Cytokine 122 (2019) 154169

Contents lists available at ScienceDirect

Cytokine

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

Potential role of IL-37 in atherosclerosis

Sara McCurdy[a], Chloe A. Liu[a], Jonathan Yap[a], William A. Boisvert[a,b,*]

[a] Center for Cardiovascular Research, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, United States
[b] Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

ARTICLE INFO

Keywords:
Atherosclerosis
IL-37
Anti-inflammatory properties

ABSTRACT

IL-37 is a member of the IL-1 family, but unlike most other members of this family of cytokines, it has wide-ranging anti-inflammatory properties. Initially shown to bind IL-18 binding protein and prevent IL-18-mediated inflammation, its known role has been expanded to include distinct pathways, both intracellular involving the transcription factor Smad3, and extracellular via binding to the orphan receptor IL-1R8. A number of recent publications investigating the role of IL-37 in atherosclerosis and ischemic heart disease have revealed promising therapeutic value of the cytokine. Although research concerning the role of IL-37 and its mechanism in atherosclerosis is relatively scant, there are a number of well-known atherosclerotic processes that this cytokine can mediate with the potential of modulating the disease progression itself. This review will probe in detail the effects of IL-37 on important pathological processes such as inflammation, dysregulated lipid metabolism, and apoptosis, by analyzing existing data as well as exploring the potential of this cytokine to influence these properties.

1. Introduction

Since its discovery in 2000 by *in silico* research, the Interleukin (IL)-1 family cytokine IL-37 has gained much attention due to its potent anti-inflammatory properties. Initially shown to prevent IL-18-induced inflammation via binding to the IL-18 binding protein (IL-18bp), the mechanisms by which IL-37 functions have been further elucidated in the last few years to include both intra- and extra-cellular pathways involving the transcription factor Smad3 and the orphan receptor IL-1R8, respectively. There are 5 known splice variants of IL-37, designated a-e [1]. The longest isoform, IL-37b, is predominantly found in immune cells, and is the most well characterized to date [2].

Notably, there is no known mouse homolog of human IL-37. However, a landmark 2010 publication by Nold et al. [3] showed that transgenic mice expressing human IL-37b are protected from acute inflammation. The finding that human IL-37 is functional in the mouse has paved the way for many studies using mouse models to investigate the role of IL-37 in a wide range of inflammatory diseases. It is worth noting that IL-37 does exist in many other rodents such as Guinea pigs and rabbits [4], which could be used to develop a knockout model for studying IL-37 deficiency *in vivo*.

Many correlation studies using human samples have shown a strong connection between IL-37 levels and various disease states. IL-37 expression has been strongly associated with many inflammatory diseases, both autoimmune [5–9], and infection-related [10,11]. Elevated plasma IL-37 levels have also been found in human patients with acute coronary syndrome [12] and atrial fibrillation [13]. Research using mouse models of pathogenic cardiovascular inflammation, including ischemia/reperfusion (I/R) injury [14,15], myocardial infarction (MI) [16,17], and vascular calcification [18,19], reveal significant benefit from IL-37 expression or treatment *in vivo*, indicating promising therapeutic value for use of the cytokine to treat the equivalent conditions in humans. Here we review the potential role of IL-37 in cardiovascular disease, with a specific focus on its protective effects against atherosclerosis development.

2. IL-37 modulates multiple atherogenic macrophage functions

The initiation of atherosclerosis occurs when circulating LDL particles become trapped within the arterial wall and become modified, eliciting an inflammatory response. Signaling molecules produced by cells of the vessel wall, such as endothelial cells and smooth muscle cells, lead to activation of the innate immune response. Although the role of IL-37 has been examined in various inflammatory diseases of both acute and chronic nature, there is limited research on the therapeutic potential of IL-37 to reduce the chronic inflammation and dysregulated cholesterol homeostasis that drive the pathogenesis of atherosclerosis. However, the known protective effects of IL-37 on the various cell types involved in atherogenesis, especially macrophages, suggest a therapeutic role for the cytokine in preventing or suppressing

* Corresponding author at: Center for Cardiovascular Research, John A. Burns School of Medicine, University of Hawaii, 651 Ilalo Street, Honolulu, HI 96813, United States.
E-mail address: wab@hawaii.edu (W.A. Boisvert).

http://dx.doi.org/10.1016/j.cyto.2017.09.025
Received 23 May 2017; Received in revised form 13 September 2017; Accepted 22 September 2017
Available online 05 October 2017
1043-4666/

atherosclerosis.

2.1. Intracellular IL-37 expression suppresses inflammatory signaling

It has been shown that IL-37, similar to other IL-1 family cytokines such as IL-1β, IL-18, and IL-33, is processed by caspase-1 within the cell to produce the mature IL-37 cytokine after the N-terminal pro-domain is cleaved. Mature IL-37 can then either be secreted to act extracellularly like other classical cytokines, or similar to the intracellular pathway of mature IL-1α and IL-33, can translocate to the nucleus and act as a transcription factor [20–22]. Various danger signals such as cholesterol crystals and modified LDL particles in the atherosclerotic plaque can activate IL-1β expression via NLRP3 inflammasome activation, subsequently leading to inflammatory cytokine signaling and secretion of additional cytokines [23]. Caspase-1-mediated cleavage is required for IL-37 to function within the nucleus as a transcription factor [22]. Cleavage is not necessary for IL-37 to function as an extracellular cytokine since both pro-IL-37 and mature IL-37 bind the IL-18Rα chain, although the mature form was shown by immunoprecipitation to bind with stronger affinity than the uncleaved form [20]. Mutation of the caspase-1 cleavage site in the first exon of IL-37b, or use of a pharmacologic caspase-1 inhibitor, leads to inhibition of nuclear translocation. This results in reversal of its suppressive effects on inflammatory gene and protein expression [21,22], implicating an important role for intracellular IL-37 signaling especially in immune cells.

