REVIEW

Department of Ultrasound[1]; Department of Cardiology[2], the People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China

IL-35: a potential target for the treatment of atherosclerosis

YING HUANG[1,*], YING-ZHONG LIN[2,*], YING SHI[2], QING-WEI JI[2]

Received December 18, 2012, accepted January 25, 2013

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.*

Pharmazie 68: 793–795 (2013) *doi: 10.1691/ph.2013.2928*

Abstract: The imbalance of anti- inflammatory/pro-inflammatory cytokines plays an important role in the process of atherosclerosis. IL-35 is an anti-inflammatory cytokine comprising the p35 subunit of IL-12 and the subunit Epstein-Barr virus (EBV) -induced gene 3(EBI3). Accumulating evidence showed that IL-35 up-regulates the expression of anti-inflammatory cytokines, induces the generation of CD4 + regulatory T cells, inhibits CD4 + effector T cells response and other target cells activity, and reduces the progression of inflammatory and autoimmune diseases. In addition, it has been found that Ebi3 and p35 strongly co-expressed in human advanced lesions. Therefore, we hypothesize that IL-35 may become a novel target for the treatment of atherosclerosis. Further studies are required to investigate the precise effect and the signaling pathway of IL-35 in atherosclerosis process.

1. Introduction

IL-35, an anti-inflammatory cytokine, was identified in 2007 (Collison et al. 2007; Niedbala et al. 2007). IL-35 is a heterodimer cytokine comprising the p35 subunit of IL-12 and the subunit Epstein-Barr virus (EBV) -induced gene 3(EBI3) which was identified in B lymphocytes based on its induction following EBV infection (Devergne et al. 1996, 1997; Collison et al. 2007; Niedbala et al. 2007). Besides CD4 + regulatory T (Treg) cells, activated dendritic cells (DCs), macrophages, endothelial cells and aortic smooth muscle cells express IL-35 (Allan et al. 2008; Bardel et al. 2008; Chaturvedi et al. 2011; Collison et al. 2010; Li et al. 2012; Kempe et al. 2009). Study results demonstrated that IL-35 is an important anti-inflammatory cytokines and can efficiently suppress the CD4 + effector T cells (Teff, including Th1, Th2, and Th17) activity, induce the generation of Treg cells, and reduce the progression of inflammatory diseases and autoimmune diseases, suggesting IL-35 may also be involved in atherosclerosis (Bettini et al. 2012; Chaturvedi et al. 2011; Collison et al. 2007, 2010; Kochetkova et al. 2010; Niedbala et al. 2007).

2. Imbalance of anti-inflammatory/pro-inflammatory cytokines in atherosclerosis

Atherosclerosis, characterized by extensive lipid deposition and atherosclerotic plaque formation in the intima, is a chronic inflammatory disease (Ross 1999). Evidence from atherosclerosis prone models showed that atherosclerosis and plaque stabilization were related to the imbalance of anti-inflammatory/pro-inflammatory cytokines (Frostegård et al. 1999; Tedgui and Mallat 2006). The interactive cascade of inflammatory cells, chemokines and pro-inflammatory cytokines plays a major role in the initiation and progression of atherosclerosis and in the development of clinical syndromes we recognize as acute coronary syndromes (Frostegård et al. 1999; Alam et al. 2004; Tedgui and Mallat 2006).

