial infarction, stroke, and peripheral artery disease. It has been proposed that proinflammatory and Th1-related cytokines such as IL-1β and IL-18 promote atherosclerosis development [11,12], whereas Treg-related anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGF-β) are anti-atherogenic [13,14]. These notions are largely supported by the findings from atherosclerotic mouse models. It has been shown that transfer of murine IL-10 in the atherogenic apolipoprotein E (ApoE$^{-/-}$) mouse model achieved 60% reduction in atherosclerotic lesion size [15]. In addition, knockout of IL-10[13] or inhibition of TGF-β signaling[16] in ApoE$^{-/-}$ mice resulted in 300% and 200% increase of atherosclerotic lesion formation, respectively. Nevertheless, current anti-inflammatory therapies are not clinically effective against atherosclerosis [17], suggesting that our understanding of the roles of immune responses during atherosclerosis development is limited,

---

* Corresponding author at: Centers of Metabolic Disease Research and Cardiovascular Research, Lewis Katz School of Medicine at Temple University, MERB 1059, 3500 North Broad Street, Philadelphia, PA 19140, USA.

E-mail address: xfyang@temple.edu (X. Yang).

https://doi.org/10.1016/j.cyto.2017.06.003
Received 4 April 2017; Received in revised form 10 June 2017; Accepted 12 June 2017

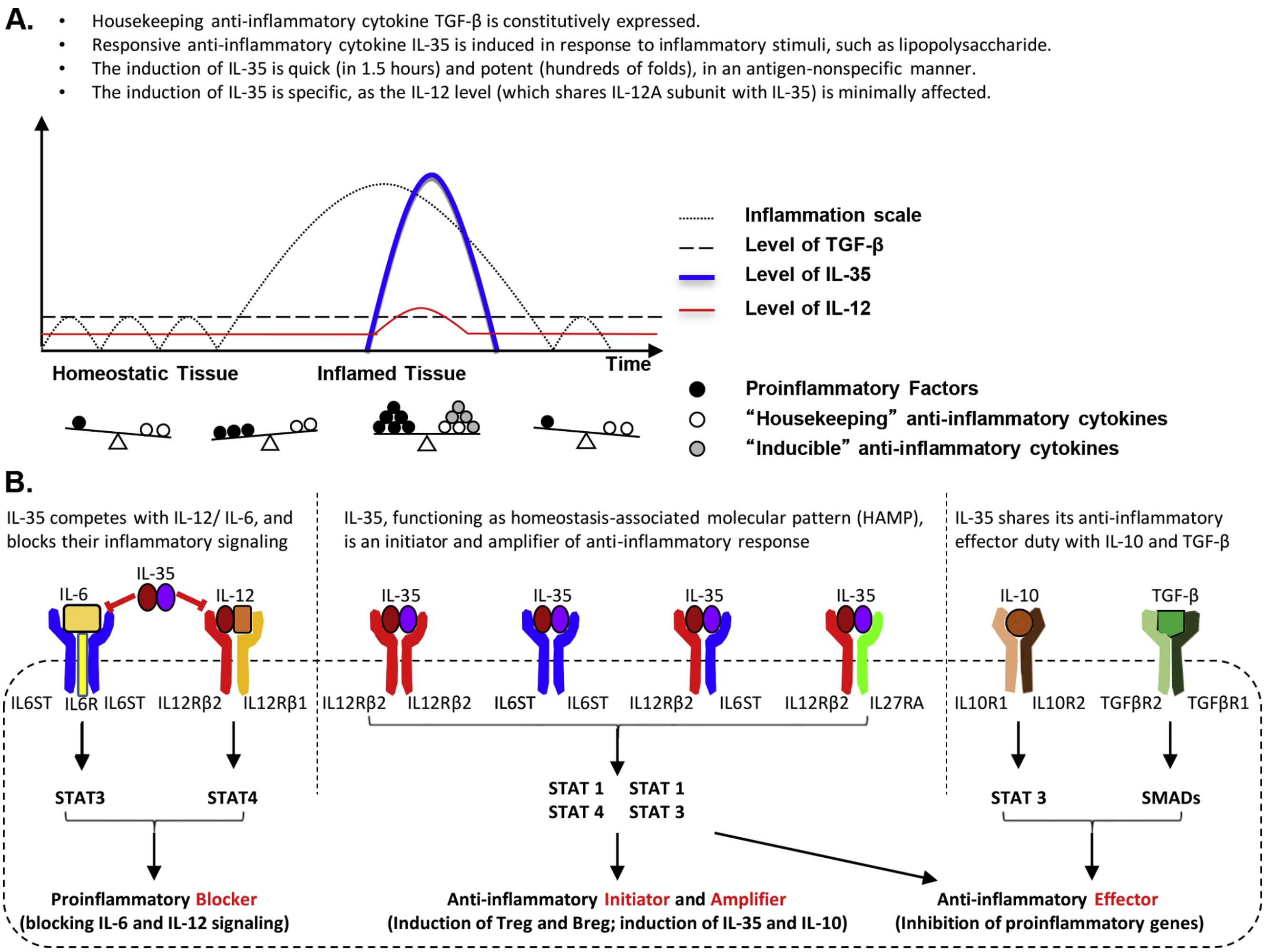

Fig. 1. IL-35, functioning as homeostasis-associated molecular pattern (HAMP), is both an initiator and effector of anti-inflammatory signaling. A. IL-35 is a responsive anti-inflammatory cytokine in an antigen-independent manner. Homeostatic tissues express "house-keeping" anti-inflammatory cytokine TGF-β to prevent it from initiation of inflammation. When tissues get inflamed, proinflammatory factors may stimulate tissues to express "responsive" anti-inflammatory cytokine IL-35 to counteract inflammation response. The induction of IL-35 is quick and potent, in an antigen-nonspecific manner. B. Three types of anti-inflammatory duties of IL-35. IL-35 functions as a HAMP, which are recognized by a variety of receptor and transcription factor formats and finetunes cellular homeostasis. Sharing its anti-inflammatory duty with IL-10 and TGF-β, IL-35 induces regulator T cells (Treg) and regulator B cells (Breg) and amplifies the production of IL-35 and IL-10. Furthermore, IL-35 could also potentially compete with IL-12 and IL-6 signaling (IL-12 and IL-35 shares IL12RB2 receptor subunit while IL-6 and IL-35 shares IL6ST receptor subunit), and blocks their proinflammatory signaling.

