Interleukin-10 protects against atherosclerosis by modulating multiple atherogenic macrophage function

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Summary
Atherosclerosis is primarily a disorder of lipid metabolism, but there is also a prominent chronic inflammatory component that drives the atherosclerotic lesion progression in the artery wall. During hyperlipidaemic conditions, there is a rapid influx of circulating monocytes into the atherosclerosis-prone areas of the arterial intima. These infiltrated monocytes differentiate into macrophages and take up the atherogenic lipoproteins in the intima of the vessel wall that have been modified within the lesion environment. Interleukin (IL)-10 is a prototypic anti-inflammatory cytokine made primarily by the macrophages and Th2 subtype T lymphocytes. In terms of atherosclerosis its major roles include inhibition of macrophage activation as well as inhibition of matrix metalloproteinase, pro-inflammatory cytokines and cyclooxygenase-2 expression in lipid-loaded and activated macrophage foam cells. Recent discoveries suggest another important role of IL-10 in atherosclerosis: its ability to alter lipid metabolism in macrophages. The current review will highlight the present knowledge on multiple ways in which IL-10 mediates atherosclerosis. As macrophages play a critical role in all stages of atherosclerosis, the review will concentrate on how IL-10 regulates the activities of macrophages that are especially important in the development of atherosclerosis.

Introduction
Cardiovascular diseases, including coronary artery disease (CAD), stroke, abdominal aortic aneurysms, and many cases of heart failure collectively account for the largest rate of mortality in the Western world (1). Atherosclerosis is the common cause of all of these diseases, which is well known as a disorder of lipid metabolism but with a prominent inflammatory component as well. Persistent chronic inflammation fuels the atherosclerotic lesion progression in the artery wall throughout the different stages of the disease (2–5), from early fatty streak to advanced fibro-fatty plaque formation. During hyperlipidaemic conditions, there is a rapid influx of circulating monocytes into the atherosclerosis-prone areas of the arterial intima which differentiate into macrophages and take up the atherogenic cholesterol ester-rich lipoproteins in the intima of the vessel wall that have been modified within the lesion environment (6–8). The accumulation of cholesterol-loaded macrophages in the arterial wall called “foam cells” is a key feature of early atherosclerotic lesions (9). The importance of these foam cells is illustrated by their participation in every stage of atherosclerosis and their ability to trigger an acute thrombotic event (1). As the most numerous inflammatory cell type in the plaque, macrophages are the most important source of cytokine production in the lesion environment (10) and can produce pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-12, IL-15, IL-18, as well as anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGF-β). Many studies have shown that pro-inflammatory cytokines promote the development of atherosclerosis (4) whereas anti-inflammatory cytokines like TGF-β (11) and IL-10, as will be discussed in detail below, can have an anti-atherogenic effect.

IL-10 is a prototypic anti-inflammatory cytokine made primarily by the macrophages and T lymphocytes of the Th2 subtype. In terms of atherosclerosis its major roles include inhibition of macrophage activation as well as inhibition of matrix metalloproteinase (MMP), pro-inflammatory cytokines and cyclooxygenase-2 expression in lipid-loaded and activated macrophage foam cells (12). More recently, another important role of IL-10 in atherosclerosis has emerged by identifying its ability to alter lipid metabolism in macrophages. The current review will highlight the present knowledge on how IL-10 mediates atherosclerosis. As macrophages play a critical role in the pathogenesis of atherosclerosis, the review will concentrate on how IL-10 regulates the activities of macrophages that are important in the development of...
atherosclerosis. Identification of the mechanisms that regulate these responses could be invaluable in the development of new therapeutic approaches to prevent and/or treat atherosclerosis.

**IL-10 modulates multiple atherogenic macrophage functions**

IL-10 exerts its anti-atherogenic effects on plaque development throughout the different stages of atherosclerosis by influencing the local inflammatory process within the atherosclerotic lesion. IL-10 is produced within the atherosclerotic lesion predominantly by macrophages where it could play a significant role in the modulation of the local inflammatory reaction on both macrophages and T cells (13).

Macrophages play a central role during all stages of atherosclerosis (14). Atherogenesis is initiated with the recruitment of monocytes to the intima, followed by inflammatory activation which differentiates the recruited monocytes into macrophages. The intimal macrophages can then take up modified low-density lipoproteins (LDL) particles such as oxidised LDL (oxLDL) through upregulated scavenger receptors, thereby promoting cholesterol loading and foam cell formation in the plaque’s core. Lipid-loaded macrophages make multiple pro-inflammatory mediators, reactive oxygen species (ROS), and pro-coagulants that promote local inflammation as well as thrombotic complications.

An early study on IL-10 and atherosclerosis showed detection of IL-10 mRNA by RT-PCR in four of five human atherosclerotic tissues but not in plaque-free aortic specimens (15). The presence of IL-10 mRNA was subsequently verified in 12 of 17 advanced human atherosclerotic plaques, mainly in macrophages (13). These studies suggest that progressive inflammation during atherosclerosis may induce macrophages to express IL-10.