Many pro-atherogenic effects of proinflammatory cytokines such as IFN-γ, TNF-α and IL-17, mainly produced by lymphocytes and macrophages, have been identified in cell culture studies and compound knockout experiments. IFN-γ enhances recruitment of T cells and macrophages to the plaques, promotes the formation of foam cells, and increases secretion of Th1-promoting cytokines, which subsequently continues to drive these processes. IFN-γ also inhibits the formation of fibrous cap, resulting in vulnerable, ruptureprone plaques. It seems that TNF-α is important in the early phase of atherosclerotic lesion development. Oxidized low density lipoprotein (LDL) stimulates release of TNF-α from monocytes/macrophages in a dose-dependent manner. TNF-α once released promotes oxidized LDL uptake from differentiating monocytes, resulting in foam cell accumulation. Clinical evidence has shown that the expression of TNF-α in atherosclerotic plaques was associated with plaque remodeling and facilitated plaque rupture and thrombus formation, suggesting that TNF-α also plays critical role in the onset of acute coronary syndromes (Loppnow et al. 2008). IL-17, a predominantly proinflammatory cytokine, is produced by Th17 cells as well as other cell types including γδ T cells, natural killer (NK) cells, NKT cells and lymph tissue inducer cells. IL-17 induces tissue inflammation mainly by stimulating the production of TNF-a, IL-6, and MCP-1 as well as adhesion molecules in inflammatory cells and this ability of IL-17 could be enhanced by costimulation with TNF-α or IL-1β. Furthermore, IL-17 could contribute to the proinflammatory milieu of atherosclerosis even in the presence of dominant Th1 immune responses (Eid et al. 2009).

During the inflammatory process, anti-inflammatory cytokines are also produced and tend to modulate the inflammatory

cellular signaling pathways. IL-10 and TGF-β1, the critical anti-inflammatory cytokines, have been widely investigated in atherosclerosis disease. IL-10 is a pleiotropic cytokine mainly produced by T cells, DCs, and monocytes/macrophages that inhibits a broad array of immune parameters including Th1 and Th2 type cytokines production, antigen presentation, and antigen-specific T-cell proliferation. IL-10 binds to the IL-10 receptor complex and plays an active role in limiting the inflammatory response in the vessel wall. The antiatherogenic effect of IL-10 has been demonstrated using gain and loss of function strategies in atherosclerosis-prone mouse models. IL-10 has an inhibitory effect on lesion size and promotes plaque stabilization. However, pro-inflammatory cytokines strongly are expressed in atherosclerotic plaques, whereas IL-10 is expressed in the minority of atherosclerotic plaques (Uyemura et al. 1996). TGF-β1 is a potent anti-inflammatory, immunosuppressive and pro-fibrotic cytokine. TGF-β1 inhibits recruitment of leukocytes into the lesion, the formation of foam cell, and Th1 type response. TGF-β1 promotes plaque stabilization through the induction of collagen and smooth muscle cell synthesis of tissue inhibitors of MMPs. In fact, both IL-10 and TGF-β1 levels were markedly decreased in patients with advanced atherosclerosis and accompanied with the onset of acute coronary syndrome, and high concentrations of IL-10 and TGF-β1 were associated with a favorable outcome in patients with coronary artery disease (Grainger et al. 1995; Heeschen et al. 2003; Ji et al. 2009).

Taken together, imbalance of anti-inflammatory/pro-inflammatory cytokines may play an important role in the initiation and progression of atherosclerosis and the onset of acute coronary syndromes.

