REVIEW

Institute of Cardiovascular Diseases[1], Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan; Department of Cardiology[2], the People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China

The potential role of IL-37 in atherosclerosis

BANG-WEI WU[1,*], QIU-TANG ZENG[1,*], KAI MENG[1], QING-WEI JI[2]

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Dr. Qing-wei Ji, Department of Cardiology, the People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China
jqw124@gmail.com
[*]*These authors contributed equally to this work*

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Atherosclerosis is an inflammatory disease characterized by extensive lipid deposition and atherosclerotic plaque formation in the intima. Interleukin (IL)-37 is anti-inflammatory cytokine in the IL-1 ligand family. Given that IL-37 plays an important function in the development and progression of inflammatory and autoimmune diseases, it may be associated with the development of atherosclerosis. IL-37, which is normally expressed at low levels in peripheral blood mononuclear cells (PBMC), mainly monocytes, and dendritic cells (DC), is rapidly up-regulated in the inflammatory context, and therefore IL-37 conversely inhibits the production of inflammatory cytokines in PBMC and DC. In addition, IL-37 effectively suppresses the activation of macrophage and DC. It is not controversial that the activation of macrophage and DC and the over-expression of inflammatory cytokines are critical component elements in inflammatory process of atherosclerosis. Therefore, IL-37 may play a protective role in atherosclerosis through inhibition of inflammatory cytokines production and suppression of macrophage and DC activation.

1. Introduction

Atherosclerosis is a chronic inflammatory disease. Pro-inflammatory cells, pro-inflammatory cytokines, as well as anti-inflammatory cells, anti-inflammatory cytokines play an essential role in this process. It is notable that the balance of the two opposites is crucial for the maintenance of normal steady-state conditions in healthy individuals. However, this balance can be broken in inducible situations, primarily hyperlipidemia, hypertension and diabetes, resulting in the occurrence and development of plaque and the onset of acute cardiovascular events (Ross 1999).

The novel anti-inflammatory cytokine interleukin (IL)-37 is a recently discovered member of the IL-1 family, (Pan et al. 2001; Nold et al. 2010; Boraschi et al. 2011). The IL-1 family consists of 11 members, including IL-1α, IL-1β, IL-1Rα, IL-18, IL-33, IL-36Rα, IL-36α, IL-36β, IL-36γ, IL-37 and IL-1F10. Most of the cytokines of the IL-1 family, such as IL-1 and IL-18, effectively promote inflammation, whereas others like IL-1Rα efficiently inhibit inflammation or trigger anti-inflammatory reaction. Evidence shows that IL-37 plays a protective role in inflammatory and autoimmune diseases in animal models *via* inhibition of the generation of pro-inflammatory cytokines and the activation of macrophage and dendritic cells (DC) (Nold et al. 2010; Sharma et al. 2008; McNamee et al. 2011; Sakai et al. 2012). However, the exact role of IL-37 in inflammatory processes has not yet been elucidated. Recently, IL-37 protein has been detected in the foam-like cells of human atherosclerotic coronary and carotid plaque (Boraschi et al. 2011), suggesting that IL-37 is involved in the development of atherosclerotic disease.

2. Characteristics of IL-37

Found in 2000, IL-37 was formerly named as IL-1F7, IL-1H and IL-1H4. Its gene is located on chromosome 2 (Kumar et al. 2000; Busfield et al. 2000; Boraschi et al. 2011). There are five splice variants of IL-37 (IL-37a-e), among them IL-37b is the largest and contains a sequence of 218 amino acids. In addition, IL-37b is the best characterised IL-37 isoform and the main IL-37 isoform that exists in peripheral blood. Evidence shows that IL-37 variants tend to be expressed in a tissue-specific fashion (Boraschi et al. 2011). IL-37a is the only isoform expressed in the brain, IL-37b is the only one present in kidney, IL-37c is the heart specific isoform, IL-37d and e are only expressed in bone marrow and testis. Like other cytokines of the IL-1 family, IL-37 is synthesized as precursor molecule that needs to be processed to produce the mature compound. *In vitro*, caspase-1 efficiently cleaves IL-37 precursor to generate mature IL-37, caspase-4 also promotes the shift of IL-37 precursor to mature IL-37 but is significantly weaker than caspase-1 (Kumar et al. 2002). How the IL-37 precursor transforms to mature body *in vivo* is still unclear. The hypotheses that caspase-1 or other proteolytic enzymes play an important role in IL-37 mature *in vivo* require further investigation.

