



Is Interleukin-38 a key player cytokine in atherosclerosis immune gene therapy?



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ABSTRACT

Atherosclerosis, a chronic inflammatory disease of the arteries associated with lipids and other metabolic alterations is a leading cause of death all around the world and its rate is raising as a result of unhealthy lifestyles. Reports by World Health Organization indicate that 31% of all death occurrences are due to heart attacks and strokes. Today, the most common medicines for treating atherosclerosis are statins which are HMG-coA reductase inhibitors. Beside their benefits in treating atherosclerosis, some side effects have been reported as well. Thus, therapeutic methods based on statins should be evaluated to result in more beneficial effects. Since atherosclerosis is an inflammatory disorder, an anti-inflammatory component can decrease the impact of this disease. Interleukin-38, a newly discovered anti-inflammatory cytokine, which acts as an Interleukin-36 receptor antagonist can block Nuclear Factor KB and Activator protein-1 signaling pathways, and block atherogenic core formation accordingly. This novel proposed immune gene therapy can be applied to atherosclerosis treatment in a trial study. In this hypothesis, Interleukin-38 gene is transferred into bone marrow Mesenchymal Stem Cells of atherosclerotic mouse model Apo E^{-/-} via an adenoviral vector. It is expected that Interleukin-38 gene expression by Mesenchymal Stem Cells can efficiently remedy atherosclerosis without the side effects of statins.

Introduction

Atherosclerosis, which impacts large- and medium-sized arteries, is a chronic immune-mediated inflammatory disease [1]. It is the major cause of myocardial infarction (MI), stroke, angina (chest pain) and heart failure. So it is one of the leading cause of death all over the world and the incidence is rising as a result of unhealthy lifestyles [2,3]. Atherosclerosis is a kind of multifactorial disease. According to epidemiological studies, atherosclerosis is associated with both genetic (susceptibility or resistance of artery to atherosclerosis) and environmental risk factors including hypertension, diabetes, tobacco smoking and blood flow pattern [4,5]. This causes altered cell metabolism and gene expression and also pro-inflammatory cytokines secretion by endothelial cells [6]. Inflammation has a key role in the initiation and development of atherosclerosis; great number of immune cells and immune mediators are involved in atherogenesis process [7–9]. Pro-inflammatory cytokines like IL-6 and Tumor Necrosis Factor- α (TNF- α) contribute to atheromatous plaque formation [8]. As reported by Canault M. et al., TNF- α induces inflammatory reactions in atheromatous

lesion of Apo-E knocked-out mice, and inactivation of TNF- α diminishes lesion size in both nutritional and genetic model of atherosclerosis [10]. In addition, an increase in IL-6 production is also associated with atherosclerosis and cardiovascular mortality [8,11]. Up-regulation of T-helper17 (Th17) is increased during atherosclerosis [12]. IL-17, a principal pro-inflammatory cytokine, is produced by Th17 and also other types of cells such as Natural Killer cells (NK cells), T cells and lymph tissue inducer cells [9]. IL-17 stimulates the production of TNF- α , IL-6 and Monocyte Chemoattractant Protein-1 (MCP-1), thus induces inflammation [9]. First step in the formation of an atheromatous plaque is the entrance of Low Density Lipoprotein (LDL) particles and their derivatives from blood to intima, where they accumulate. Then LDL particles will be modified by enzymes like myeloperoxidase, sphingomyelinase and secretory phospholipase which in turn will activate innate inflammatory system [4,5]. Inflammation initiates when Endothelial Cells (ECs) are activated in lesion-prone sites [5]. Activated ECs express adhesion molecules like Vascular Cell Adhesion Molecule-1 (VCAM-1), E-selectin, P-selectin [13] and Intercellular Adhesion Molecule-1 (ICAM-1) [14] which recruit monocytes [13].