3. IL-35 and atherosclerosis

IL-35, an anti-inflammatory cytokine, is an IL-12 family member cytokine composed of an α chain p35 and a β chain Ebi3 (Collison et al. 2007; Niedbala et al. 2007). The p35 subunit is also associated with the p40 subunit to form IL-12 and Ebi3 is also associated with the p28 subunit to form IL-27, another member of the IL-12 family. Both IL-12 and IL-27 has been found to promote Th1 cells development, suggesting their atherogenic effect (Uyemura et al. 1996; Jankowski et al. 2010; Jafarzadeh et al. 2011). The EBI3/p35 heterodimer has been confirmed to suppress Teff cells activity, expand Treg cells effect, attenuate established collagen-induced arthritis, and eventually was designated as IL-35 (Niedbala et al. 2007). It has shown that IL-35 was constitutively secreted by mouse Foxp3+ Treg cells but not activated Teff cells (Collison et al. 2007). Furthermore, the regulatory activity of *Ebi3* or *p35* knockout mice Treg cells was significantly reduced than that of wild-type Treg cells *in vivo* and *in vitro* and does not ameliorate insufficiently the process of colitis, suggesting that IL-35 is critical to the regulatory activity of Treg cells in mice (Collison et al. 2007; Liu et al. 2012; Yang et al. 2008). IL-35 may also play pivotal role in human Treg cells. Stimulated by anti-CD3 and anti-CD28, the expression of both EBI3 and p35 in human Treg cells were higher significantly than that in Teff cells. In addition, neutralizing anti–IL-35 completely abolished the suppression of human Treg cells (Chaturvedi et al. 2011). Especially, the conversion of suppressed target Teff cells into Foxp3-independent Treg population, namely iTr35, could be induced efficiently by IL-35 in both human and mice (Chaturvedi et al. 2011; Collison et al. 2010, 2012; Seyerl et al. 2010). When co-cultured with DC activated by human rhinovirus (R-DC), iTr35 also can be induced and secret IL-35. This effect could be reverted by blocking the inhibitory receptors B7-H1 and sialoadhesin on R-DC, suggesting an important mechanism in regulating the IL-35 expression (Seyerl et al. 2010). Besides inducing the generation of iTr35 cells and suppressing the proliferation of Teff cells, IL-35 performs its biological effect via up-regulating the expression of anti-inflammatory cytokines such as IL-10 and IL-35, and inhibiting directly the other target cells activity.

In healthy humans, EBI3 is expressed at high levels in placental trophoblast cells, activated DCs and lymphocytes, and at lower levels in macrophages and endothelial cells but not in normal resting CD3+T cells, whereas the p35 gene is constitutively expressed at low levels in the majority of cell types, and both of them do not express in heart or vessels (Devergne et al. 1996; Bardel et al. 2008; Li et al. 2012). Under inflammatory conditions, IL-35 could be up-regulated to control fully-blown inflammation in all the tissue of human. T cells are easily found in human lesions. EBV-specific T lymphocytes were frequently observed in human atherosclerotic plaques indicating the ability of these T lymphocytes to secret IL-35 and the association between IL-35 and atherosclerosis (de Boer et al. 2006). Apart from T cells, human non-T cells, such as microvascular endothelial cells and aortic smooth muscle cells express IL-35 after stimulation by the pro-inflammatory cytokines TNF-a, IL-1β, and/or IFN-γ (Li et al. 2012). Kempe et al. (2009) obtained plaque samples from patients with symptomatic carotid plaques. They found Ebi3 and p35 strongly co-expressed in endothelial cells, macrophages, and vascular smooth muscle cells in almost all advanced lesions, whereas p28 weakly stained, suggesting that not IL-27 but IL-35 is critical in atherosclerosis process. The study also demonstrated that vascular smooth muscle cells could be the source of IL-35 in inflammatory context.

More recently, we recruited 161 patients with coronary artery disease (CAD) (including 43 patients with stable angina pectoris, 62 patients with unstable angina pectoris and 56 patients with acute myocardial infarction) and 47 chest pain syndrome patients without coronary stenosis verified by coronary angiography (Lin et al. 2012). We found that the anti-inflammatory cytokines IL-35, IL-10, and TGF-β1 levels were significantly decreased in patients with CAD, whereas the pro-inflammatory cytokines IL-12 and IL-27 levels were significantly increased in patients with CAD. In addition, we found that lower IL-35 levels were positively correlated with LVEF in CAD patients, suggesting that the plasma IL-35 level is not only a potential predictor for symptoms onset of CAD, but also plays a critical role in myocardial function of CAD.