The expression of IL-37 is at low levels in physiological state and can be up-regulated in an inducible manner. After treatment with phorbol myristoyl acetate (PMA), the mRNA expression of IL-37 was increased 2-fold in peripheral blood mononuclear cells (PBMC, primarily monocytes) and 4.5-fold in DC (Pan et al. 2001). *In vitro*, toll-like receptor (TLR) agonist, a variety of cytokines such as IL-1β, IL-18, TNF-α, IFN-γ, and TGF-β, also significantly up-regulate the expression of IL-37,

whereas IL-12, IL-32, IL-4 plus granulocyte-macrophage colony-stimulating factor (GM-CSF) can inhibit the expression of IL-37 (Nold et al. 2010).

IL-18Rα, IL-18 binding protein (IL-18BP) are two receptors of the pro-inflammatory cytokine IL-18. IL-18 binds to the IL-18Rα and recruits the accessory protein IL-18Rβ to initiate cell activation and IL-18-dependent inflammation, whereas IL-18 binds to the IL-18BP resulting in suppression of IL-18 activity. Although IL-37 also binds to the IL-18Rα, the affinity of IL-37 for IL-18Rα is 50 times lower than that of IL-18. In addition, the binding of IL-37 to the IL-18Rα has nothing to do with the IL-18-dependent or independent IFN-γ expression, indicating that IL-37/IL-18Rα complex is not related to the effect of IL-37 (Kumar et al. 2002; Pan et al. 2001; Bufler et al. 2002). Interestingly, both immature and mature IL-37 can bind to IL-18BP, but only the mature IL-37 significantly inhibited the secretion of IFN-γ induced by IL-18, and this effect was observed at lower concentrations of IL-18BP situation rather than high concentrations of IL-18BP (Bufler et al. 2002). The investigators speculated that the IL-37/IL-18BP complex is able to recruit the IL-18Rβ accessory chain into an inactive complex, thus decreasing its availability to form active receptor complexes with IL-18/IL-18Rα, thereby blocking the pro-inflammatory effect of IL-18. This hypothesis, however, has yet been proven thus far.

In order to clarify the effect of endogenous IL-37 on inflammatory response induced by lipopolysaccharide (LPS), investigators transfected the human IL-37 gene into mouse macrophage RAW264.7 cells (named as RAW-IL-37), in which the expression of human IL-37 was more stable and could increase dose dependently upon stimulation with LPS (Nold et al. 2010). The results showed that the over-expression of IL-37 up-regulated by LPS significantly dampened the levels of TNF-α, IL-1α, IL-1β, IL-6, monocytes inflammatory protein-2, and IL-10, but increased the secretion of Th2-type cytokine IL-13, Th17-type cytokine IL-17, and monocyte chemoattractant protein-1 in culture supernatant of RAW-IL-37. In addition, the over-expression of IL-37 also effectively inhibited the inflammatory response induced by IL-1β, TNF, or various TLR ligands in RAW-IL-37 cells. The same results are observed in other cell lines such as THP-1 cells and the alveolar epithelial cell line A549. These data indicate that IL-37 is a powerful anti-inflammatory cytokine and this function of IL-37 does not depend on the anti-inflammatory cytokine IL-10. Mature IL-37 translocates to the nucleus by caspase-1 and then binds with the phosphorylated Smad3 to form an IL-37/Smad3 complex in perinuclear, which affects gene transcription (Sharma et al. 2008; Nold et al. 2010). Using the specific inhibitor SIS3 to block the activation of Smad3 or using siRNA to silence Smad3 significantly suppressed the activity of IL-37, indicating that the anti-inflammation effect of IL-37 depends on the activation of Smad3 (Nold et al. 2010).