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Intimal macrophages replicate and also secrete some cytokines and Growth factors which contribute in atheromatous lesion complication [13]. Primarily, researchers had supposed that macrophages were the only leukocytes which are present in an atheromatous plaque, but further studies indicated that other immune cells including regulatory T-cells (T-regs), CD4⁺ T cells, myeloid cells and mast cells during atherosclerosis should also be considered [15]; MCP-1 (which is important in initial steps), MCP-4, MIP-1a, Macrophage Inflammatory Protein-1b (MIP-1b), IL-8 are a few examples [14]. Interferons (IFN) which guide T-cells and monocytes to the lesion site and increase T-helper 1 promoting cytokines secretion, TNF- (which have an important role in early stages of atherosclerosis by stimulating monocytes to collect oxidized LDL (ox-LDL) and forming foam cells) [9]. In addition, IL-4 and IL-6 levels are raised in patients with cardiovascular diseases [16]. According to the role of inflammation in atherosclerosis, some novel anti-inflammatory therapies are introduced as potential adjunct drugs to current therapy [17]. For example, Canakinumab which acts as IL-1 β neutralizing monoclonal antibody [18,19], Adalimumab which is a TNF- α blocker antibody [18–20], Darapladib which is a phospholipase A2 (PLA2) inhibitor [21] and other drugs like Methotrexate, Colchicine, Ustekinumab, briakinumab, varespladib and terutroban can be considered as such. There are also some drugs on clinical trial [18] like Anakinra or interleukin-1 receptor antagonist (IL-1Ra) which reduces IL-6 and C-Reactive Protein (CRP) levels [17,22]. Other drugs with no anti-inflammatory effect are Statins. The most common and best-selling drug (in the USA) in treating cardiovascular diseases and atherosclerosis, is HMG-CoA reductase which is a statin [23,24]. Statins have these pleiotropic effects [13]; they reduce atherogenic lipoprotein levels [4], stabilize plaque and retard plaque progression [5]. They also reduce leukocyte adhesion, and prevent macrophages from activation and proliferation. Additionally, statins prevent Matrix Metalloproteinase (MMP) production by macrophages. Similar to any drug, statins have some side effects [23], where it inhibits HMG-CoA reductase at an early stage in the mevalonate pathway. In addition to cholesterol, other products are generated by this pathway, too including heme-A, coenzyme Q10, isoprenylated proteins, which have vital roles in natural physiology of body.

Above this, cholesterol is not solely a final product, but also a mediator for series of products which have fundamental role in health; such as sex steroids, corticosteroid, vitamin D, bile acids, etc. which can be affected by statin treatment [23]. Additionally, the main adverse effects of statins are myopathy and impaired insulin resistance with a risk of diabetes [24]. As reported by Koga *et al.* “cholesterol lowering therapy in patients with CRP \geq 2 mg/l, decreased nearly half of major Cardiovascular Diseases (CVDs) and other half of CVDs are not prevented yet” [25]. So novel preventive and treatment methods are required and they should be able to confront CVDs which are resulted from all types of risk factors, not only high LDL-cholesterol. Interleukin-38 (IL-38) has also been introduced as IL1HY2, IL-1F10, IL-10 and FIL-10 [5], which is a novel anti-inflammatory cytokine, and was first identified in 2001 [26]. The 10th member of IL-1 family is IL-38 which belongs to IL-36 subfamily. The gene is positioned on human chromosome 2q13-14 in the IL-1 family cluster except IL-18 and IL-33, between IL-1Ra and IL-36Ra [26–28]. This protein with 16.9 kD molecular mass [29] contains 152 amino acids and histidine is not present in IL-38 [28]. As other IL-1 family members, including IL-1 α , IL-1Ra, IL-1 β , IL-33, IL-18, IL-36 α , IL-36 β , IL-36 γ , IL-36Ra and IL-37, IL-38 lacks a signal peptide and caspase 1 consensus site [26,29]. However, the N terminus for IL-38 is not defined yet [30]. Generally, inflammatory cells express IL-1 family members and these cytokines connect to the cell-surface receptor IL-1R to induce signaling, like downstream NF- κ B and AP-1 [28]. IL-38 protein is mainly expressed in the basal epithelia of skin [31], proliferating B cells of tonsils and also heart, placenta, fetal liver, spleen and thymus [26,27]. Surprisingly, there is IL-38 mRNA in patients with human atheromatous plaques of coronary artery disease [32]. IL-38 binds to IL-36 receptor (also called IL-1Rrp2) like IL-36Ra so