and that there is an urgent need in identifying novel therapeutic targets for the treatment of CVDs. IL-35 is a particularly promising target in this setting, since it is an initiator anti-inflammatory cytokine, which potently regulates the function of multiple immunosuppressive cell types, including Treg and Breg [18]. In this review, we summarize current understandings of several compelling issues: (1) How is IL-35 produced and regulated; (2) What signaling pathways are activated by IL-35 in the cells; and (3) The roles of IL-35 in CVDs including atherosclerosis, diabetes, and sepsis. Systemic characterizations of physiological and pathophysiological roles of IL-35 are significant towards the development of novel anti-inflammatory therapeutics against CVDs.

2. IL-35 is an initiator anti-inflammatory cytokine by inducing Treg and Breg, an effector cytokine in suppressing both innate and adaptive immune Responses, and a blocker cytokine in limiting the Accessibility of proinflammatory cytokines to their receptors

In 1997, it was demonstrated for the first time that IL-12A could heterodimerize with EBI3 to form a potentially naturally occurring protein [19]. In 2007, this protein was designated IL-35, which was shown to be highly expressed and secreted by Treg [2]. Deficiency of either subunit of IL-35 impaired the regulatory activity in Treg, while ectopic expression of IL-35 conferred suppressive activity on naïve T cells. In 2014, IL-35 production by B cells in mice was found to be also critical for the protection against experimental autoimmune encephalomyelitis and experimental autoimmune uveitis [8,20]. In addition, IL-35 induced the conversion of both mouse and human B cells into Breg, similarly to its role in the Treg [8,21]. Unlike Treg that is characterized by its specific transcription factor forkhead box P3 (Foxp3) expression, the identity of Breg is poorly understood [22,23]. Nevertheless, it has been reported that IL-35 could amplify its anti-inflammatory signal by inducing itself as well as IL-10 in T cells and B cells [8,21]. These unique initiator properties of IL-35 in inducing Treg and Breg, by which IL-35 immunosuppression is indirectly enhanced, as well as IL-35 self-amplification are unique and different from other anti-inflammatory cytokines such as IL-10 and TGF-β. It should be noted that IL-35 is not strictly required for the initiation of IL-10-mediated immunosuppression of Treg and of *in vivo* generated Breg [20,24], whereas the function of IL-35-induced Treg is independent of IL-10, Foxp3, or TGF-β [6]. In addition, TGF-β could also induce Treg [25]. Thus, other anti-inflammatory cytokines including IL-10 and TGF-β share their duty with IL-35 to initiate distinct anti-inflammatory programs.

Despite these significant discoveries, however, the roles of IL-35 in humans have been challenged since it was found that human T cells, including Treg, do not constitutively express IL-35 [26]. This apparent discrepancy could be reconciled by our observations that IL-35 is not constitutively expressed in human tissues, but it is inducible in response to inflammatory stimuli [27]. The genes encoding IL-35 subunits are induced by vascular endothelial cells, smooth muscle cells, and monocytes after activation with proinflammatory stimuli. IL-35 gene promoters can be bound by proinflammatory transcription factors including NF-kB; and IL-35 subunit transcripts are targeted by AU-rich elements and microRNAs, by which IL-35 may achieve its non-constitutive expression status and upregulation in response to inflammatory stimuli. Our findings were confirmed by our experimental report showing that IL-35 is induced in the plasma of mice after lipopolysaccharide (LPS) injection as well as in the plasma of sepsis patients [4]. Furthermore, recent discoveries from our laboratory suggested that IL-35 is also induced during early atherosclerosis in the plasma and aortas of atherosclerotic mouse model, ApoE$^{-/-}$ mice as well as in the plasma of human atherosclerotic patients (Li X and Yang X, unpublished observations). Taken together, these results indicated that IL-35 is a responsive anti-inflammatory cytokine, which can be inducible during acute inflammatory response and chronic inflammatory diseases, such as atherosclerosis (Fig. 1A).

In adaptive immune cells, such as naïve Treg co-activated by T cell antigen receptor-CD3 complex/T cell co-stimulation molecule CD28 and effector T cells stimulated by IL-35, the induction of IL-35 takes 9 days [27]. This contrasts with the observations that IL-35 subunits could be induced in a few hours in innate immune cell types such as dendritic cells and monocytes [27]. In one study of LPS-stimulated monocytes, it was found that IL-35 subunits are potently induced in less than 3 h [28]. This agrees with our previously findings that plasma IL-35 level in LPS-challenged mice could be upregulated by hundreds of folds in less than 1.5 h [7], indicating that IL-35 is induced by LPS from innate immune cell types in an antigen-independent manner, rather from the previously characterized adaptive immune cell types including Treg and Breg (Fig. 1A). Importantly, IL-12 levels in the same mice were minimally affected after LPS challenge, even though IL-12 and IL-35 shares the same IL-12A subunit [7]. These observations not only suggest the specificity of IL-35 rapid induction during inflammatory responses (Fig. 1A), but also indicate that competition of dimerization of IL-35 subunits against dimerization of IL-12/IL-27 subunits may be one of the novel mechanisms underlying IL-35′s anti-inflammatory cytokine function. Nevertheless, the residues in IL-12A and EBI3 that are critical for IL-12 and IL-27 dimerization do not mediate IL-35 dimer formation [29]. Therefore, an affinity/structure-based competition among the pairing of the IL-12 family subunits is currently an unlikely hypothesis. Regardless, this competition working model of IL-35 may apply to its receptor level. Since IL-35 shares its receptor and downstream transcription factors with proinflammatory cytokines such as IL-6 family cytokines (sharing IL6ST), IL-12 (sharing IL12RB2), and IL-27 (sharing IL6ST), engagement of IL-35 with its receptors could potentially limit the availability of these receptors binding to related proinflammatory cytokines including IL-6s, IL-12 and IL-27, thus dampening cellular signaling of those proinflammatory cytokines (Fig. 1B).