IL-37 is not only related to the cytokines of innate immunity but also involved in the adaptive immunity. Evidence shows that IL-37 significantly inhibits the LPS-induced dendritic cell activation and the average expression of CD86 and MHC II on DC was 30 percent reduction in IL-37 transgenic mice (Nold et al. 2010). Therefore, IL-37 may reduce the activation of T lymphocytes through inhibition of the DC activation. In addition, the activation of macrophages induced by LPS is also suppressed by IL-37 (Nold et al. 2010).

3. The relationship between IL-37 and diseases

Thus far, knowledge of IL-37 in human diseases is still limited. Like atherosclerosis, the occurrence of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE) is associated with the imbalance of proinflammatory/anti-inflammatory response. The IL-37 protein is highly expressed in synovial cells of patients with rheumatoid arthritis, but expressed at low levels in healthy human synovial cells (Nold et al. 2010). Using RT-PCR method, Boraschi et al. (2011) found that high expression of IL-37 is observed in PBMC of patients with SLE. More recently, a clinical study from Song et al. (2013) showed that plasma IL-37 levels were significantly increased in newly diagnosed patients with SLE, accompanied with higher levels of IL-18, IL-18BP, IFN-γ, and IL-6. After 2 weeks of treatment with dexamethasone, the levels of the five cytokines were decreased, but only the reduction in the levels of IL-37 reached statistical significance, suggesting that the plasma IL-37 levels may serve as biomarker to assess the treatment effect of newly diagnosed patients with SLE. In addition, IL-37 expression was also significantly increased in the skin lesions of patients with psoriasis and in macrophages of Crohn's disease lesions (Boraschi et al. 2011). These studies only showed that IL-37 is involved in the disease process. However, the exact role of IL-37 in human diseases still remains uncertain and should be investigated in the future.

IL-37 transgenic (IL-37tg) mice were used to investigate whether endogenous IL-37 attenuates shock induced by LPS *in vivo* (Nold et al. 2010). Although IL-37 is stably expressed in IL-37tg mice in the absence of LPS, the amount of IL-37 is very low. After stimulation with LPS, the amount of IL-37 significantly increased in IL-37tg mice. It is notable that although non-lethal shock was also successfully induced by LPS, the effects of endotoxemia such as hypothermia, metabolic acidosis, dehydration, electrolyte imbalance and liver damage were significantly ameliorated in IL-37tg mice, especially in homozygotes IL-37tg mice, accompanied with a reduction in various cytokines secretion and DC activation. Recently, McNamee et al. (2011) found that endogenous IL-37 effectively protects mice from dextran sulfate sodium-induced colitis through inhibition of IL-1β and TNF-α production and of leukocytes recruitment. Although the production of IL-10 significantly increased in IL-37tg mice, blockade of IL-10 signaling, however, did not abrogate the protective role of IL-37.

Exogenous IL-37 plays a protective role in hepatic ischemia/reperfusion (I/R) injury (Sakai et al. 2012). Mice were injected intraperitoneally with recombinant human IL-37 or phosphate buffered saline at the time of reperfusion and these mice were killed after 1 or 8 h of reperfusion. The results showed that IL-37 treatment markedly attenuated hepatocyte injury and neutrophil accumulation in the liver after I/R, accompanied with the decrease of IL-1β, TNF-α and hepatic reactive oxygen species. Because I/R injury is also easy to occur and contributes to adverse cardiovascular outcomes after myocardial ischemia, herein we hypothesize that IL-37 treatment protect patients with acute myocardial infarction from myocardial I/R injury injury.

4. The potential role of IL-37 in atherosclerosis

More recently, Boraschi et al. (2011) found that IL-37 was expressed in the foam-like cells of atherosclerotic coronary and carotid artery plaques, suggesting that IL-37 is associated with the activation of macrophages and the shift in macrophages to foam cells in atherosclerosis. Activated macrophages accumulate in atherosclerotic lesions and play an indispensable role throughout the different stages of atherosclerosis, from the occurrence of fatty streaks to plaque rupture and thrombosis (Boyle 2005). Macrophages are activated without antigenic specificity and many atherosclerotic risk factors such as oxidized low density lipoprotein (oxLDL), advanced glycosylation end products (AGEs) of diabetes, angiotensin II and endothelin effectively promote the activation of macrophages. Activated