Gene expression of IL-37 has been suggested to be co-regulated by the same transcriptional machinery that activates IL-1β and IL-1α during inflammation, as the promoters of all three genes are in a cluster on chromosome 2, and come in close proximity following LPS stimulation in monocytes [24]. It has been postulated that secretion of both the full length and mature IL-37 occurs in stimulated macrophages in an effort by the immune cells to control excessive inflammatory response [21]. Nold et al. show that IL-37 is expressed in monocytes treated with the inflammatory mediators IL-1b, IL-18, IFNg, TNF, and LPS, and also with TGFβ [3]. Other cell types are known to express IL-37, although the main source of IL-37b, the most abundant and biologically active isoform, is immune cells and epithelial cells, a topic that is discussed further by Dinarello et al. [2].

Further investigation into the intracellular role of IL-37 was inspired by the identification of IL-37 through a proteomics screen as a potential binding partner of the transcription factor, Smad3 [25]. This link was confirmed through co-localization and immunoprecipitation experiments using an epithelial cell line, demonstrating that binding between IL-37 and Smad3 occurs *in vitro* after IL-1β stimulation [3]. In the same study, IL-37 was shown to act through a Smad3-dependent mechanism in both human THP-1, and mouse RAW264.7 macrophages *in vitro*. It is known that Smad3 has a specific preferred DNA binding sequence and acts as a transcription factor in response to TGF-b signaling [26], but is unclear whether IL-37 can also bind DNA. Since IL-37 does not have a nuclear localization sequence, it is likely that it enters the nucleus bound to Smad3, and acts by enhancing the anti-inflammatory activity of Smad3 rather than direct DNA binding. Inhibition of Smad3 expression *in vitro* by siRNA, or activity by the Smad3 inhibitor SIS3, led to reversal of the anti-inflammatory effects of intracellularly expressed IL-37b [3]. Additionally, Smad3 knockdown *in vivo* by siRNA treatment of IL-37-Tg mice challenged with LPS led to increased inflammatory gene and protein expression compared to control IL-37-Tg mice treated with scrambled siRNA [3], supporting an essential role of Smad3 in the anti-inflammatory mechanism of IL-37.

The Smad3-TGFβ pathway has been suggested to be protective in the context of atherogenesis due to its anti-inflammatory signaling [27,28], however the fibrosis associated with TGFβ activation has made its function in cardiovascular disease a double-edged sword [29]. Expression of Smad3 in cardiovascular tissues by adenovirus resulted in reduced inflammation, decreased macrophage infiltration, and attenuated lipid accumulation and atherosclerosis development compared to control mice, without inducing pathogenic fibrosis [30]. Although the role of Smad3 and IL-37 signaling in atherosclerosis has not yet been investigated, Smad3 phosphorylation and nuclear translocation downregulates expression of oxidized-LDL induced adhesion molecules via thioredoxin-1 [31], potentially aiding in the prevention of both the initiating and progressive stages of atherosclerosis development.

2.2. Secreted IL-37 suppresses inflammation via extracellular signaling

The study of the extracellular function of IL-37 gained momentum after it was discovered that IL-37b binds the IL-18Rα chain and also the IL-18 binding protein (IL-18bp). IL-37 is structurally similar to IL-18 and fits within the binding site of IL-18Rα to competitively inhibit binding of IL-18 and subsequent recruitment of the co-receptor, IL-18Rβ [32]. It is not surprising that IL-37 also shares significant homology with another anti-inflammatory member, IL-1 receptor antagonist (IL-1Ra), which competitively inhibits IL-1β from binding the IL-1R [33]. A similar antagonistic role for extracellular IL-37 was investigated and has now been confirmed by multiple studies. Treating IL-37-Tg mice with neutralizing antibodies against IL-37 resulted in reversal of its anti-inflammatory effect on levels of pro-inflammatory cytokines in the serum after stimulation with LPS [21,34], supporting a clear role for IL-37 in quelling systemic inflammation though an extracellular mechanism.

Recently it has been discovered that a complex consisting of IL-37, IL-18Rα, and the orphan receptor IL-1R8 (also known as SIGIRR), is necessary for IL-37 to inhibit inflammatory signaling [34]. Although a specific receptor for IL-37 has not been found, there still exists the possibility that IL-37 may also act through an extracellular pathway apart from SIGIRR and IL-18Rα. However, complexing of the three proteins was visualized by bioluminescence resonance energy transfer in fresh human PBMCs [18], and appears to be the main mechanism through which IL-37 acts extracellularly. The disruption of the complex formation by inhibiting either IL-18Rα or SIGIRR in THP-1 macrophages or PBMCs resulted in significant reversal of the anti-inflammatory effects of IL-37 *in vitro* and *in vivo* [34]. Nold-Petry et al. compared total phosphorylated proteins in LPS-stimulated macrophages and dendritic cells isolated from wild-type, IL-37-Tg, or IL-37-Tg/SIGIRR-deficient mice. They demonstrated that effective IL-37 binding to the IL-18Rα and SIGIRR receptor complex leads to reduced phosphorylation of TAK1, Fyn, and the NF-κB pathways mediators IκBε, p65 and p105. Additionally, extracellular IL-37 signaling was shown to increase phosphorylation of the anti-inflammatory mediators Stat3, Mer, PTEN and p62 [34]. This set of changes in phosphorylation of key signaling proteins acts in suppressing the inflammatory response. Interestingly, Nold-Petry et al. also observed anti-inflammatory effects of IL-37 that were independent of extracellular signaling through SIGIRR, including reduced phosphorylation of various MAP kinases, the focal adhesion kinase FADK, and the NF-κB kinase IKKβ. This supports the hypothesis that IL-37 plays a dual role in preventing inflammation through both extracellular as well as intracellular mechanisms.

