Role of IL-18 and its signaling in atherosclerosis

Owais Mohammad Bhat, Veena Dhawan

Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh

Correspondence: Veena Dhawan
E-mail: veenad2001@yahoo.com
Received: March 11, 2015
Published online: April 22, 2015

Cardiovascular diseases (CVD) including coronary artery disease (CAD) and stroke are the largest cause of worldwide morbidity and mortality, where atherosclerosis is the underlying pathology. Recent investigations of atherosclerosis have focused on the role of inflammation, providing new insights into the mechanism of the disease. Macrophages and T-lymphocytes present in the atherosclerotic lesions produce a wide array of cytokines that can exert both pro- and anti-inflammatory effects. Pro-inflammatory cytokines of the interleukin category are considered to be key players in the chronic vascular inflammation that is typical for atherosclerosis. Various studies support the concept that interleukin-18 (IL-18) is a pro-inflammatory cytokine with pro-atherogenic properties. Previous data in Apo E-/- mice demonstrated that IL-18 accelerates atherosclerosis via interferon gamma (IFN-γ) and CXCL16 expression. IL-18 binds to its receptor IL-18R complex which is a heterodimer with α (IL-1Rrp) chain responsible for extracellular binding of IL-18 and a nonbinding, signal-transducing β (AcPL) chain. By binding to IL-18Rα, IL-18 upregulates IL-1R-associated kinase (IRAK) and TRAF-6 thus, results in nuclear translocation of nuclear factor kappa-B (NF-κB). Activated NF-κB is shown to be present in coronary arteries of pigs fed a hypercholesterolemic diet, in rat arterial smooth muscle cells after balloon injury and in unstable coronary atherectomies. Peroxisome proliferator-activated receptor-γ (PPAR-γ) and Liver-X-receptor-α (LXR-α) genes are involved in the lipid uptake and cholesterol efflux in macrophages and regulate the expression of many key genes that are involved in the development and progression of atherosclerosis e.g. cytokines, matrix metalloproteinases (MMPs) and scavenger receptors (CD36, SR-A, SR-B1). MMP-9/MMP-2 (members of gelatinase family) are shown to be one of the effector genes of these nuclear receptors (PPAR-γ and LXR-α). MMP-9 is specifically implicated in atherosclerosis, plaque instability and rupture during arterial lesion progression. Thus IL-18 can be strongly viewed as a proatherogenic and pro-inflammatory cytokine, as IL-18 signaling results in upregulation of various pro-inflammatory genes and development of atherosclerotic lesions, thus could be envisaged as a target for therapeutics.

Keywords: Atherosclerosis; inflammation; Recombinant IL-18; Apo E-/-; CD36; NF-κB

One of the original concepts for the pathogenesis of atherosclerosis was proposed by Virchow (1856), who suggested that inflammation plays a primary role in initiating the atherogenic process [4]. Any kind of injury to the vascular wall results in endothelial injury thereby, leading to endothelial dysfunction (Russell Ross, 1973) [5]. Due to endothelial dysfunction and presence of oxidative stress, excess lipids and lipoproteins are oxidized resulting in the formation of oxidized low density lipoprotein (Ox-LDL).

Atherosclerotic plaques are characterized by accumulation of lipids in the arterial wall together with the infiltration of immunocytes. Monocytic recruitment, egress of macrophage, and the balance among proliferation, survival and apoptosis in the arterial walls determines the degree of influx of inflammatory cells to atherosclerotic lesions [6]. The up-regulation of scavenger receptors in plaque-activated macrophages results in the uptake of modified lipoprotein particles (mLp), and transforms them into cholesterol-laden foam cells, characteristic of fatty-streak type lesion, that progressively may evolve to advanced fibro-lipid plaque and advanced lesions [7]. Therefore, understanding the principle of the inflammatory processes is crucial for deciphering the complex mechanism involved in atherosclerosis progression.