3. IL-35 and its receptors are novel Homeostasis-Associated molecule pattern (HAMP) and HAMP receptors

The composition and signaling of the IL-35 receptor are firstly studied in T cells [6]. This was based on the findings that IL-35 could suppress T cell proliferation and converts naïve T cells into IL-35-producing induced-Treg (iTr35 cells). Using T cells deficient in IL12RB2 and IL6ST, it was shown that either IL12RB2 or IL6ST is sufficient for mediating partial IL-35-mediated T cell suppression. Nevertheless, both IL12RB2 and IL6ST are required for the conversion of naïve T cells into iTr35 cells, since naïve T cells that lacks either subunit fail to upregulate IL-35 and develop suppressive activity. Thus, IL-35 could signal through either heterodimer of the two receptor subunits or homodimers of each subunit in T cells. Furthermore, IL-35 receptor signaling requires a heterodimer of STAT1 and STAT4, which could bind to the promoter regions of IL-35 subunits IL-12A and EBI3, contributing to its self-amplification.

Although it is not confirmed whether aortic endothelial cells (ECs) could secret IL-35, as we reported, IL-35 could potently inhibit leukocyte adhesion and the induction of EC activation marker gene vascular cell adhesion molecule (VCAM-1) in response to proinflammatory stimuli in ECs [7]. Like the scenario in T cells, the suppressive activity of IL-35 in ECs requires heterodimer or homodimer IL12RB2/IL6ST receptor. Both IL12RB2 and IL6ST are present in human aortic endothelial cells, and blocking either subunit reversed the suppressive effects of IL-35 on VCAM1 gene expression. Notably, engagement of IL-35 receptors leads to the suppression of mitogen-activated protein kinase (MAPK)-transcription factor activator protein-1 (AP-1) signaling pathway in ECs.

In B cells, knockdown or neutralizing of IL-35 receptor subunit IL6ST does not affect IL-35-mediated suppression of B cell proliferation or IL-10 production [8]. In contrast, silencing either IL-35 receptor subunit IL12RB2 or IL27RA completely abolished the inhibitory effects of IL-35 in B cells. Moreover, IL-35 activates STAT1/STAT3, which is required for IL-35-mediated suppression of B cell proliferation and IL-10 production. Collectively, these results indicated that IL-35 receptor has at least 4 types of different formats (two homodimers IL12RB2-IL12RB2, IL6ST-IL6ST, and two heterodimers IL12RB2-IL-6ST, and IL12RB2-IL27RA) and elicit at least 2 sets of transcription factor complex (STAT1-STAT4 and STAT1-STAT3).

As we pointed out in our previous report [30], it is significant for us to propose a new model [31] to recognize novel anti-inflammatory and homeostatic signals derived from endogenous metabolites. Recent advances in immunology have clearly demonstrated the well-accepted “two arms model”. This model states that in addition to pro-inflammatory effector and T cell co-stimulatory mechanisms, there are several anti-inflammatory mechanisms present in the immune system. These anti-inflammatory mechanisms include T cell co-inhibition/co-suppression pathways, T cell anergy, regulatory T cells [18], and secretion of anti-inflammatory/immunosuppressive cytokines such as TGF-β, IL-10, IL-35 [7,27], and IL-37 as we and others reported. We have reported two types of lysophospholipids such as lysophosphatidylserine (LysoPS), lysophosphatidylethanolamine (LPE) [30], a few uremic toxins as anti-inflammatory homeostasis-associated molecular patterns (HAMPs), which is a new concept proposed in our recent report [32]. Moreover, most nuclear receptor family members could also be characterized as HAMPs (Wang L and Yang X, unpublished observations). Similar to other endogenous metabolites that we reported[30], here we propose for the first time that IL-35, along with its IL-12 cytokine family members, are HAMPs for anti-inflammatory functions and Danger-Associated Molecular Patterns (DAMPs) for pro-inflammatory functions. IL-35 does not specifically bind to one type of receptor, but rather loosely possesses a “pattern” feature that is promiscuously recognized by different IL-12 family receptors, which serve as HAMP receptors [30] (Fig. 1B). This feature of IL-35 is very similar to high mobility group box 1 (HMGB1), which is a molecular pattern recognized by at least 11 distinct receptors [33]. Of note, IL-35 is not evolutionally conserved and needs to be transcriptionally induced for its action, which is different from classical molecular patterns. The potential advantages for IL-35 to act as a HAMP and bind those pattern types of homodimer- and heterodimer- receptors may include the following aspects: (1) IL-35 inhibits broad spectrum of inflammation and immune responses no matter which receptor(s) is expressed and functional in different cell types during various pathogenic inflammatory processes; (2) IL-35 blocks and interferes with IL-6, IL-12 and IL-27 receptor signaling; and (3) IL-35 maintains systemic and tissue homeostasis in a highly responsive and effective manner. This unique property of IL-35 might be shared by other IL-12 family members, since a recent study has found that EBI3 (receptor subunit for IL-35 and IL-27) could also mediate IL-6 *trans*-signaling, different from its classical IL6ST/IL6R receptor format [34].