Macrophages that stably express IL-37b *in vitro* show significant reduction in inflammatory cytokine production, decreased lipid uptake, and reduced macrophage transmigration towards the chemoattractant MCP-1 [74]. However, it is unclear what contribution the intracellular mechanism has compared to the autocrine signaling that may occur from secreted IL-37b. It is clear that IL-37b-expressing macrophages secrete the protein into the circulation of mice that have undergone bone marrow transplantation of HSC transduced with IL-37b [74]. This may have played a significant role in the reduction of systemic inflammation, particularly concerning endothelial cell activation, which is generally accepted as an initiating event in the development of atherosclerosis.

Many of the same inflammatory pathways that are suppressed by IL-

37b in immune cells are likely affected by a similar mechanism in cell types such as endothelial cells and vascular smooth muscle cells. It has recently been shown that cardiac microvascular endothelial cells from IL-37-Tg mice show significantly less inflammatory response following LPS stimulation, with decreased NF-kB signaling and reduced MCP-1 production [35], further supporting a protective role for IL-37 in preventing the initiation of atherosclerotic plaque development. In patient samples of calcified human aortas, IL-37 was found to be significantly elevated compared to normal aortic tissues [36]. Immunohistochemical staining revealed that macrophages as well as vascular smooth muscle cells appeared to be the main sources of IL-37 within the calcified plaque areas, indicating that both cell types express IL-37 under conditions of chronic inflammation. Human dendritic cells and peripheral blood mononuclear cells (PBMC) have also been shown to secrete IL-37 [3]. We have found that IL-37b expressed specifically in macrophages attenuates atherosclerosis development *in vivo* using *ldlr*$^{-/-}$ mice [74], although further investigation is needed to determine whether intracellular expression of IL-37 specifically in other immune cells, VSMC, or aortic endothelial cells is also protective against atherogenesis.

2.3. Modulation of lipid metabolism by IL-37

Elevated levels of lipids in circulation result in their accumulation within the extracellular matrix of the subendothelial space [37]. The trapped LDL particles are modified and taken up by monocyte-derived macrophages, which are unable to metabolize the modified lipid species and undergo a pathological transformation into lipid-laden foam cells [38,39]. Foam cell accumulation beneath the endothelium is the hallmark of early stage atherosclerotic plaque formation termed “fatty streak”. This unrestricted uptake of lipoproteins and fatty acids by macrophages leads to cellular dysfunction, apoptosis and necrosis [40–42]. It was reported that IL-37 expression was found in foam cells within atherosclerotic plaques [1], implicating a potential role in macrophage lipid homeostasis in the context of cardiovascular disease.

The discovery that IL-37 activity is dependent on the transcription factor Smad3 and that IL-37 expression is induced by TGFβ provides evidence for a potential role of IL-37 in regulating macrophage cholesterol uptake. Activation of the TGFβ-Smad3 signaling pathway has been shown to reduce macrophage foam cell formation [43,44], and has also been shown to play a critical role in the resolution of the inflammatory response [45,46]. Resolution of inflammation provides an additional avenue for preventing foam cell formation and secondary necrosis caused by defective cholesterol efflux after lipid loading [47].

Recently it has been shown that IL-37 and Smad3 are expressed in macrophage foam cells of rabbit atherosclerotic plaques after vascular balloon injury [48], confirming that the pathway is activated in atherosclerotic macrophages *in vivo*. Macrophage inflammatory response to Ox-LDL was dampened by the parallel treatment with recombinant IL-37b [49] or when macrophages were manipulated to express IL-37b [74]. Furthermore, IL-37-Tg mice fed a high-fat diet (HFD) for 16 weeks show reduced plasma levels of cholesterol, and trended towards reduced free fatty acids and triglycerides, compared to wild-type controls [50], further implicating a role for IL-37 in the regulation of cholesterol homeostasis. These investigators also showed that AMP-activated kinase (AMPK) is an important signaling protein downstream of IL-37, providing a putative mechanism for IL-37 involvement in macrophage lipid handling [50]. The reduced serum cholesterol levels observed in IL-37-Tg mice might be an effect of AMPK activation in the liver in response to IL-37, although this remains to be investigated. Activation of AMPK attenuates accumulation of oxidized LDL (ox-LDL) in mouse macrophages by upregulating the expression of ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1), the predominant mediators of macrophage cholesterol efflux [51]. Furthermore, AMPK deficiency led to decreased fatty acid oxidation and increased inflammatory response in mouse macrophages [52]. Interestingly, we have found that IL-37b expression in mouse bone marrow-derived macrophages leads to increased gene and protein expression of ABCA1 and ABCG1 [74]. Thus, IL-37 may have an effect on reverse cholesterol transport through AMPK, which would aid in the resolution of aberrant macrophage accumulation of modified lipids and prevent foam cell formation [73]. Taken together, these findings provide strong support for a protective role of IL-37 in the regulation of macrophage inflammation as well as lipid homeostasis, both key drivers of atherosclerosis.

2.4. Anti-apoptotic properties of IL-37

Apoptotic cells are rapidly cleared by macrophages under normal conditions through a process termed efferocytosis. This important mechanism of cell clearance throughout the body has been shown to be defective in atherosclerotic plaques, leading to increased necrosis and inflammation [53]. ROS production as well as various pro-inflammatory cytokines are known to induce macrophage apoptosis [54]. Additionally, excessive loading of macrophages with materials they cannot effectively digest has been shown to cause impaired engulfment of apoptotic cells [55], implicating the massive accumulation of lipids found in macrophage foam cells within the plaque microenvironment as a major cause of the impaired efferocytosis seen in atherosclerosis.

In addition to its potential role in preventing foam cell formation and the subsequent apoptosis and/or necrosis that follows in the plaque microenvironment *in vivo*, IL-37 has been shown to attenuate apoptosis of cardiomyocytes *in vitro* and *in vivo,* as shown by reduced Caspase-3 cleavage and TUNEL staining [14]. Furthermore, it was recently demonstrated that IL-37 downregulates many of the signaling pathways involved in cardiovascular inflammation and apoptosis [56,57], including JNK, JAK/STAT, MAP kinases, and c-Jun [34]. Additionally, we have found that macrophages expressing recombinant human IL-37 show decreased apoptosis when challenged with DMSO or the apoptosis inducer, camptothecin [74].