macrophages express scavenger receptors, resulting in the uncontrolled uptake of oxLDL and the formation of foam cells. Activated macrophages also act as antigen presentation cells to promote T lymphocyte activation, exacerbating the process of atherosclerosis. In addition, activated macrophages secrete an enormous battery of effector molecules such as inflammatory cytokines, metalloproteinases, reactive oxygen species and death-inducing molecules that play a vital role in atherosclerosis and lead to plaque instability. Inhibition of macrophages activation has been regarded as a therapeutic approach in atherosclerotic diseases. In addition, the activation of mature DC promotes the secretion of pro-inflammatory cytokines and is critical for T-cell activation and the production of Th1 and Th17 cytokines, which possesses potentially pathogenic properties in atherosclerosis and atherosclerosis related disease, whereas immature DC have been found to secrete anti-inflammatory cytokine IL-10, induce the generation of regulatory T cells and therefore effectively ameliorate atherosclerosis (Bobryshev 2010; Gautier et al. 2009; Methe et al. 2005; Eid et al. 2009; Hermansson et al. 2011). Given that IL-37 is a critical regulator in macrophage and DC activation, IL-37 may attenuate atherosclerosis and atherosclerosis related diseases through the inhibition of macrophage and DC activation.

IL-18 is produced by monocytes/macrophages, DC and several nonhematopoeitic cell types, and further increases the expression of certain inflammatory cytokines such as IFN-γ and MMPs in endothelial cells, T cells and monocytes/macrophages, promotes Th1 and natural killer (NK) cell activity, leading to amplification of systemic inflammatory responses. The atherogenic effect of IL-18 has widely been studied in atherosclerosis. Blocking the effects of IL-18 reduces the atherosclerotic lesion size and induces a switch to a stable plaque phenotype, whereas both endogenous and exogenous IL-18 accelerated atherosclerosis development (de Nooijer et al. 2004; Elhage et al. 2003; Mallat et al. 2001; Whitman et al. 2002). Furthermore, IL-18 administration did not affect lesion development in IFN-γ-deficient mice, suggesting that the atherogenic effect of IL-18 depends on the induction of IFN-γ (Whitman et al. 2002). It is notable that IL-18 was originally defined as IFN-γ-inducing factor, because IL-18 can effectively induce the expression of IFN-γ in various cells such as T cells, NK cells and macrophages. IFN-γ is a characteristic cytokine of Th1 cells and its atherogenic properties are well accepted. More recently, unpublished results from our group showed that the plasma levels of IL-37, IL-18 and C-reactive protein (CRP) were significantly increased in patients with unstable angina pectoris and acute myocardial infarction compared with the control group and the stable angina pectoris group, and the levels of IL-37 positively correlated with IL-18 and CRP, indicating that the up-regulation of IL-37 may result from inflammatory activation and that change of IL-37 is associated with the onset of acute coronary syndrome symptoms. Because IL-37 shares the same IL-18BP and IL-18Rα receptors with IL-18 and IL-37 effectively inhibit the IL-18-induced inflammatory response, we measured the IL-37/IL-18 ratio. The results showed that the plasma IL-37/IL-18 ratio sharply decreased in patients with coronary artery disease, suggesting that increases in IL-37 fail to suppress the excessive activation of the inflammatory responses. Therefore, it is reasonable to hypothesize that IL-37 may play a protective role in atherosclerotic diseases *via* inhibition of the inflammatory response induced by IL-18.

5. Perspective

In summary, the anti-inflammatory cytokine IL-37 plays a protective role in inflammatory and autoimmune diseases. Data from Boraschi et al. and our unpublished study reveal that IL-37 may be involved in the development of atherosclerotic disease. However, whether IL-37 plays a protective role and how it works in atherosclerosis are still unknown. Combination of all the data mentioned above, we propose at least two mechanisms that could explain the protective role of IL-37 in atherosclerosis: (1) at the molecular level, IL-37 ameliorates atherosclerosis via inhibition of the secretion of inflammatory cytokines; (2) at the cellular level, IL-37 attenuates atherosclerosis through the inhibition of macrophage and DC activation. We are convinced that an in-depth study of IL-37 in atherosclerosis not only deepens our understanding of the nature of atherosclerosis but also provides a new therapeutic approach in atherosclerotic diseases in the future.

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