4. IL-35 and atherosclerosis

Atherosclerosis is a chronic inflammatory disorder of the arteries, involving both inflammation and autoimmune processes [35]. Due to the immunomodulatory nature of IL-35, considerate interest has been developed to investigate its role in the development of atherosclerosis. One of the earliest report investigating the role of IL-35 in atherosclerosis development comes from examination of the expression of IL-35 subunits in human atheromatous lesions by immunohistochemistry. It was found that one of the IL-35 subunit, EBI3, is detectable in lesional endothelial cells, smooth muscle cells, and macrophages [36]. In addition, the other subunit of IL-35, IL-12A, was also present at high levels throughout the atheroma samples and co-expressed with EBI3. Another study examined plasma IL-35 levels in patients with acute coronary syndrome (ACS, including unstable angina pectoris and acute myocardial infarction) and stable angina pectoris, in comparison to chest pain syndrome controls [37]. The plasma levels of IL-35, alongside with other anti-inflammatory cytokines such as IL-10 and TGF-β, were found to be significantly decreased in the stable angina pectoris, and further decreased in ACS patients, compared with those in the chest pain syndrome control group. By contrast, the plasma levels of IL-27 (which shares EBI3 subunit with IL-35) and IL-12 (which shares IL-12A subunit with IL-35) were increased in the ACS patients in comparison with patients with stable angina pectoris and chest pain syndrome, indicating the specificity of IL-35 level changes in these patients. Furthermore, there was a positive correlation between plasma IL-35 levels and left ventricular ejection fraction, while IL-27 negatively correlated with the heart function feature in these patients [37]. Genetics study also provided strong evidence that IL-35 plays an important role in the development of atherosclerotic disease. The *IL-12A* rs2243115 and *EBI3* rs428253 polymorphisms were found to be associated with decreased risk of developing premature coronary artery disease (CAD) in the Genetics of Atherosclerotic Disease (GEA) study of 1162 patients with CAD and 873 controls [38]. However, it should be noted that these two associated polymorphisms did not correlate with plasma IL-35 level changes; and that two other polymorphisms, EBI3 rs4740 and rs4905, were associated with different levels of IL-35 only in the healthy control groups. Taken together, these results indicate that IL-35 is a promising biomarker for atherosclerotic disease, and may also serve as a potential therapeutic target for the treatment of disease. This notion was supported by the findings that exogenous treatment of IL-35 significantly attenuates atherosclerotic lesions in ApoE$^{-/-}$ mice, which is a mouse model for atherosclerosis [39]. The inhibitory effect of IL-35 in atherogenesis was attributed to the findings that IL-35 significantly decreased plasma levels of total cholesterol and total triglyceride; and that it also significantly upregulated Foxp3 expression in the plasma and lesion, an indication of enhanced activity of regulatory T cells. Despite these results, the roles of endogenous IL-35 in atherosclerosis development remain unclear. This is related to the fact that IL-35 shares its subunit with IL-12 and IL-27, and that knockout of either subunit of IL-35 will also result in the loss of either IL-12 or IL-27, respectively. Nevertheless, it has been found that deficiency of EBI3 in atherosclerosis-prone low density lipoprotein receptor knockout mice resulted in significantly larger atherosclerotic lesions, due to enhanced accumulation and activation of macrophages in arterial walls [40]. These results indicated that the gene EBI3 itself (which is shared by both IL-35 and IL-27) suppresses the development of atherosclerosis. Although IL-27 has been reported to inhibit atherosclerosis [40,41], further studies are still needed to dissect the role of IL-35 and IL-27 in atherosclerosis.

5. IL-35 and type 1 diabetes

As many as three million Americans suffer from type 1 diabetes (T1D); and T1D is associated with around 10-fold increased risk for cardiovascular diseases (CVDs) [42]. We previously reported that prolongation of Treg survival enhances Treg suppression of T1D [43,44]. Due to the autoimmune nature of T1D, significant efforts have been made to investigate the role of anti-inflammatory cytokine IL-35 in T1D. Early reports showed that ectopic expression of IL-35 by pancreatic β-cells led to substantial, long-term protection against autoimmune diabetes in nonobese diabetic (NOD) mice, which is a mouse model for human T1D [45]. These mice exhibited decreased islet infiltration of lymphocytes, including antigen-specific reactive T cells, which were attributed to diminished T-cell proliferation and G1 arrest. Another study showed that ectopic IL-35 expression by splenic dendritic cells from NOD mice also induced a delayed and less severe form of diabetes, an effect accompanied by the increase of suppressive T cells in pancreatic lymph nodes [46]. A third study investigated the role of systemic application of IL-35 in the multiple dose streptozotocin induced (MLDSTZ) T1D model [47]. This report demonstrated that in MLDSTZ mice, Treg were induced, and showed decreased production of anti-inflammatory cytokines such as IL-35 but increased production of proinflammatory cytokines. IL-35 administration both prevented and counteracted established MLDSTZ T1D, likely through upregulating IL-35 production in Treg. Systemic IL-35 treatment also reversed established T1D in the NOD mouse model. Furthermore, plasma IL-35 levels were found to be decreased in human T1D patients compared to age-matched controls. Moreover, the serum and vitreous levels of IL-35 were significantly decreased in proliferative diabetic retinopathy patients compared to those in the controls. Lastly, the capacity of IL-35 to suppress ongoing autoimmunity in NOD mice was tested by gene therapy method. An adeno-associated virus vector containing IL-35 transgene expression was selectively targeted to β cells via an insulin promoter. Vaccination of this construct towards NOD mice at a late preclinical stage of type 1 diabetes not only successfully suppressed β-cell autoimmunity but also blocked T1D onset [48]. This was accompanied by reduced numbers of islet-infiltrating lymphocytes and dendritic cells. Taken together, these results demonstrated promising therapeutic potential of targeting IL-35 in the treatment of T1D and its associated CVD complications.