it can influence the pro-inflammatory function of IL-36 [26,33], but it doesn't bind to the immobilized IL-1RI, IL-18R and IL-1R accessory proteins (IL-1RAP, IL-1RAcP) [29]. IL-1RI was once supposed to be a receptor for IL-38 based on IL-38 amino acid homology to IL-1Ra [27,30]. However, its binding affinity is significantly lower than IL-1Ra and IL-1 β . So the anti-inflammatory functions of IL-38 are related to blocking IL-1R and IL-36R pathways [33]. However, the exact molecular mechanism by which IL-38 controls inflammation is still disputable and further studies are needed [34]. A novel study has illustrated that IL-38 inhibited Candida Albicans-induced Th17 (IL-17 and IL-22) production in Peripheral Blood Mononuclear Cells (PBMCs). This inhibition was absent in IFN- γ (Th1 cytokine). By comparison, LPS-induced IL-6 production was notably higher in the presence of IL-38 (26, 28). Van de Veerdonk, has reported that it is unlikely that IL-38 suppresses IL-17 by affecting IL-18 or its receptor [33]. Quoted from Xianli Yuan, IL-38 gene polymorphisms are associated with psoriatic arthritis (PsA), ankylosing spondylitis and cardiovascular disease suggesting that IL-38 is strongly correlated with these inflammatory diseases [28]. Recent studies have demonstrated a correlation between IL-38 and autoimmune diseases like rheumatoid arthritis [35]. IL-38 also takes part in immune regulation and is involved in MAPK activation. Recently, Kazuki takada, indicated that “IL-38 is involved in carcinogenesis and tumor progression through the regulation of tumor associated transcriptional factors” [36]. Contrary to biological effects of IL-38 as an inhibitor of IL-22 and IL-17 production, IL-38 is not a classic receptor antagonist in comparison with IL-1Ra. Notably, IL-36Ra is not a classic receptor antagonist, as well. These data indicate that IL-36Ra and IL-38 are partial receptor antagonists which act as antagonists when their concentration is high. Furthermore, above linking to IL-36R, the co-receptor that IL-38 and IL-36Ra recruit are different at high and low concentrations; as we see an inhibitory co-receptor at low concentrations and a signaling co-receptor at high concentrations [33]. As a result, IL-38 may have dose-dependent effects, i.e. the efficacy of IL-38 at low concentrations were more than high concentrations [26,28]. Some effects of IL-38 and IL-36Ra are still unclear; for instance, it is yet ambiguous why they increase pro-inflammatory cytokines in dendritic cells whereas decrease pro-inflammatory cytokines in peripheral blood mononuclear cells [27]. So further studies are required to complete our understanding of IL-38. In this novel hypothesis, we introduced IL-38 as a gene therapy candidate which is a newly-identified anti-inflammatory cytokine for the treatment of atheromatous plaque formation in infected individuals.

IL-38 signaling pathway

According to amino acid homology between IL-38 and IL-36, IL-36 receptor (IL-1Rrp2) is the specific receptor for IL-38. IL-1 family members connect to IL-1 receptors on the surface of inflammatory cells so they induce downstream signals including AP1 and NF- κ B, two nuclear transcripts. These signals induce the expression of cyclooxygenase, nitric oxide (NO) synthesis and other inflammatory mediators. IL-38 blocks IL-36 receptor and prevents inflammatory functions of IL-36 cytokines (IL-36 α , IL-36 β , IL-36 γ) (Fig. 1) [28].

Hypothesis

Atherosclerosis is one of the leading causes of death in western countries [37]. So atherosclerosis requires efficient preventive and treatment strategies. Nowadays, various kinds of drugs like statins are widely applied in clinics for these patients, with several side effects beside their remedial effects. Therefore, using gene therapy and cell therapy approaches, which have been accepted in many diseases and also malignancies [38–45], can be useful in prevention and treatment of atherosclerosis as favorable therapeutic methods. In this hypothesis, we introduce the blockage of IL-36 pathway method as a novel target for atherosclerosis prevention and treatment. In fact, IL-38 functions as an

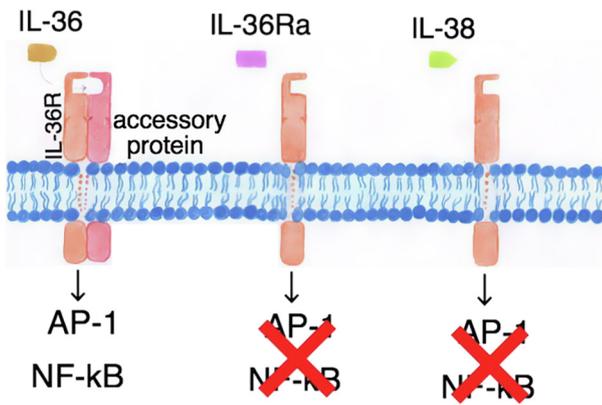


Fig. 1. IL-38 and IL-36 Signaling Pathway.