Cytokines and atherosclerosis

Atherosclerosis is not only a disorder of lipid metabolism, but is also considered as a chronic inflammatory disease [8]. Presence of inflammatory cells, pro- and anti-inflammatory cytokines and their receptors in the atheromatous plaque demonstrates a positive correlation with coronary artery disease and its consequences. A critical balance between pro-inflammatory and anti-inflammatory cytokines in atherosclerotic plaque is essential for the development and progression of the lesion. The pro-inflammatory cytokines secreted by type 1 CD4+ T-helper cells (TH1 cells) include interleukin-2 (IL-2), IL-12, IFN-γ, TNF-α and TNF-β that exacerbate the atherosclerotic process, whereas TH2 cytokines e.g. IL-4, IL-5, IL-10 and IL-13 are known to exert atheroprotective actions and can counteract TH1 cytokine activity [9-11]. Animal studies in mice have revealed the pivotal role of interleukins, the macrophage-associated cytokines and the colony stimulating factors in atherogenesis.

Interleukin-18

IL-18, a potent pro-inflammatory cytokine with pleotropic properties is a member of the IL-1 family of cytokines and was originally described as an IFN- γ inducing factor [12]. Produced constitutively in many different cell types [13, 14], Evidence shows that IL-18 is involved in T cell and natural killer cell maturation, and production of other inflammatory chemokines, cytokines, and cell adhesion molecules [15].

Interleukin-18 and cardiovascular disease

In two large studies carried out in 1111 randomly (Perth, Australia) selected community subjects and 2231 subjects (Dallas County, USA), elevated levels of IL-18 were found to be associated with carotid intima-media thickness in univariate analyses, but not after adjustment for traditional risk factors [16, 17]. Two large prospective studies, which included 10,600 healthy European men [18] and 253 healthy women [19] demonstrated that elevated IL-18 levels were associated with future cardiovascular disease.

Interleukin-18 and atherosclerosis

IL-18 is shown to be highly expressed in unstable atherosclerotic lesions, mainly in the plaque macrophages [20]. IL-18 exerts its pro-atherogenic effects through IFN-γ production that amplifies the inflammatory process leading to thinning of the fibrous cap formation causing rupture-prone plaques [21, 22]. Further, IL-18 is shown to indirectly cause plaque destabilization by increasing the expression of matrix metalloproteinases in macrophages and vascular cells [14, 21]. IL-18 overexpression is shown to enhance the collagenolytic activity of smooth muscle cells resulting in reduced intimal collagen and thickness of the fibrous cap, leading to vulnerable plaques in an Apo E−/− mice [23]. Increased mRNA expression of cardiac IL-18 and a subsequent reduction in myocardial contractility was reported in a mouse model with myocardial infarction [24]. We also observed significantly augmented IL-18 gene expression and circulating levels in the IL-18-treated Apo E−/− mice [25]. In an earlier study, IL-18 administration increased serum cholesterol and lipoprotein-cholesterol distribution in these mice.

Chronic systemic inflammation through IL-18 may give rise to dysfunctional or pro-inflammatory HDL and therefore may cause reverse cholesterol transport dysregulation leading to increased cholesterol levels. Increase in cholesterol levels could also be attributed to isoprenoid generation by endogenous cellular cholesterol synthesis as well as by cholesterol synthesis in activated monocytes during the inflammatory response. Isoprenoids are an integral component of the signaling pathway for IL-6-mediated inflammation and there is plenty of evidence in the literature demonstrating that IL-18 induces IL-6 production both in vitro and in vivo [26, 27]. Increased IL-6 production may induce C-reactive protein (CRP) that may also trigger cholesterol levels and in this regard statin like drugs are shown to lower LDL-C and non-HDL-C and thereby lower levels of CRP.
IL-18 Receptor and atherosclerosis

Upon binding of IL-18 to its receptor IL-18Rα, IL-18Rα is recruited to form a high-affinity complex-inducing signaling pathway shared with other IL-1R family members. Gerdes et al. [14], reported increased expression of functional IL-18 and IL-18 receptor on endothelial cells, SMCs and macrophages present in the human-atheroma, and induced IFN-γ expression in SMCs, thereby suggesting simpering inflammation during atherogenesis. The induction of IL-18Rα/β by IFN-γ in association with the IL-18-induced IFN-γ expression in the vasculature via IL-18 signaling suggests the presence of a positive feedback loop that might contribute to the dysregulated inflammatory response in atheroma [14].

In our study, a significantly augmented IL-18 Rα expression was observed on peripheral blood mononuclear cells and circulatory levels of IL-18 in IL-18-treated Apo E-/− mice as compared to the controls [25]. Our study also demonstrated that IL-18 induced IL-18 Rα expression via NF-κB, as a significant downregulation IL-18 and IL-18 Rα was observed using a specific NF-κB inhibitor i.e. pyrrolidine dithiocarbamate (PDTC) [25, 28]. Various in vitro studies reported that IL-18 binding to IL-18 Rα results in upregulation of IL-1R-associated kinase (IRAK) and TRAF-6 resulting in nuclear translocation of NF-κB and also activates p38 mitogen activated protein kinase (p38 MAPK) signaling [29-31].