6. IL-35 and sepsis

Sepsis, caused by bacterial infection, strikes about 700,000 people annually. Epidemiologic evidence shows that endotoxemia at the levels as low as 50 pg/ml constitutes a strong risk factor for the development of atherosclerosis [49,50]. In addition, chronic infections conferred an increased risk of atherosclerosis even in low-risk populations who lacked classic vascular disease risk factors. We have reported previously that in human patients with sepsis, plasma IL-35 levels were also significantly higher than that of healthy controls [7]. Moreover, in mice with LPS-induced endotoxemia, plasma concentration of IL-35 was dramatically increased, which occurred as early as 1.5 h after LPS challenge and lasted for 24 h. Similarly, plasma IL-27 level was also quickly induced after LPS challenge in mice, but decreased after 24 h. These results strongly support the notion that both IL-35 and IL-27 are responsive cytokines, which are upregulated in response to inflammation and play distinct roles during different stage of the inflammatory response. Another independent group reported similar findings. IL-35 concentration was found to be significantly higher in serum samples from adult or child patients with sepsis when comparing with that from healthy controls [49]. Furthermore, plasma IL-35 concentrations also positively correlated with sepsis severity, with patients who suffer from septic shock having the highest amount of IL-35 levels in their blood samples. After effective treatment of sepsis, IL-35 levels were also found to return to normal values. These results indicated IL-35 may serve as an ideal biomarker for sepsis diagnostics. Another recent study examined this issue and found that the diagnostic performance of IL-35 was superior to that of procalcitonin, C-reactive protein, and other commonly used markers for early onset sepsis [51].

In terms of its role in vascular inflammation during sepsis, it was found that IL-35 could inhibit leukocyte adhesion to the endothelium in the mouse vessels of lung and cremaster muscle induced by LPS [7]. Mechanistically, IL-35 was shown to suppress LPS-induced endothelial cell activation by engaging its receptor IL6ST and IL12RB2 and subsequently inhibiting MAPK-AP-1 signaling pathway. The role of endogenous IL-35 during sepsis was also examined by studying EBI3 gene knockout mice [7]. EBI3$^{-/-}$ mice showed similar levels of leukocyte adhesion in the vessels of lung and cremaster muscle with or without LPS challenge. Nevertheless, exogeneous IL-35 could still inhibit LPS-induced leukocyte adhesion in EBI3-/- mice. Furthermore, we have shown that IL-27, which shares EBI3 subunit with IL-35, could significantly induce endothelial cell activation. These results indicate that EBI3 gene itself is neither proinflammatory nor anti-inflammatory during acute vascular response to LPS stimulation, due to the opposing roles of IL-35 and IL-27. Thus, fine-tuning of the production of IL-35 and IL-27 is important in controlling the scale of vascular inflammation in sepsis.

7. Concluding remarks and future directions

During the last 10 years since the discovery of IL-35, significant progress has been made to characterize the biology of IL-35 and its implication in different diseases such as CVDs. As a type of responsive anti-inflammatory cytokine that potently suppresses multiple pro-inflammatory cell types while promoting suppressive immune cells, IL-35 holds great promise for the treatment of inflammatory diseases. Nevertheless, before translational studies of IL-35 could occur, further characterizations of both physiological and pathological role of IL-35 are required. Firstly, although IL-35 can promote the function of certain Treg and Breg subsets, block pro-inflammatory signaling and inhibit innate immune responses, currently it is unclear when these effects take place temporally and spatially relative to each other and within a CVD-specific context. More specifically, IL-35 could be found in the atherosclerotic lesions, but it remains unknown what cells are producing IL-35 and what cells are recruited to the plaque by IL-35. Moreover, it is not known what cell types are affected by IL-35. Secondly, although IL-35-regulated genes have been reported in cancer cells [52], the signaling pathways activated/inhibited by IL-35 and the genes regulated by IL-35 in the primary cell types relevant to CVDs remain poorly characterized. Lastly, the precise endogenous roles and underlying mechanisms of IL-35 in CVDs, such as atherosclerosis and myocardial infarction, need to be established using IL-35 subunit knockout mouse models. Given the unique dual initiator and effector anti-inflammatory roles of IL-35 during inflammatory response, clinical expectation is high towards new IL-35-targeted therapies in CVDs and other inflammatory/autoimmune diseases.

Competing interests

The authors declare that they have no competing interests.

Author’s contribution

XL carried out the primary literature search and drafted the manuscript. PF, WYY and HW provided material input and helped critically reading the manuscript. XFY supervised the study and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This work was partially supported by the National Institutes of Health – United States Grants to XFY and HW.