IL-36 receptor antagonist which connects to IL-36 receptor and suppresses the inflammation pathway. Overexpression of IL-38 will block IL-36 signaling pathway and this will prevent atheromatous plaque formation. Thus, in this study, IL-38 is suggested for treatment purposes in an atherosclerotic Apo-E knocked out mouse model. AAV, as a gene therapy vector, which can infect both dividing and quiescent cells and remain in an extrachromosomal environment without integrating into the host cell genome is introduced to this study. These applicable characteristics make AAV an interesting choice as a viral vector for gene therapy [46,47].

Evaluation of hypothesis

To examine the hypothesis, the authors suggest the following steps in male Apo lipoprotein E knocked out (Apo E^{-/-}) mice [48] which has been shown in Fig. 2.

- (1) The MSCs are separated from the mentioned mouse models.
- (2) IL-38 gene clone into Adeno Associated Virus vector (according to IL-38 gene properties (gene ID:84639)) and then transfected to MSCs in a PLTmax media.
- (3) 40 male Apo E^{-/-} mouse models are selected. Then they are divided into four equal groups:
 - a. The first group receives nothing as a control group of the atherogenic model.
 - b. Second group receives MSCs only.
 - c. Third group receives MSCs infected with AAV only.
 - d. Fourth group receives MSCs infected with adeno associated virus vector carrying IL-38 Gene.
- (4) Overexpression of RNA and IL-38 protein are checked by RT-PCR and ELISA, respectively.
- (5) Levels of IL-38 in all groups are measured by real-time PCR.
- (6) Histopathological studies for atheromatous plaque formation and molecular assays of NF-KB and AP-1 pathways are needed.

Discussion

Atherosclerosis is a chronic inflammatory disease. Many immune cells and immune mediators are involved to form an atheromatous