The mechanisms by which IL-10 may protect against atherogenesis can be categorised into five general aspects of macrophage function as follows: 1) anti-inflammatory properties, 2) inhibition of matrix metalloproteinases (MMPs) and tissue factor (TF), 3) anti-apoptotic feature, 4) effects on macrophage polarisation, and 5) modulation of lipid metabolism. IL-10’s role in each of these properties of macrophages is described in detail below.

**Anti-inflammatory properties of IL-10**

As a potent anti-inflammatory cytokine, increased IL-10 serum level is a beneficial prognostic determinant in patients with acute coronary syndromes (16). A number of studies have shown that IL-10 expression by plaque macrophages limits the inflammatory response and promotes plaque healing by inhibiting IL-12 (15) and iNOS production (13, 17). Attenuation of atherogenesis by IL-10 is attributed to its anti-inflammatory effects, most notably its ability to inhibit the release of several pro-inflammatory cytokines (including IL-1β, TNF-α, and IL-8) from monocyctic cells, and to induce the production of IL-1 receptor antagonist (18). As IL-10 receptor blockade in macrophages results in significantly higher nuclear factor (NF)-κB activation (19), IL-10’s ability to suppress the expression of inflammatory mediators such as TNF-α, MCP-1 (monocyte chemotactic protein 1) and ICAM-1 (intracellular adhesion molecule 1) is likely attributed to the inhibition of NF-κB activity (20). IL-10 also suppresses the production of the chemokine, KC/GRO-α (21) which is implicated in intimal macrophage accumulation and the progression of complex atherosclerotic lesions in advanced disease (22). Inhibition of both MCP-1 and ICAM-1 by IL-10 (23–25) is an important property of IL-10 that may inhibit monocyte influx into the plaque area and thereby curb the disease development. Recently, microRNA-155 has been shown to promote atherosclerosis by inhibiting the expression of BCL6 in macrophages (26). As IL-10 has been shown to suppress microRNA-155 (27), it is possible that one of the ways that IL-10 mediates its anti-atherogenic role is by inhibiting this microRNA in macrophages.

**Inhibition of matrix metalloproteinases and tissue factor**

Extracellular matrix content of a plaque is intimately associated with how vulnerable the plaque is to rupture. Clinically unstable atherosclerosis is associated with the activation of local inflammatory and immune cells with increased expression of MMPs (28) and TF (29) in the culprit plaque as well as increased systemic production of MMPs (30) and thrombin (31). Within atherosclerotic lesions macrophages are important sources of MMPs, including MMP-2, MMP-8, MMP-9, MMP-12, MMP-13, and MMP-14 (4, 32). MMPs influence lesion progression by degrading extracellular matrix proteins which can lead eventually to the development of unstable, rupture-prone atherosclerotic lesions (4, 33). However, it appears that not all MMPs promote unstable plaques and there are conflicting reports of their effects on plaque stability in the literature. While an early study suggests that remodelling of the neointimal extracellular matrix by MMP-1 is beneficial in the progression of lesions (34), other studies suggest that MMP-1 and MMP-9 contribute to the weakening of fibrous caps and plaque disruption leading to the destabilisation of atherosclerotic plaques (35, 36). Another study highlighting the widely differing effects of MMPs on atherogenesis shows MMP-3 and MMP-9 to play protective roles by limiting plaque growth and promoting a stable plaque phenotype, while MMP-12 supports lesion expansion and destabilisation, and MMP-7 does not have effect on plaque growth or stability (37).

Some studies suggest that IL-10 may have protective effects against plaque rupture and thrombus formation. IL-10 can inhibit the secretion of MMP9 (38, 39), the synthesis of TF (40), and the production of thrombin (41) from PBMC and macrophages. Low collagen synthesis and increased activity of macrophage-derived MMPs are responsible for fibrous cap thinning and fragility. Therefore, low levels of IL-10 may lead to augmented MMP activity which may in turn promote plaque instability and acute cardiovascular events in certain individuals (38). On the other hand, pro-inflammatory cytokines like interferon (IFN)-γ may destabilise plaques by inhibiting collagen production (42) in human vascular smooth muscle cells, and also by stimulating MMP production in...
macrophages (28) and modulating the fibrinolytic response of endothelial cells (43).

**Anti-apoptotic properties**

Macrophages and other cell types in the atherosclerotic plaque constantly undergo apoptosis and necrosis which may accelerate atherosclerosis by releasing lipids and inflammatory mediators from the macrophages to the plaque and thereby contributing to the formation of the necrotic core. IL-10’s anti-apoptotic properties have been reported in cultured macrophages (24, 25) and in T lymphocytes (44). The production of ROS is increased in atherosclerotic arteries (45), leading to endothelial damage, oxidation of lipid components (46), and recruitment of inflammatory cells to the site of injury. Inflammatory nitric oxide has apoptotic effects (47) and can induce cell death, at least in part through local peroxynitrite formation (48). IL-10 can also activate signal transducer and activator of transcription 3 (STAT3), which suppresses endoplasmic reticulum stress-induced apoptosis in lipid-laden macrophages by increasing the expression of anti-apoptotic genes like Bfl-1 and Mcl-1 (49, 50). As excessive accumulation of free cholesterol in cells causes apoptosis, one other way in which IL-10 may exert its anti-apoptotic effects is by stimulating ABCA1/ABCG1 production which increases cholesterol efflux from lipid-laden foam cells (24, 25, 51).