4. Hypothesis

Not only the imbalance of anti-inflammatory/pro-inflammatory cytokines but the imbalance of Teff cells and Treg cells is involved in atherosclerosis. So far, the atherogenic effects of Th1 and Th17 have been confirmed in cell culture studies and animal experiments. Blocking the effect of Th1 and/or Th17 could efficiently attenuate atherosclerosis in atherosclerosis prone models. Evidence from clinical studies showed that Th1 and Th17 dominated both in human atherosclerotic plaque and circulation (Eid et al. 2009). Although Treg cells could inhibit Teff cells response and play a protective role in the progression of atherosclerosis, the suppressive function of peripheral Treg cells was significantly reduced in acute coronary syndromes (Ait-Oufella et al. 2006; Mor et al. 2006; Zhong et al. 2012).

Based on these studies, we hypothesize that IL-35 may become a novel target for the treatment of atherosclerosis. IL-35 may ameliorate the atherosclerosis process and improve the prognosis of CAD by up-regulating the expression of anti-inflammatory cytokines, inducing the generation of Treg cells, and inhibiting Teff cells response and other target cells (such as endothelial cell, macrophage, and vascular smooth muscle cell) activity.

However, the exact role of IL-35 still remains uncertain and further studies are required to investigate the precise effect and the signaling pathway of IL-35 in atherosclerosis process.

Acknowledgments: This work was supported by National Natural Science Foundation of China (No. 81160045).

References

Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, Merval R, Esposito B, Cohen JL, Fisson S, Flavell RA, Hansson GK, Klatzmann D,Tedgui A, Mallat Z (2006) Natural regulatory T cells control the development of atherosclerosis in mice. Nat Med 12: 178–180.

Alam SE, Nasser SS, Fernainy KE, Habib AA, Badr KF (2004) Cytokine imbalance in acute coronary syndrome. Curr Opin Pharmacol 4: 166 –170.

Allan SE, Song-Zhao GX, Abraham T, McMurchy AN, Levings MK (2008) Inducible reprogramming of human T cells into Treg cells by a conditionally active form of FOXP3. Eur J Immunol 38: 3282–3289.

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

Bettini M, Castellaw AH, Lennon GP, Burton AR, Vignali DA (2012) Prevention of Autoimmune Diabetes by Ectopic Pancreatic β-Cell Expression of Interleukin-35. Diabetes 61: 1519–1526.

Chaturvedi V, Collison LW, Guy CS, Workman CJ, Vignali DA (2011) Cutting edge: Human regulatory T cells require IL-35 to mediate suppression and infectious tolerance. J Immunol 186: 6661–6666.

Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA (2007) The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 450: 566 –569.

Collison LW, Chaturvedi V, Henderson AL, Giacomin PR, Guy C, Bankoti J, Finkelstein D, Forbes K, Workman CJ, Brown SA, Rehg JE, Jones ML, Ni HT, Artis D, Turk MJ, Vignali DA (2010) IL-35-mediated induction of a potent regulatory T cell population. Nat Immunol 11: 1093 –1101.

Collison LW, Delgoffe GM, Guy CS, Vignali KM, Chaturvedi V, Fairweather D, Satoskar AR, Garcia KC, Hunter CA, Drake CG, Murray PJ, Vignali DA (2012). The composition and signaling of the IL-35 receptor are unconventional. Nat Immunol 13: 290–299.

de Boer OJ, Teeling P, Idu MM, Becker AE, van der Wal AC (2006). Epstein Barr virus specific T-cells generated from unstable human atherosclerotic lesions: implications for plaque inflammation. Atherosclerosis 184: 322–329.

Devergne O, Hummel M, Koeppen H, Le Beau MM, Nathanson EC, Kieff E, Birkenbach M (1996). A novel interleukin-12 p40-related protein induced by latent Epstein-Barr virus infection in B lymphocytes. J Virol 70: 1143–1145.

Devergne O, Birkenbach M, Kieff E (1997). Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. Proc Natl Acad Sci U S A 94: 12041–12046.

Eid RE, Rao DA, Zhou J, Lo SF, Ranjbaran H, Gallo A, Sokol SI, Pfau S, Pober JS, Tellides G (2009) Interleukin-17 and interferon-gamma are produced concomitantly by human coronary artery-infiltrating T cells and act synergistically on vascular smooth muscle cells. Circulation 119: 1424–1432.