Nuclear Factor-kappa B (NF-κB) and atherosclerosis

Activation of NF-κB signaling is initiated by extracellular stimuli which are recognized by the receptors and transmitted into the cell, where the adaptor signaling proteins initiate a signaling cascade culminating in IκB kinase (IKK) activation. IKK phosphorylates the inhibitory IκB subunit of the NF-κB-IκB complex in the cytoplasm that marks IκB for degradation by the proteasome and releases NF-κB from the inhibitory complex [32]. The freed NF-κB proteins are then translocated into the nucleus and binds to the promoter regions of various genes thereby activates their transcription.

Atherosclerosis and inflammatory diseases involve activation of NF-κB, which is now considered to be a major transcription factor and a master regulator regulating many functions of the vessel wall. Moreover, NF-κB activation is thought to lie downstream of many of the stimuli proposed to be involved in atherosclerosis, such as mLp, cytokines and infectious agents.

Activated NF-κB has been identified in situ in human atherosclerotic plaques and seems to be more prominent in acute complications of atherosclerosis, which includes acute coronary syndrome. Nuclear NF-κB binding activity has been found in peripheral blood mononuclear cells and myocardial biopsies of patients with unstable angina [33, 34]. Nuclear translocation of relA is reported to be higher in unstable coronary atherectomies, and is also present instable angina patients [35]. The majority of our knowledge is gathered from studies using animal models of atherosclerosis despite evidence in human atherosclerosis. Activated NF-κB was detected in coronary arteries of pigs fed a hypercholesterolemic diet and in arterial SMCs after balloon injury in a rat model [36, 37]. In our study, we also demonstrated a significant augmentation of NF-κB in IL-18-treated mice [25]. Further, a significant downregulation of IL-18-induced molecules like IL-18 Rα, CD36, MMP-9, NF-κB and upregulation of LXR-α was observed with PDTC demonstrating that IL-18 acts through NF-κB pathway. Significant neutralization of IL-18-induced signaling by PDTC as observed via blockage of IL-18 as well as NF-κB activation was found to be responsible for these observations. Also, PDTC displays robust hypolipidemic effects in our study [25]. PDTC is shown to attenuate plasma TG and VLDL-C and restore HDL-C levels in obese db/db mice via reduction in TNF-α and IL-6 levels, thus reduces inflammation [38]. Our findings are further supported by the finding of Jawien et al. [28], who observed that inhibition of NF-κB activity reduces atherosclerosis in Apo E/LDLR- double knockout (KO) mice model.

Bone marrow transfers from p50-deficient mice to LDL-R-deficient mice demonstrated an astonishing shift towards a different plaque phenotype, characterized by reduced number of foam cells, increased macrophages and T cells, and the appearance of B cells (traditionally not present at the lesion site besides reduction in atherosclerotic lesion size) [39]. These targeted gene deletion studies highlight that NF-κB is involved in atherogenesis in a far more complex manner than already predicted. In order to identify suitable therapeutic targets, that may likely to interfere with the whole pathway, future research is therefore needed to identify the key stimuli leading to the activation of NF-κB signaling.

Cholesterol efflux regulators

Expression of genes involved in lipid uptake and cholesterol efflux in macrophages is controlled by nuclear receptors i.e. peroxisome proliferator-activated receptors (PPARs) like PPAR-α, PPAR-δ, PPAR-γ and liver x receptor alpha (LXR-α), which are not only implicated in the modulation of macrophage inflammatory gene expression in vascular diseases, but are also involved in the cholesterol efflux via macrophages [40, 41].
PPAR-γ is shown to induce the expression of LXR-α and thereby stimulate ABCA1-dependent cholesterol efflux to apo A-I [42]. Alleva et al [43] reported that ligand-activated PPAR-γ complexes suppress murine macrophage production of the proinflammatory cytokines by binding critical transcription factors such as NF-κB, AP-1, STAT-1 and Ets (E26 transformation-specific), suggesting that these ligands use PPAR-γ-mediated suppressor pathway. A large number of inflammatory responses are shown to be negatively regulated by PPAR-γ agonists [44]. These nuclear receptors also regulate the expression of many genes involved in the production of pro-inflammatory mediators e.g. cell adhesion molecules, cytokines and scavenger receptors (CD36, SR-A, SR-B1) that control the native and modified LDL uptake that is an important step in the formation of foam cells [45]. The pathogenic role of Ox-LDL in atherosclerosis is shown to be largely dependent on CD36 expression.