A link between apoptosis and autophagy has been suggested [58], and it is possible that IL-37 affects both processes during atherosclerotic plaque development. Macrophages with defective autophagy show increased apoptosis after lipid loading compared to controls, and mice with defective autophagy develop more severe atherosclerosis [59]. Extracellular IL-37 treatment has been shown to decrease mTOR [34] and increase AMPK phosphorylation [50], which have both been shown to activate autophagy [60], further supporting a protective role of IL-37 in the context of atherosclerosis.

3. Protective effects of IL-37 in animal models of cardiovascular diseases

There are numerous publications to date investigating the expression and effects of IL-37 in the context of cardiovascular disease, covering disease states such as tissue injury due to ischemia, vascular calcification and atherosclerotic plaque development. The unifying theme in all of these studies is that IL-37 has a protective role against these cardiovascular pathologies.

Reperfusion with recombinant IL-37 protein immediately following a period of ischemia *in vivo* has been shown to reduce damage and improve organ function in mice after ischemia/reperfusion (I/R) injury [14,15]. In a mouse model of cardiac I/R injury, IL-37-treated mice showed improved outcomes including decreased infarct size, inflammatory signaling, neutrophil infiltration and ROS production compared to controls. The authors speculate that these factors likely account for the observed decrease in cardiomyocyte apoptosis in IL-37-treated mice [17]. Given the causal link between JNK-mediated inflammatory signaling and apoptosis in hepatic ischemic injury [61], and the known downregulation of JNK signaling by IL-37 [34], it is possible that the protective effects of IL-37 in this context are due to suppression of such pathways.

Similar protective effects were observed in two different studies

using a mouse model of myocardial infarction (MI) [16,17]. Recombinant IL-37-treated mice showed decreased MPO and TLR-4 expression, and NF-kB activity in heart tissue, while exhibiting improved cardiac function following MI compared to controls [17]. Interestingly, the IL-37-treated mice showed significant upregulation of the anti-inflammatory cytokine IL-10 in the serum, indicating that IL-37 not only suppresses expression of inflammatory mediators, but also promotes the production of anti-inflammatory mediators [17]. In another mouse model of MI, IL-37 expression by dendritic cells after adoptive transfer significantly increased the number of regulatory T cells, decreased inflammation and fibrosis within the heart, and improved cardiac function [16]. Zhu et al. were the first to investigate the effects of IL-37 on the adaptive immune system after myocardial infarction, although previous studies have shown that IL-37 induces a tolerogenic DC phenotype that suppresses the adaptive immune response [62]. It is interesting to note that monocytes and DCs were identified as the primary sources of IL-37 among human immune cells [63]. Interestingly, while monocytes were responsible for a majority of the expression, DCs were the only cell type to show steady state IL-37 secretion without inflammatory stimulus such as LPS [63], indicating their role in constitutive immune suppression and potential for preventing cardiovascular disease complications.

The protective effects of IL-37 on myocardium also extends to aged mice (20–24 months old), including both IL-37-Tg as well as mice treated with recombinant IL-37 [35]. Following challenge with LPS, mice were analyzed for cytokine levels in myocardial tissue, as well as secretion in the serum. NF-kB activation and MCP-1 production were measured in isolated cardiac microvascular endothelial cells from IL-37-Tg and control mice, revealing decreased levels of both inflammatory mediators by IL-37. Wild-type mice treated with recombinant IL-37 showed significantly improved left ventricle function compared to controls after challenge with endotoxemia. Although protective in both IL-37 treatment scenarios, the different results observed when IL-37 is expressed by the cells affected, versus mice treated systemically by injection with recombinant IL-37 protein implies distinct intra- and extracellular mechanisms of IL-37 on its target cells.

The effectiveness of recombinant IL-37 treatment in preventing atherosclerosis was tested in atherosclerosis-prone, diabetic *apoE*$^{-/-}$ mice. Treatment with recombinant IL-37 injections for 16 weeks resulted in reduced atherosclerotic plaque size and vulnerability, decreased inflammatory cytokines in the serum, and decreased vascular calcification compared to control mice [19]. We have also observed an atheroprotective role of IL-37 in mice using the *ldlr*$^{-/-}$ mouse model. Following bone marrow transplantation of hematopoietic stem cells (HSC) transduced with either IL-37b or EGFP control, we found that atherosclerotic plaque development was mitigated due to macrophage-specific expression of IL-37b after 10 weeks of HFD [74]. We found that although most circulating cytokines measured were not different between groups, macrophage IL-37 expression *in vivo* led to reduced circulating M-CSF levels compared to control mice, potentially effecting the recruitment of monocytes/macrophages to the growing plaque. Another atherosclerosis study, using *ldlr*$^{-/-}$ mice transplanted with IL-37-Tg hematopoietic stem cells, showed no difference in plaque size after 8 weeks of HFD, although there was a reduction in circulating immune cells, and inflammatory response of peritoneal macrophages [64]. One difference between this study and others is that female mice were used, which are known to develop less severe atherosclerosis than males [65]. The authors propose that the severity of inflammation was not great enough to elicit significant IL-37 expression to affect atherosclerosis development, although its concentration in the sera of the mice was not reported. The authors observed reduced expression of the neutrophil chemoattractant KC, as well as reduced circulating neutrophil levels, indicating that IL-37 might have effected early atherosclerosis, when neutrophils are thought to play a larger role.