Frostegård J, Ulfgren AK, Nyberg P, Hedin U, Swedenborg J, Andersson U, Hansson GK (1999) Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis 145: 33–43.

Grainger DJ, Kemp PR, Metcalfe JC, Liu AC, Lawn RM, Williams NR, Grace AA, Schofield PM, Chauhan A (1995) The serum concentration of active transforming growth factor-beta is severely depressed in advanced atherosclerosis. Nature Med 1: 74–79.

Heeschen C, Dimmeler S, Hamm CW, Fichtlscherer S, Boersma E, Simoons ML, Zeiher AM; CAPTURE Study Investigators (2003) Serum level of the antiinflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation 107: 2109–2114.

Jafarzadeh A, Nemati M, Rezayati MT (2011) Serum levels of interleukin (IL)-27 in patients with ischemic heart disease Cytokine 56:153–156.

Jankowski M, Kopiński P, Goc A (2010) Interleukin-27: biological properties and clinical application. Arch Immunol Ther Exp (Warsz) 58: 417–425.

Ji QW, Guo M, Zheng JS, Mao XB, Peng YD, Li SN, Liang ZS, Dai ZY, Mao Y, Zeng QT (2009) Downregulation of T helper cell type 3 in patients with acute coronary syndrome. Arch Med Res 40: 285–293.

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

Kochetkova I, Golden S, Holderness K, Callis G, Pascual DW (2010) IL-35 stimulation of CD39+ regulatory T cells confers protection against collagen II-induced arthritis via the production of IL-10. J Immunol 184: 7144–7153.

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

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

Liu JQ, Liu Z, Zhang X, Shi Y, Talebian F, Carl JW Jr, Yu C, Shi FD, Whitacre CC, Trgovcich J, Bai XF (2012) Increased Th17 and regulatory T cell responses in EBV-induced gene 3-deficient mice lead to marginally enhanced development of autoimmune encephalomyelitis. J Immunol 188: 3099–3106.

Loppnow H, Werdan K, Buerke M (2008) Vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. Innate Immun 14: 63–87.

Mor A, Luboshits G, Planer D, Keren G, George J (2006) Altered status of CD4+CD25+ regulatory T cells in patients with acute coronary syndromes. Eur Heart J 27: 2530–2537.

Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB, Liew FY (2007) IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Eur J Immunol 37: 3021–3029.

Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340: 115–126.

Seyerl M, Kirchberger S, Majdic O, Seipelt J, Jindra C, Schrauf C, Stöckl J (2010) Human rhinoviruses induce IL-35-producing Treg via induction of B7-H1 (CD274) and sialoadhesin (CD169) on DC. Eur J Immunol 40: 321–329.

Tedgui A, Mallat Z (2006) Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev 86: 515–581.

Uyemura K, Demer LL, Castle SC, Jullien D, Berliner JA, Gately MK, Warrier RR, Pham N, Fogelman AM, Modlin RL (1996) Cross-regulatory roles of interleukin (IL)-12 and IL-10 in atherosclerosis. J Clin Invest 97: 2130–2138.

Yang J, Yang M, Htut TM, Ouyang X, Hanidu A, Li X, Sellati R, Jiang H, Zhang S, Li H, Zhao J, Ting AT, Mayer L, Unkeless JC, Labadia ME, Hodge M, Li J, Xiong H (2008). Epstein-Barr virus-induced gene 3 negatively regulates IL-17, IL-22 and RORgamma t. Eur J Immunol 38: 1204–1214.

Zhong Y, Wang X, Ji Q, Mao X, Tang H, Yi G, Meng K, Yang X, Zeng Q (2012) CD4+LAP + and CD4 +CD25 +Foxp3 + regulatory T cells induced by nasal oxidized low-density lipoprotein suppress effector T cells response and attenuate atherosclerosis in ApoE(−/−) Mice. J Clin Immunol 32: 1104–1117.