Liver X receptor-α (LXR-α)

LXRs belong to the orphan nuclear receptor superfamily, first identified in the liver and hence their name [46]. LXR-α and LXR-β isoforms have already been well characterized. The latter is ubiquitously expressed [47], while the expression of the former is more restricted in the skeletal muscle, adipose tissue, spleen, kidney, intestine, lung and macrophages [48]. Intracellular cholesterol levels stimulate the production of LXR specific physiological ligands [45], such as 24(S), 25-epoxycholesterol, 24(S)-hydroxycholesterol, and 22(R)-hydroxycholesterol as the most abundant and potent oxysterols capable of activating LXRs in the cell [49]. Similar to PPARs, ligand-bound LXRs tend to form heterodimers with their obligate partner RXR, and the activated LXR-RXR heterodimers are capable of binding to specific DNA binding sites known as LXR response elements (LXREs) [46]. LXREs have been recognized in the promoter regions of several genes regulated by LXRs including ABCA1 [50], ABCG1 [51], PPAR-γ [52] and Apo E [53].

LXR agonists increase reverse cholesterol transport (RCT) from macrophages by increasing the expression of macrophage Apo E and cholesterol efflux transporters ABCA1 and ABCG1 [40]. Excessive accumulation of cholesterol within macrophages at the sites of atherosclerotic lesions converts them into foam cells and accounts for the major fraction of the cholesterol deposited in the lesions [54]. Thus, by stimulating reverse cholesterol transport, LXR reduces foam cell formation and cholesterol content in the lesion directly. LXRs and their agonists act as negative regulators of macrophage inflammatory gene expression. An in vitro study demonstrated that in response to bacterial infection or lipopolysaccharide (LPS), LXR agonists reduce the expression of inflammatory molecules such as, cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS) and interleukin-6 (IL-6) whereas, in vivo, these agonists decrease inflammation in a model of contact dermatitis and also inhibit inflammatory gene expression in the aortas of atherosclerotic mice. This study concludes that genes inhibited by LXR are well known targets of NF-κB, as iNOS and COX-2 were inhibited through antagonism of NF-κB [55].

In a previous study [25], we observed significant downregulation of LXR-α expression in IL-18-treated Apo E/- mice. Studies carried out in the literature using LXR knockouts and LXR agonists suggest that LXR-α is an important regulator of cholesterol efflux in macrophages [51]. All these reports outlined above support our observations of finding decreased LXR-α expression in IL-18-treated Apo E/- mice. Our observation that IL-18 attenuates LXR-α in a NF-κB dependent manner is the first report in the literature.

Scavenger Receptor CD36

CD36, a transmembrane glycoprotein, is one of the most critical scavenger receptors on macrophages that cross the membrane twice. CD36 is expressed on multiple cells and has several physiologic functions that include its role as a high-affinity receptor for specific oxidized phospholipids found within Ox-LDL [56]. An interesting aspect of CD36 biology is that its expression on macrophages is increased when the cells are exposed to Ox-LDL. Amongst the changes that occur in the lipid components of LDL, subsequent to oxidation, is the formation of oxidized fatty acids such as 9- and 13-hydroxy octadecadienoic acid (HODE). These oxidized fatty acids are ligands for the PPAR-γ which regulates expression of many genes, including CD36. Hence, Ox-LDL induces CD36 expression and further cellular uptake of Ox-LDL. This feed-forward loop, most likely accelerates foam cell formation in the arterial neointima. The pathogenic role of Ox-LDL in atherosclerosis is largely dependent on CD36. Studies employing macrophages from genetically altered mice lacking CD36 have demonstrated that the absence of CD36 expression was associated with a lack of foam cell formation in vitro when cells were treated with Ox-LDL. In contrast, formation of foam cells was observed after 12 to 24 hours in wild-type mice [57].