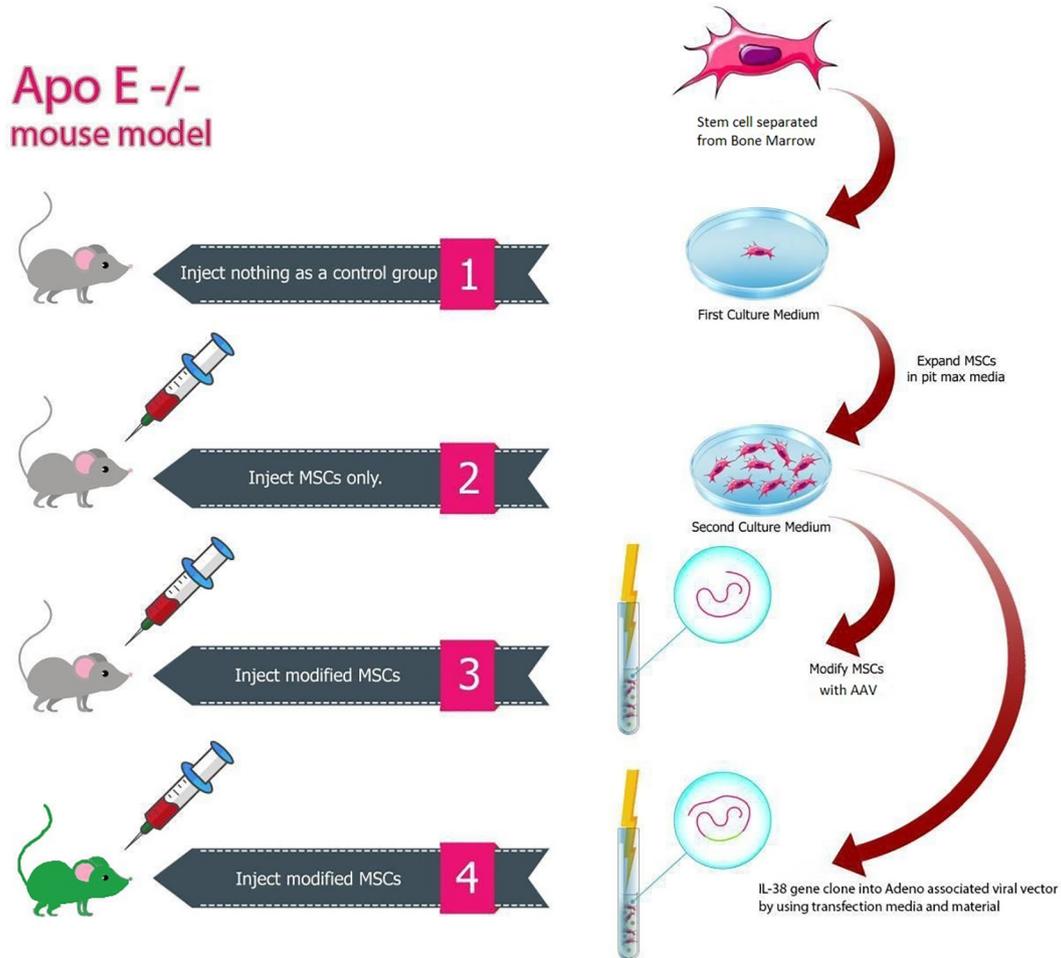


Fig. 2. To test the hypothesis, the authors have proposed this experiment.

lesion [7,8]. Atheromatous plaque formation initiates with the accumulation of LDL Particles and derivatives into intima. Modification of these LDL particles, activate inflammatory system [4,5] and subsequent to inflammatory system activation, pro-inflammatory cytokines and adhesion molecules take part in the process [13,14]. Today, the most common drugs to confront atherosclerosis are statins which are HMG-Co A reductase inhibitors [24,47]. Statins suppress plaque progression from different ways such as lowering atherogenic lipoproteins [4] and a reducing monocyte adhesion [5]. Statins, inhibit HMG-Co A reductase. This enzyme is important in producing pathway of some physiological products like Heme-A and co-enzyme Q [23]. Reduction of co-enzyme Q levels will reduce cell energy but elevate oxidation and apoptosis. Statins inhibit Mevalonate pathway, which in turn produces heme-A which has an important role in electron transportation [23]. Statins increase the risk of diabetes mellitus because they can disturb the function of B cells of Langerhans islets of pancreas and insulin signaling pathway [49]. They also have some muscular side effects where myalgia is the most common and myositis is the least common ones. Beside these, fatigue, myoglobinuria, renal impairments and serum electrolyte abnormalities can be mentioned as statins' adverse effects [23,49]. These adverse effects can minimize statins' usefulness, so better therapeutic methods should be developed. Based on inflammatory processes during atheromatous plaque progression, it seems that anti-inflammatory drugs and methods can be effective in atherosclerosis treatment. Some articles have introduced a number of these anti-inflammatory substances like Canakinumab [18,19], Adalimumab [18,20], Anakinra [17,22], etc. each of which has certain advantages and disadvantages. Recently cell and gene therapy are as novel methods to treat AIDS and HIV [38–40], Hodgkin's lymphoma [41], Behçet's disease [42], Pancreatic cancer [43], thyroid carcinoma [44] and multiple sclerosis (MS) [45]. In this article, authors suggest gene therapy of atherosclerosis with IL-38 Gene in order to suppress inflammation involved in this disease. IL-38 can be introduced as a novel anti-inflammatory therapeutic cytokine which is the newest member of IL-1 family [26–28]. It is located on human chromosome 2q13-14.1. IL-38 connects to IL-36 receptor and acts as an IL-36Ra by preventing inflammatory signals like AP-1 and NF-kB [27,28,33]. So it can prevent inflammation involved in many stages of atheromatous plaque formation. Recently, gene therapy has been suggested as a novel strategy for treating many chronic inflammatory diseases [50]. Here, we designed a study using IL-38 in a mouse model using its anti-inflammatory effects for atherosclerosis cases. Meanwhile, further studies are suggested especially in clinical fields.

Conflicts of interest statements

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.02.048>.

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