The beneficial effects of IL-37 treatment in cell types other than immune cells have also been documented. Aortic valve interstitial cells (AVIC) isolated from diseased human aortic valves stimulated with TLR agonists show decreased IL-37, with increased bone morphogenic protein-2 and alkaline phosphatase expression, and presence of calcified deposits via NF-κB and ERK1/2 signaling, compared to healthy control AVIC [18]. When AVIC were treated with IL-37, these pathogenic effects were reversed. Similar protective findings were observed in IL-37-Tg mice compared to controls challenged with a TLR4 agonist or prolonged high fat diet, revealing decreased thickening of the aortic valve as well as reduced lipid deposition and calcification of aortic valve leaflets [18]. Additionally, IL-37-treated human coronary artery endothelial cells stimulated with a TLR2 agonist showed reduced expression of the adhesion molecule ICAM1 as well as decreased NF-κB activity [66]. These findings suggest a potential therapeutic value of IL-37 in treating a range of cardiovascular pathologies.

4. IL-37 in human diseases

The recognition of IL-37 as a potential therapeutic cytokine has led to its investigation in human populations. Through analysis of data from the 1000 Genomes Project, 114 modern human haplotypes of the *IL-37* gene have been identified in the general population [4]. Sequence variants have been shown to affect IL-37 protein stability [67], potentially contributing to different levels of protection or susceptibility from inflammatory diseases [68]. Elevated IL-37 expression has been strongly associated with many human diseases including colitis [5], psoriasis [6], rheumatoid arthritis [7], systemic lupus erythematosus [8] asthma [69], inflammatory bowel disease [9] and viral infection [10,11]. It is speculated that the increased expression of IL-37 is part of a compensatory mechanism to keep the excessive, and often damaging, inflammation of these various pathologies in check.

Concerning cardiovascular disease patients, elevated plasma IL-37 has been found in individuals with acute coronary syndrome [12,70] and atrial fibrillation [13]. Recently it was shown that a human genetic variant of IL-37 in the population is associated with an increased risk of coronary artery disease (CAD) [71]. This allele variant, SNP rs3811047 of *IL-37*, is associated with decreased expression of IL-37, indicating that IL-37 expression in humans has a protective role in the prevention of inflammation and cardiovascular pathologies.

5. Therapeutic considerations with special emphasis on dimerization of IL-37

The intracellular signaling pathways of IL-37b in preventing inflammatory response in cardiovascular disease are important when considering direct manipulation of immune cells, especially macrophages. It might be worth considering gene therapy options to express IL-37 intracellularly, especially given the differences between its intra- and extracellular signaling pathways. However, the most straightforward therapeutic routes would involve injection of IL-37 protein intravenously, or use of a targeted drug delivery system that would most likely act through the extracellular signaling pathway to accomplish its anti-inflammatory objective within the body.

Recently, IL-37b has been crystallized and shown to form head-to-head homodimers [72], which significantly reduces its anti-inflammatory capacity. The IL-37b protein exists in a pro and mature state, which have different estimated dimerization association constants of 5nM and 4uM, respectively [1,20]. In various cell types, the molecular weight of IL-37b protein was 25 kDa, characteristic of the monomer, but in PBMCs, the molecular weight of IL-37b has been reported to vary from ~25 kDa to 45 kDa, indicating dimerization [3]. This head-to-head orientation of IL-37 dimers is unique in the IL-1 superfamily of cytokines, and may act to diminish the anti-inflammatory potency of IL-37 by hindering formation of the IL-37/IL-18Ra/IL-1R8 complex at the cell surface. Ellisdon et al. developed two variants of IL-37b, which have mutations within the homodimer binding region at either Tyr 85 (Y85A) or Asp 73 (D73K) [72]. Both mutations completely