References

[1] L.W. Collison, D.A. Vignali, Interleukin-35: odd one out or part of the family? Immunol. Rev. 226 (2008) 248–262.
[2] L.W. Collison, C.J. Workman, T.T. Kuo, K. Boyd, Y. Wang, K.M. Vignali, R. Cross, D. Sehy, R.S. Blumberg, D.A. Vignali, The inhibitory cytokine IL-35 contributes to regulatory T-cell function, Nature 450 (7169) (2007) 566–569.
[3] D.A. Vignali, V.K. Kuchroo, IL-12 family cytokines: immunological playmakers, Nat. Immunol. 13 (8) (2012) 722–728.
[4] D.V. Sawant, K. Hamilton, D.A. Vignali, Interleukin-35: Expanding Its Job Profile, J. Interferon Cytokine Res. 35 (7) (2015) 499–512.
[5] J. Zhang, Y. Lin, C. Li, X. Zhang, L. Cheng, L. Dai, Y. Wang, F. Wang, G. Shi, Y. Li, Q. Yang, X. Cui, Y. Liu, H. Wang, S. Zhang, Y. Yang, R. Xiang, J. Li, D. Yu, Y. Wei, H. Deng, IL-35 Decelerates the Inflammatory Process by Regulating Inflammatory Cytokine Secretion and M1/M2 Macrophage Ratio in Psoriasis, J. Immunol. 197 (6) (2016) 2131–2144.
[6] L.W. Collison, G.M. Delgoffe, C.S. Guy, K.M. Vignali, V. Chaturvedi, D. Fairweather, A.R. Satoskar, K.C. Garcia, C.A. Hunter, C.G. Drake, P.J. Murray, D.A. Vignali, The composition and signaling of the IL-35 receptor are unconventional, Nat. Immunol. 13 (3) (2012) 290–299.
[7] X. Sha, S. Meng, X. Li, H. Xi, M. Maddaloni, D.W. Pascual, H. Shan, X. Jiang, H. Wang, X.F. Yang, Interleukin-35 Inhibits Endothelial Cell Activation by Suppressing MAPK-AP-1 Pathway, J. Biol. Chem. 290 (31) (2015) 19307–19318.
[8] R.X. Wang, C.R. Yu, I.M. Dambuza, R.M. Mahdi, M.B. Dolinska, Y.V. Sergeev, P.T. Wingfield, S.H. Kim, C.E. Egwuagu, Interleukin-35 induces regulatory B cells that suppress autoimmune disease, Nat. Med. 20 (6) (2014) 633–641.
[9] A. Virtue, C. Johnson, J. Lopez-Pastrana, Y. Shao, H. Fu, X. Li, Y.F. Li, Y. Yin, J. Mai, V. Rizzo, M. Tordoff, Z. Bagi, H. Shan, X. Jiang, H. Wang, X.F. Yang, MicroRNA-155 Deficiency Leads to Decreased Atherosclerosis, Increased White Adipose Tissue Obesity, and Non-alcoholic Fatty Liver Disease: A NOVEL MOUSE MODEL OF OBESITY PARADOX, J. Biol. Chem. 292 (4) (2017) 1267–1287.
[10] E.J. Benjamin, M.J. Blaha, S.E. Chiuve, M. Cushman, S.R. Das, R. Deo, S.D. de Ferranti, J. Floyd, M. Fornage, C. Gillespie, C.R. Isasi, M.C. Jimenez, L.C. Jordan, S.E. Judd, D. Lackland, J.H. Lichtman, L. Lisabeth, S. Liu, C.T. Longenecker, R.H. Mackey, K. Matsushita, D. Mozaffarian, M.E. Mussolino, K. Nasir, R.W. Neumar, L. Palaniappan, D.K. Pandey, R.R. Thiagarajan, M.J. Reeves, M. Ritchey, C.J. Rodriguez, G.A. Roth, W.D. Rosamond, C. Sasson, A. Towfighi, C.W. Tsao, M.B. Turner, S.S. Virani, J.H. Voeks, J.Z. Willey, J.T. Wilkins, J.H. Wu, H.M. Alger, S.S. Wong, P. Muntner, C. American Heart Association Statistics, S. Stroke Statistics, Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association, Circulation 135 (10) (2017) e146–e603.
[11] F. Merhi-Soussi, B.R. Kwak, D. Magne, C. Chadjichristos, M. Berti, G. Pelli, R.W. James, F. Mach, C. Gabay, Interleukin-1 plays a major role in vascular inflammation and atherosclerosis in male apolipoprotein E-knockout mice, Cardiovascular Res. 66 (3) (2005) 583–593.
[12] Z. Mallat, A. Corbaz, A. Scoazec, P. Graber, S. Alouani, B. Esposito, Y. Humbert, Y. Chvatchko, A. Tedgui, Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability, Circulation Res. 89 (7) (2001) E41–E45.
[13] G. Caligiuri, M. Rudling, V. Ollivier, M.P. Jacob, J.B. Michel, G.K. Hansson, A. Nicoletti, Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice, Mol. Med. 9 (1–2) (2003) 10–17.
[14] N.N. Singh, D.P. Ramji, The role of transforming growth factor-beta in atherosclerosis, Cytokine & Growth Factor Rev. 17 (6) (2006) 487–499.
[15] Z. Mallat, S. Besnard, M. Duriez, V. Deleuze, F. Emmanuel, M.F. Bureau, F. Soubrier, B. Esposito, H. Duez, C. Fievet, B. Staels, N. Duverger, D. Scherman, A. Tedgui, Protective role of interleukin-10 in atherosclerosis, Circulation Res. 85 (8) (1999) e17–e24.
[16] Z. Mallat, A. Gojova, C. Marchiol-Fournigault, B. Esposito, C. Kamate, R. Merval, D. Fradelizi, A. Tedgui, Inhibition of transforming growth factor-beta signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice, Circulation Res. 89 (10) (2001) 930–934.
[17] I.F. Charo, R. Taub, Anti-inflammatory therapeutics for the treatment of atherosclerosis nature reviews, Drug Discovery 10 (5) (2011) 365–376.
[18] W.Y. Yang, Y. Shao, J. Lopez-Pastrana, J. Mai, H. Wang, X.F. Yang, Pathological conditions re-shape physiological Tregs into pathological Tregs, Burns & trauma 3 (1) (2015).
[19] O. Devergne, M. Birkenbach, E. Kieff, Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin, Proc. Natl. Acad. Sci. USA 94 (22) (1997) 12041–12046.
[20] P. Shen, T. Roch, V. Lampropoulou, R.A. O'Connor, U. Stervbo, E. Hilgenberg, S. Ries, V.D. Dang, Y. Jaimes, C. Daridon, R. Li, L. Jouneau, P. Boudinot, S. Wilantri, I. Sakwa, Y. Miyazaki, M.D. Leech, R.C. McPherson, S. Wirtz, M. Neurath, K. Hoehlig, E. Meinl, A. Grutzkau, J.R. Grun, K. Horn, A.A. Kuhl, T. Dorner, A. Bar-Or, S.H. Kaufmann, S.M. Anderton, S. Fillatreau, IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases, Nature 507 (7492) (2014) 366–370.
[21] L.W. Collison, V. Chaturvedi, A.L. Henderson, P.R. Giacomin, C. Guy, J. Bankoti, D. Finkelstein, K. Forbes, C.J. Workman, S.A. Brown, J.E. Rehg, M.L. Jones, H.T. Ni, D. Artis, M.J. Turk, D.A. Vignali, IL-35-mediated induction of a potent regulatory T cell population, Nat. Immunol. 11 (12) (2010) 1093–1101.
[22] R. Madan, F. Demircik, S. Surianarayanan, J.L. Allen, S. Divanovic, A. Trompette,

N. Yogev, Y. Gu, M. Khodoun, D. Hildeman, N. Boespflug, M.B. Fogolin, L. Grobe, M. Greweling, F.D. Finkelman, R. Cardin, M. Mohrs, W. Muller, A. Waisman, A. Roers, C.L. Karp, Nonredundant roles for B cell-derived IL-10 in immune counter-regulation, J. Immunol. 183 (4) (2009) 2312–2320.

[23] L.L. Teichmann, M. Kashgarian, C.T. Weaver, A. Roers, W. Muller, M.J. Shlomchik, B cell-derived IL-10 does not regulate spontaneous systemic autoimmunity in MRL.Fas(lpr) mice, J. Immunol. 188 (2) (2012) 678–685.

[24] L.W. Collison, M.R. Pillai, V. Chaturvedi, D.A. Vignali, Regulatory T cell suppression is potentiated by target T cells in a cell contact, IL-35- and IL-10-dependent manner, J. Immunol. 182 (10) (2009) 6121–6128.

[25] W. Chen, W. Jin, N. Hardegen, K.J. Lei, L. Li, N. Marinos, G. McGrady, S.M. Wahl, Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3, J. Exp. Med. 198 (12) (2003) 1875–1886.

[26] E. Bardel, F. Larousserie, P. Charlot-Rabiega, A. Coulomb-L'Hermine, O. Devergne, Human CD4+ CD25+ Foxp3+ regulatory T cells do not constitutively express IL-35, J. Immunol. 181 (10) (2008) 6898–6905.

[27] X. Li, J. Mai, A. Virtue, Y. Yin, R. Gong, X. Sha, S. Gutchigian, A. Frisch, I. Hodge, X. Jiang, H. Wang, X.F. Yang, IL-35 is a novel responsive anti-inflammatory cytokine–a new system of categorizing anti-inflammatory cytokines, PLoS One 7 (3) (2012) e33628.

[28] S. Pflanz, J.C. Timans, J. Cheung, R. Rosales, H. Kanzler, J. Gilbert, L. Hibbert, T. Churakova, M. Travis, E. Vaisberg, W.M. Blumenschein, J.D. Mattson, J.L. Wagner, W. To, S. Zurawski, T.K. McClanahan, D.M. Gorman, J.F. Bazan, R. de Waal Malefyt, D. Rennick, R.A. Kastelein, IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells, Immunity 16 (6) (2002) 779–790.

[29] L.L. Jones, V. Chaturvedi, C. Uyttenhove, J. Van Snick, D.A. Vignali, Distinct subunit pairing criteria within the heterodimeric IL-12 cytokine family, Mol. Immunol. 51 (2) (2012) 234–244.

[30] L.M. Ferrer, A.M. Monroy, J. Lopez-Pastrana, G. Nanayakkara, R. Cueto, Y.F. Li, X. Li, H. Wang, X.F. Yang, E.T. Choi, Caspase-1 plays a critical role in accelerating chronic kidney disease-promoted neointimal hyperplasia in the carotid artery, J. Cardiovasc. Transl. Res. (2016).

[31] T.S. Kuhn, The structure ofscientific revolutions, 3rd ed., University of Chicago Press, Chicago, 1996.

[32] Y. Sun, Candice Johnson, Jun Zhou, Luqiao Wang, Ya-Feng Li, Yifan Lu, Gayani Nanayakkara, Hangfei Fu, Ying Shao, Claudette Sanchez, William Y. Yang, Xin Wang, Eric T. Choi, Rongshan Li, Hong Wang, Xiao-Feng Yang, Uremic toxins are conditional danger- or homeostasis-associated molecular patterns, Frontiers in Bioscience 22 (2017).

[33] H. Yang, H. Wang, S.S. Chavan, U. Andersson, High mobility group box protein 1 (HMGB1): the prototypical endogenous danger molecule, Mol. Med. 21 (Suppl 1) (2015) S6–S12.