Though, we did not determine Ox-LDL levels, a significantly increased scavenger receptor (CD36) expression with IL-18 administration in our study could be due to raised Ox-LDL that may lead to foam cell formation and thus development and progression of atherosclerosis [25]. Inflammatory cytokines such as granulocyte macrophage colony–stimulating factor (GM-CSF) and macrophage colony–stimulating factor (M-CSF) upregulates
mRNA expression of CD36 in macrophages, and blocking CD36 expression or its downstream signaling is shown to inhibit Ox-LDL uptake and limit experimental atherosclerosis in mice [58, 59].

Further, studies have shown that the proatherogenic role of CD36 was likely mediated by CD36 on macrophages, since bone marrow transplantation from CD36-null mice into Apo E-null mice, had the same effect on atherosclerosis as seen in the Apo E/CD36-double-null mice [60].

Matrix Metalloproteinases (MMPs)

Macrophage-derived MMPs are involved in the thinning of the fibrous cap [61]. MMPs are a family of protease-activated enzymes that degrade extracellular matrix (ECM) proteins. Presently, 26 members of the MMP family have been identified in vertebrates out of which 23 have been found in humans [62, 63].

Among MMPs, MMP-2 and MMP-9 have stimulated great interests for their potential roles in plaque rupture [64]. Clinical and experimental studies clearly demonstrate that MMPs are directly involved in the atherosclerotic plaque destabilization and show that members of the MMP family have widely differing effects on atherogenesis. A prominent role of MMP-2/9 has been demonstrated in atherosclerosis, plaque instability as well as rupture during arterial lesion progression [62]. Galis et al [64] demonstrated that over-expression of activated MMPs can lead to the promotion of destabilization of atherosclerotic plaques. Gough et al [65] demonstrated that retroviral overexpression of an active form of MMP-9 in macrophages induces morphological appearances interpreted as plaque disruption.

MMP-9 is known to process a number of inflammatory chemokines (CXCL5, CXCL8) and cytokines (TNF-α, IL-1β) through the proteolysis involved in vascular remodeling and reorganization of ECM [66]. IL-18 is shown to induce MMPs in murine peritoneal macrophages and human monocytes [14, 67]. Another mechanism of lipid independent atherosclerotic plaque development due to IL-18 could be due to increased MMP-9 expression in an earlier study [25]. Our study revealed that atherosclerotic plaques were rich in inflammatory cells, smooth muscle cells and collagen, thus induction of MMP-9 via IL-18 may be a crucial link in the chain of events promoting plaque rupture, thrombosis and myocardial infarction [25]. Bond et al [68] reported that NF-κB activity was required for upregulation and production of MMP-9 in rabbit and human VSMCs. Both MMP-2 and MMP-9 were found to be significantly decreased with PDTC treatment in spontaneously hypertensive rats [69]. These studies support our findings that inhibition of NF-κB downregulates IL-18-induced pro-inflammatory genes.

Scientific evidence suggests that local overexpression of MMP-9 promotes intravascular thrombus formation through increased tissue factor expression and tissue factor-mediated activation of the coagulation cascade [70]. Studies using various genetic manipulations in animal models have also been used to determine which MMPs are relevant in the progression of atherosclerosis. Galis et al [71] used MMP-9 knockout mouse carotid artery model to demonstrate that MMP-9 deficiency lead to a decrease intimal hyperplasia, lumen loss and also caused accumulation of interstitial collagen. Inhibition of MMP-9 increases the mechanical stability of arteries by increasing their collagen content and decreasing lumen loss. Therefore, the MMPs may represent as potential prognostic markers as well as therapeutic targets for the prevention of atherosclerotic lesion development and progression.

Our study clearly demonstrated that exogenous administration of a pro-inflammatory cytokine such as IL-18 significantly upregulates CD36 and MMP-9 expression via NF-κB pathway and suggested that a significant crosstalk involved in the progression and destabilization of atherosclerotic plaques (Figure 1).

In conclusion, this review highlights IL-18 and NF-κB as important therapeutic targets for prevention of atherosclerotic plaque development and its complications. Our findings open up a new horizon for future research in human subjects to explore not only the molecular targets of IL-18 and NF-κB-mediated signaling, but also that these targets could be specifically manipulated for therapeutic benefits.
Acknowledgements

We thank Department of Biotechnology, New Delhi, India, for providing the financial support.

Conflicting interests

The authors declared that no competing interests exist.

References


56. Raffetto JD, Khailil RA. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem