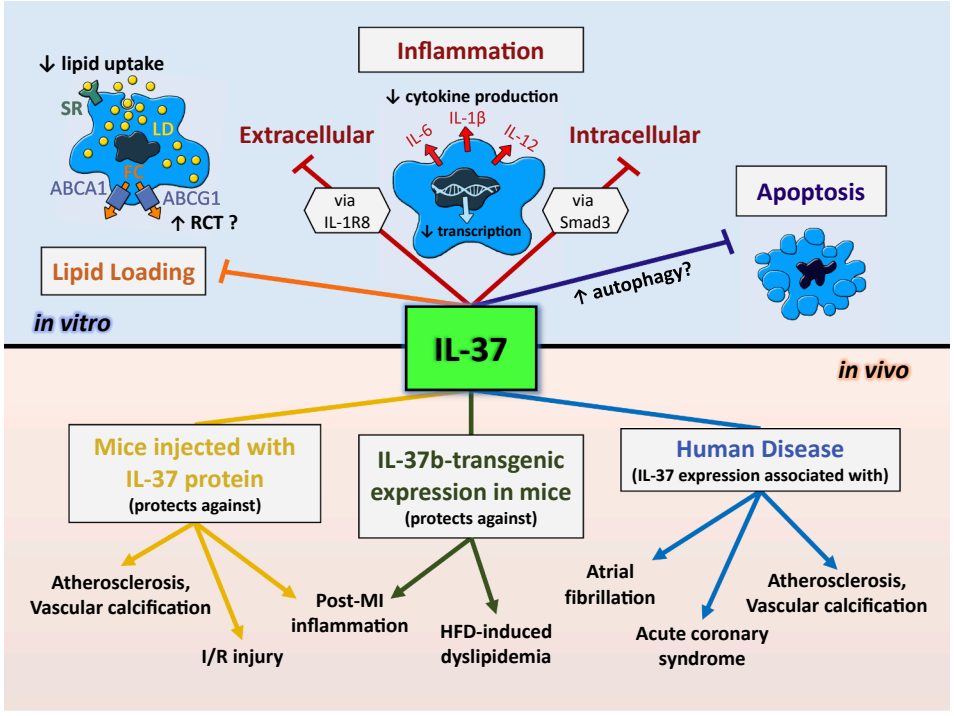

Fig. 1. IL-37's role in modulating atherosclerotic macrophage function as well as in various inflammatory cardiovascular pathologies. ***In vitro***: IL (Interleukin)-37, *in vitro*, acts as a key anti-inflammatory cytokine that decreases inflammation extracellularly via the orphan receptor, IL-1R8 (IL-1 Receptor Family Member 8), and intracellularly via the transcription factor, Smad3. Macrophage inflammatory gene expression is reduced by IL-37, leading to decreased secretion of key cytokines such as IL-6, IL-1β, and IL-12. IL-37 also decreases lipid loading by reducing uptake of modified LDL via scavenger receptors (SR) and storage in lipid droplets (LD). IL-37 could potentially increase reverse cholesterol transport (RCT) by mediating the efflux of free cholesterol (FC) via ATP-binding cassette A1 (ABCA1) or ABCG1. Macrophage apoptosis may be inhibited by IL-37 via increased autophagy. ***In vivo:*** Mice injected with IL-37 recombinant protein (yellow) show protection against atherosclerosis development, vascular calcification, ischemia/reperfusion (IR) injury, and post-myocardial infarction (MI) inflammation (yellow arrows). IL-37b-transgenic mice (green) are protected against post-MI inflammation and high fat diet (HFD)-induced dyslipidemia (green arrows). In humans (blue), IL-37 has been associated with atrial fibrillation, acute coronary syndrome, atherosclerosis, and vascular calcification (blue arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ablate dimer formation and restore its anti-inflammatory capacity. Treatment of THP-1 cells or PBMCs with monomeric IL-37b mutants reduced the expression of inflammatory cytokines and conferred markedly greater protection against endotoxic shock in mice.

Regulating the ratio between IL-37 monomers versus homodimers should be considered when developing potential therapeutic treatments using IL-37 to suppress excessive inflammation. The dimerization (and possibly oligomerization) capabilities of IL-37 may diminish the desired anti-inflammatory effect *in vivo*, as the shifts in the monomer-to-dimer ratio could lead to reduced bioactivity. Recent findings suggest that the greatest anti-inflammatory effects are seen with concentrations of IL-37b ranging from 1 pg/ml to 10 ng/ml, while essentially ineffective at higher concentrations [3]. Moreover, unmodified, mature IL-37 becomes pro-inflammatory at a concentration of 100 ng/ml while the mutated, monomeric variants remain anti-inflammatory at this high concentration [72]. Further investigation into the stability, safety and efficacy of IL-37 treatment options needs to be carried out before it can be used to treat human disease. However, the mounting evidence in favor of a protective role in many diverse inflammatory diseases makes IL-37 a very promising therapeutic option for use in the future.

6. Conclusion

Although it has been only a few years since its discovery, the research looking into the potential role of IL-37 in fighting many different types of inflammatory diseases has been robust. In the context of atherosclerosis, IL-37 has the potential to modulate several pathways that lead to atherosclerosis development, including inflammation, lipid homeostasis and cell survival. Several murine studies have shown the atheroprotective effects of IL-37. However, most of the studies involving human populations have been associative rather than testing the direct effects of IL-37 on the disease process. A cartoon depicting IL-37's role in modulating atherosclerotic macrophage function as well as its role in various inflammatory cardiovascular pathologies is summarized in Fig. 1. Although the evidence thus far is strong that IL-37 has the potential to be a promising therapeutic target in preventing or treating atherosclerosis, many more studies, especially involving its efficacy and safety for long-term treatment, are needed to validate this potential.

Acknowledgements

Some of the work contained herein was funded by NIH grants R01HL075677 and R01HL081863, and by Innovative Research Grant 16IRG27540027 from the American Heart Association to WAB. Part of the work was performed within the Russian Government Program of Competitive Growth of Kazan Federal University.

References

[1] D. Boraschi, et al., IL-37: a new anti-inflammatory cytokine of the IL-1 family, Eur. Cytokine Netw. 22 (2011) 127–147.
[2] C.A. Dinarello, et al., Suppression of innate inflammation and immunity by interleukin-37, Eur. J. Immunol. 46 (2016) 1067–1081.
[3] M.F. Nold, et al., IL-37 is a fundamental inhibitor of innate immunity, Nat. Immunol. 11 (2010) 1014–1022.
[4] B. Kang, S. Cheng, J. Peng, J. Yan, S. Zhang, Interleukin-37 gene variants segregated anciently coexist during hominid evolution, Eur. J. Hum. Genet. 23 (2015) 1392–1398.
[5] S. Weidlich, et al., Intestinal expression of the anti-inflammatory interleukin-1 homologue IL-37 in pediatric inflammatory bowel disease, J Pediatr Gastroenterol Nutr (2014).
[6] X. Teng, et al., IL-37 ameliorates the inflammatory process in psoriasis by suppressing proinflammatory cytokine production, J. Immunol. 192 (2014) 1815–1823.
[7] P.W. Zhao, et al., Plasma levels of IL-37 and correlation with TNF-alpha, IL-17A, and disease activity during DMARD treatment of rheumatoid arthritis, PLoS One 9 (2014) e95346.
[8] L. Ye, et al., IL-37 inhibits the production of inflammatory cytokines in peripheral blood mononuclear cells of patients with systemic lupus erythematosus: its correlation with disease activity, J Transl Med 12 (2014) 69.
[9] H. Imaeda, et al., Epithelial expression of interleukin-37b in inflammatory bowel disease, Clin. Exp. Immunol. 172 (2013) 410–416.
[10] J.F. Hojen, et al., Interleukin-37 expression is increased in chronic HIV-1-infected individuals and is associated with inflammation and the size of the total viral reservoir, Mol. Med. 21 (2015) 337–345.
[11] Z. Huang, et al., IL-37 Expression is upregulated in patients with tuberculosis and induces macrophages towards an M2-like phenotype, Scand. J. Immunol. 82 (2015) 370–379.
[12] Q. Ji, et al., Elevated plasma IL-37, IL-18, and IL-18BP concentrations in patients with acute coronary syndrome, Mediators Inflamm. 2014 (2014) 165742.
[13] W. Li, S. Li, X. Li, S. Jiang, B. Han, Interleukin-37 elevation in patients with atrial fibrillation, Clin. Cardiol. 40 (2017) 66–72.
[14] B. Wu, et al., Interleukin-37 ameliorates myocardial ischaemia/reperfusion injury in mice, Clin. Exp. Immunol. 176 (2014) 438–451.
[15] Y. Yang, et al., IL-37 inhibits IL-18-induced tubular epithelial cell expression of pro-inflammatory cytokines and renal ischemia-reperfusion injury, Kidney Int. 87