[34] S. Chehboun, J. Labrecque-Carbonneau, S. Pasquin, Y. Meliani, B. Meddah, W. Ferlin, M. Sharma, A. Tormo, J.F. Masson, J.F. Gauchat, Epstein-Barr virus-induced gene 3 (EBI3) can mediate IL-6 trans-signaling, J. Biol. Chem. 292 (16) (2017) 6644–6656.

[35] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, Nature 473 (7347) (2011) 317–325.

[36] S. Kempe, P. Heinz, E. Kokai, O. Devergne, N. Marx, T. Wirth, Epstein-barr virus-induced gene-3 is expressed in human atheroma plaques, Am. J. Pathol. 175 (1) (2009) 440–447.

[37] Y. Lin, Y. Huang, Z. Lu, C. Luo, Y. shi, Q. Zeng, Y. Cao, L. Liu, X. Wang, Q. Ji, Decreased plasma IL-35 levels are related to the left ventricular ejection fraction in coronary artery diseases, PLoS One 7 (12) (2012) e52490.

[38] R. Posadas-Sanchez, N. Perez-Hernandez, J. Angeles-Martinez, F. Lopez-Bautista, T. Villarreal-Molina, J.M. Rodriguez-Perez, J.M. Fragoso, C. Posadas-Romero, G. Vargas-Alarcon, Interleukin 35 polymorphisms are associated with decreased risk of premature coronary artery disease, metabolic parameters, and IL-35 levels: the genetics of atherosclerotic disease (GEA) study, Mediators Inflamm. 2017 (2017) 6012795.

[39] L. Tao, J. Zhu, Y. Chen, Q. Wang, Y. Pan, Q. Yu, B. Zhou, H. Zhu, IL-35 improves Treg-mediated immune suppression in atherosclerotic mice, Exp. Ther. Med. 12 (4) (2016) 2469–2476.

[40] T. Hirase, H. Hara, Y. Miyazaki, N. Ide, A. Nishimoto-Hazuku, H. Fujimoto, C.J. Saris, H. Yoshida, K. Node, Interleukin 27 inhibits atherosclerosis via immunoregulation of macrophages in mice, Am. J. Physiol. Heart Circ. Physiol. 305 (3) (2013) H420–H429.

[41] E.K. Koltsova, G. Kim, K.M. Lloyd, C.J. Saris, S. von Vietinghoff, M. Kronenberg, K. Ley, Interleukin-27 receptor limits atherosclerosis in Ldlr-/- mice, Circulation research 111 (10) (2012) 1274–1285.

[42] S.D. de Ferranti, I.H. de Boer, V. Fonseca, C.S. Fox, S.H. Golden, C.J. Lavie, S.N. Magge, N. Marx, D.K. McGuire, T.J. Orchard, B. Zinman, R.H. Eckel, Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association, Diabetes Care 37 (10) (2014) 2843–2863.

[43] Y. Yan, Z. Xiong, S. Zhang, J. Song, Y. Huang, A.M. Thornton, H. Wang, X.F. Yang, CD25high T cells with a prolonged survival inhibit development of diabetes, International J. Immunopathol. Pharmacol. 21 (4) (2008) 767–780.

[44] X.F. Yang, Factors regulating apoptosis and homeostasis of CD4+CD25highFOXP3+ regulatory T cells are new therapeutic targets, Front Biosci 13 (2008) 1472–1499.

[45] M. Bettini, A.H. Castellaw, G.P. Lennon, A.R. Burton, D.A. Vignali, Prevention of autoimmune diabetes by ectopic pancreatic beta-cell expression of interleukin-35, Diabetes 61 (6) (2012) 1519–1526.

[46] G. Mondanelli, C. Volpi, R. Bianchi, M. Allegrucci, V.N. Talesa, U. Grohmann, M.L. Belladonna, Islet antigen-pulsed dendritic cells expressing ectopic IL-35Ig protect nonobese diabetic mice from autoimmune diabetes, Cytokine 75 (2) (2015) 380–388.

[47] K. Singh, E. Kadesjo, J. Lindroos, M. Hjort, M. Lundberg, D. Espes, P.O. Carlsson, S. Sandler, L. Thorvaldson, Interleukin-35 administration counteracts established murine type 1 diabetes–possible involvement of regulatory T cells, Sci. Rep. 5 (2015) 12633.

[48] F. Manzoor, M.C. Johnson, C. Li, R.J. Samulski, B. Wang, R. Tisch, beta-cell-specific IL-35 therapy suppresses ongoing autoimmune diabetes in NOD mice, Eur. J. Immunol. 47 (1) (2017) 144–154.

[49] C.J. Wiedermann, S. Kiechl, S. Dunzendorfer, P. Schratzberger, G. Egger, F. Oberhollenzer, J. Willeit, Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study, J. Am. Coll. Cardiol. 34 (7) (1999) 1975–1981.

[50] X.F. Yang, Y. Yin, H. Wang, VASCULAR INFLAMMATION AND ATHEROGENESIS ARE ACTIVATED VIA RECEPTORS FOR PAMPs AND SUPPRESSED BY REGULATORY T CELLS, Drug Discov. Today Ther. Strateg. 5 (2) (2008) 125–142.

[51] W.X. Du, Y. He, H.Y. Jiang, Q. Ai, J.L. Yu, Interleukin 35: A novel candidate biomarker to diagnose early onset sepsis in neonates, Clin. Chim. Acta 462 (2016) 90–95.

[52] C. Huang, N. Li, Z. Li, A. Chang, Y. Chen, T. Zhao, Y. Li, X. Wang, W. Zhang, Z. Wang, L. Luo, J. Shi, S. Yang, H. Ren, J. Hao, Tumour-derived Interleukin 35 promotes pancreatic ductal adenocarcinoma cell extravasation and metastasis by inducing ICAM1 expression, Nature Commun. 8 (2017) 14035.