(2015) 396–408.
[16] R. Zhu, et al., Interleukin-37 and dendritic cells treated with interleukin-37 plus troponin I ameliorate cardiac remodeling after myocardial infarction, J Am Heart Assoc 5 (2016).
[17] D. Xu, A. Wang, F. Jiang, J. Hu, X. Zhang, Effects of interleukin-37 on cardiac function after myocardial infarction in mice, Int. J. Clin. Exp. Pathol. 8 (2015) 5247–5251.
[18] Q. Zeng, et al., Interleukin-37 suppresses the osteogenic responses of human aortic valve interstitial cells in vitro and alleviates valve lesions in mice, Proc. Natl. Acad. Sci. USA 114 (2017) 1631–1636.
[19] M. Chai, et al., The protective effect of interleukin-37 on vascular calcification and atherosclerosis in apolipoprotein E-deficient mice with diabetes, J. Interferon Cytokine Res. 35 (2015) 530–539.
[20] S. Kumar, et al., Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production, Cytokine 18 (2002) 61–71.
[21] A.M. Bulau, et al., Role of caspase-1 in nuclear translocation of IL-37, release of the cytokine, and IL-37 inhibition of innate immune responses, Proc. Natl. Acad. Sci. USA 111 (2014) 2650–2655.
[22] S. Sharma, et al., The IL-1 family member 7b translocates to the nucleus and down-regulates proinflammatory cytokines, J. Immunol. 180 (2008) 5477–5482.
[23] T. Karasawa, M. Takahashi, Role of NLRP3 inflammasomes in atherosclerosis, J. Atheroscler. Thromb. 24 (2017) 443–451.
[24] N. Sharaf, M.J. Nicklin, F.S. di Giovine, Long-range DNA interactions at the IL-1/IL-36/IL-37 gene cluster (2q13) are induced by activation of monocytes, Cytokine 68 (2014) 16–22.
[25] S. Grimsby, et al., Proteomics-based identification of proteins interacting with Smad3: SREBP-2 forms a complex with Smad3 and inhibits its transcriptional activity, FEBS Lett. 577 (2004) 93–100.
[26] A. Kurisaki, S. Kose, Y. Yoneda, C.H. Heldin, A. Moustakas, Transforming growth factor-beta induces nuclear import of Smad3 in an importin-beta1 and Ran-dependent manner, Mol. Biol. Cell 12 (2001) 1079–1091.
[27] Z. Mallat, A. Tedgui, The role of transforming growth factor beta in atherosclerosis: novel insights and future perspectives, Curr. Opin. Lipidol. 13 (2002) 523–529.
[28] M.W. Feinberg, M.K. Jain, Role of transforming growth factor-beta1/Smads in regulating vascular inflammation and atherogenesis, Panminerva Med. 47 (2005) 169–186.
[29] G.J. Prud'homme, Pathobiology of transforming growth factor beta in cancer, fibrosis and immunologic disease, and therapeutic considerations, Lab. Invest. 87 (2007) 1077–1091.
[30] H. Zhu, et al., AAV2/8-hSMAD3 gene delivery attenuates aortic atherogenesis, enhances Th2 response without fibrosis, in LDLR-KO mice on high cholesterol diet, J. Transl. Med. 12 (2014) 252.
[31] B. Chen, W. Wang, T. Shen, R. Qi, Thioredoxin1 downregulates oxidized low-density lipoprotein-induced adhesion molecule expression via Smad3 protein, PLoS One 8 (2013) e76226.
[32] P. Bufler, F. Gamboni-Robertson, T. Azam, S.H. Kim, C.A. Dinarello, Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region responsive to lipopolysaccharide, Biochem. J. 381 (2004) 503–510.
[33] C.A. Dinarello, Biologic basis for interleukin-1 in disease, Blood 87 (1996) 2095–2147.
[34] C.A. Nold-Petry, et al., IL-37 requires the receptors IL-18R[alpha] and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction, Nat. Immunol. 16 (2015) 354–365.
[35] J. Li, et al., Interleukin-37 suppresses the inflammatory response to protect cardiac function in old endotoxemic mice, Cytokine 95 (2017) 55–63.
[36] K. Yu, et al., Increased IL-37 concentrations in patients with arterial calcification, Clin. Chim. Acta 461 (2016) 19–24.
[37] J.R. Guyton, K.F. Klemp, B.L. Black, T.M.A. Bocan, Extracellular lipid deposition in atherosclerosis, Eur. Heart J. 11 (1990) 20–28.
[38] A.C. Li, et al., Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARα, β/δ, and γ, J. Clin. Investig. 114 (2004) 1564–1576.
[39] N. Gaudreault, et al., ApoE suppresses atherosclerosis by reducing lipid accumulation in circulating monocytes and the expression of inflammatory molecules on monocytes and vascular endothelium, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 264–272.
[40] S. Solanki, P.R. Dube, L. Birnbaumer, G. Vazquez, Reduced necrosis and content of apoptotic M1 macrophages in advanced atherosclerotic plaques of mice with macrophage-specific loss of Trpc3, Sci. Rep. 7 (2017) 42526.
[41] Gwendalyn J. Randolph, Macrophage supply and demand at the core of the necrotic granuloma, Cell Host & Microbe 18 (2015) 3–4.
[42] T. Seimon, I. Tabas, Mechanisms and consequences of macrophage apoptosis in atherosclerosis, J. Lipid Res. 50 (2009) S382–S387.
[43] D.R. Michael, R.C. Salter, D.P. Ramji, TGF-beta inhibits the uptake of modified low density lipoprotein by human macrophages through a Smad-dependent pathway: a dominant role for Smad-2, Biochim. Biophys. Acta 1822 (2012) 1608–1616.
[44] P.A. Pioli, K.E. Goonan, K. Wardwell, P.M. Guyre, TGF-beta regulation of human macrophage scavenger receptor CD163 is Smad3-dependent, J. Leukoc. Biol. 76 (2004) 500–508.
[45] F. Werner, et al., Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3, J. Biol. Chem. 275 (2000) 36653–36658.
[46] D. Gong, et al., TGFbeta signaling plays a critical role in promoting alternative macrophage activation, BMC Immunol. 13 (2012) 31.
[47] T.A. Seimon, et al., Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress, Cell Metab. 12 (2010) 467–482.
[48] C. Shaoyuan, D. Ming, H. Yulang, F. Hongcheng, Increased IL-37 in atherosclerotic disease could be suppressed by atorvastatin therapy, Scand. J. Immunol. 82 (2015) 328–336.
[49] J. Huang, F.L. Hou, A.Y. Zhang, Z.L. Li, Protective effect of the polarity of macrophages regulated by IL-37 on atherosclerosis, Genet. Mol. Res. 15 (2016).
[50] D.B. Ballak, et al., IL-37 protects against obesity-induced inflammation and insulin resistance, Nat. Commun. 5 (2014) 4711.
[51] A. Ma, J. Wang, L. Yang, Y. An, H. Zhu, AMPK activation enhances the anti-atherogenic effects of high density lipoproteins in apoE$^{-/-}$ mice, J. Lipid Res. 58 (2017) 1536–1547.
[52] S. Galic, et al., Hematopoietic AMPK beta1 reduces mouse adipose tissue macrophage inflammation and insulin resistance in obesity, J. Clin. Invest. 121 (2011) 4903–4915.
[53] Y. Kojima, I.L. Weissman, N.J. Leeper, The role of efferocytosis in atherosclerosis, Circulation 135 (2017) 476–489.
[54] U. Forstermann, N. Xia, H. Li, Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis, Circ. Res. 120 (2017) 713–735.
[55] D.M. Schrijvers, G.R. De Meyer, M.M. Kockx, A.G. Herman, W. Martinet, Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1256–1261.
[56] N. Adhikari, N. Charles, U. Lehmann, J.L. Hall, Transcription factor and kinase-mediated signaling in atherosclerosis and vascular injury, Curr. Atheroscler Rep. 8 (2006) 252–260.
[57] R.J. Hoefen, B.C. Berk, The role of MAP kinases in endothelial activation, Vasc. Pharmacol. 38 (2002) 271–273.
[58] T.T. Wu, W.M. Li, Y.M. Yao, Interactions between autophagy and inhibitory cytokines, Int. J. Biol. Sci. 12 (2016) 884–897.
[59] I. Sergin, et al., Inclusion bodies enriched for p62 and polyubiquitinated proteins in macrophages protect against atherosclerosis, Sci Signal. 9 (2016) ra2.
[60] J. Kim, M. Kundu, B. Viollet, K.L. Guan, AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1, Nat. Cell Biol. 13 (2011) 132–141.
[61] T. Uehara, et al., JNK mediates hepatic ischemia reperfusion injury, J. Hepatol. 42 (2005) 850–859.
[62] Y. Luo, et al., Suppression of antigen-specific adaptive immunity by IL-37 via induction of tolerogenic dendritic cells, Proc. Natl. Acad. Sci. USA 111 (2014) 15178–15183.
[63] I. Rudloff, et al., Monocytes and dendritic cells are the primary sources of interleukin 37 in human immune cells, J. Leukoc. Biol. 101 (2017) 901–911.
[64] G. Hoeke, et al., The effects of selective hematopoietic expression of human IL-37 on systemic inflammation and atherosclerosis in LDLr-deficient mice, Int. J. Mol. Sci. 18 (2017).
[65] G.S. Getz, C.A. Reardon, Animal models of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 1104–1115.
[66] Y. Xie, Y. Li, X. Cai, X. Wang, J. Li, Interleukin-37 suppresses ICAM-1 expression in parallel with NF-kappaB down-regulation following TLR2 activation of human coronary artery endothelial cells, Int. Immunopharmacol. 38 (2016) 26–30.
[67] J. Yan, et al., Common genetic heterogeneity of human interleukin-37 leads to functional variance, Cell Mol. Immunol. 14 (2016) 783–791.
[68] N. Yan, et al., Polymorphism of IL37 gene as a protective factor for autoimmune thyroid disease, J. Mol. Endocrinol. 55 (2015) 209–218.
[69] R. Charrad, et al., Anti-inflammatory activity of IL-37 in asthmatic children: correlation with inflammatory cytokines TNF-alpha, IL-beta, IL-6 and IL-17A, Immunobiology 221 (2016) 182–187.
[70] T. Yang, et al., Elevated plasma interleukin-37 playing an important role in acute coronary syndrome through suppression of ROCK activation, Oncotarget 8 (2017) 9686–9695.
[71] D. Yin, et al., Genomic variant in IL-37 confers a significant risk of coronary artery disease, Sci. Rep. 7 (2017) 42175.
[72] A.M. Ellisdon, et al., Homodimerization attenuates the anti-inflammatory activity of interleukin-37, Sci. Immunol. 2 (2017).
[73] R.A.K. Srivastava, et al., AMP-activated protein kinase: an emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases: Thematic Review Series: New Lipid and Lipoprotein Targets for the Treatment of Cardiometabolic Diseases, J. Lipid Res. 53 (2012) 2490–2514.
[74] S. McCurdy, Y. Baumer, E. Toulmin, B.H. Lee, W.A. Boisvert, Macrophage-specific expression of interleukin-37 in hyperlipidemic mice attenuates atherosclerosis, J. Immunol. (2017) in press.