**Effects on macrophage polarisation**

When responding to certain stimuli such as inflammatory mediators or microbial products, macrophages have the ability to be polarised into one of two subtypes: classically activated M1 and alternatively activated M2 form (52). Macrophages are polarised into M1 subtype by IFN-γ, microbial stimuli (e.g. lipopolysaccharide) or cytokines such as TNF-α and GM-CSF, whereas M2 macrophages are induced by IL-4, IL-13, immune complexes, glucocorticoid or secosteroid (vitamin D3) hormones (53, 54). M1 macrophages express low levels of IL-10 and high levels of IL-12 and IL-23. By contrast, M2 macrophages express abundant IL-10 and low levels of IL-12 and IL-23. A recent publication shows that IL-10 induces macrophage polarisation toward the M2 phenotype (55).

M1 and M2 macrophages can both be detected in atherosclerotic lesions (56, 57). Anti-inflammatory M2 macrophages are more susceptible to foam cell formation than pro-inflammatory M1 macrophages, and exposure of macrophages to oxLDL renders M2 macrophages pro-inflammatory (58). Although it is not entirely clear at this point how macrophage polarisation affects atherosclerosis, there is an indication that M2 phenotype may exert an athero-protective action in experimental atherosclerosis (56). Although M2 macrophages dominate at the initial stages of atherosclerosis, macrophage phenotypic switch from M2 to M1 occurs with lesion progression (57). Furthermore, M1 macrophages dominate over M2 macrophages in the rupture-prone shoulder regions of the plaque, whereas M2 polarised cells are found in stable plaques (57). This concept is further supported by a recent finding that thioredoxin-1, an oxidative stress-limiting protein with anti-inflammatory and anti-apoptotic properties, promotes M2 macrophage polarisation and antagonises atherosclerosis (59). Another study suggests that helminth-derived antigens reprogram macrophages to M2 phenotype which reduces murine atherosclerosis (60).

**Modulation of lipid metabolism**

As macrophages accumulate lipid and become foam cells during atherosclerosis, their properties can change. OxLDL can promote immune activation by inducing pro-inflammatory cytokines IL-12 and TNF-α as well as IL-10 production by mononuclear leukocytes from human atherosclerotic plaque (61). Induction of IL-10 in lipid-laden macrophages may be an indication that IL-10 may be involved in lipid metabolism in these cells. During foam cell formation, two steps are critical in maintaining lipid homeostasis in macrophages: cholesterol uptake mediated by scavenger receptors, and cholesterol efflux mediated by ABCA1/ABCG1. Scavenger receptors such as CD36 and scavenger receptor A (SR-A) on macrophages mediate the uptake of modified lipoproteins from the vessel wall (62). On the other hand, reverse cholesterol transport via ABCA1 and ABCG1 is critical to export the cytotoxic cellular free cholesterol to lipid-poor apoA1 and apolipoprotein (HDL) particles (63). It is well documented that cholesterol efflux via ABCA1 and ABCG1 is essential to slow the development of atherosclerosis by decreasing lipid loading (64, 65). Although the role of scavenger receptors appears confusing because of conflicting results from gene knockout or transgenic mouse studies as reviewed by Hansson and Hermansson (66), several recent publications demonstrated that these receptors are protective against atherosclerosis due to their ability to remove modified LDL from the vessel wall (67, 68). There is a wealth of evidence that IL-10 can influence cellular lipid metabolism by facilitating both cholesterol uptake and cholesterol efflux (reverse cholesterol transport). In 2005, it was reported that IL-10 enhances oxLDL-induced formation of macrophage foam cells as well as inhibits apoptosis, albeit indirectly, by increasing the expression of anti-apoptotic genes Bfl-1 and Mcl-1 (49). Soon after, it was demonstrated that IL-10 not only stimulates ABCA1/ABCG1 function, aiding in cholesterol efflux from lipid-laden foam cells, but inhibits CD36-mediated oxLDL uptake by macrophages, both of which would lead to preventing foam cell formation (51). These seemingly conflicting findings were somewhat clarified by our study in 2009 in which we reported that IL-10 modulates lipid metabolism in macrophages by facilitating both cholesterol uptake and efflux (24). Our results showed that IL-10 can concomitantly up-regulate ABCA1 in a PPAR-γ-dependent mechanism and increase the expression of scavenger receptors (SR-A and CD36). In support of this another group reported that IL-10 stimulates the expression of scavenger receptors and enhances foam cell formation (69). Collectively, these findings support the hypothesis that enhanced cholesterol uptake mediated by IL-10 may be athero-protective by actively removing the highly atherogenic modified lipoproteins from the artery wall. At the
same time, IL-10-mediated increase in ABCA1-dependent cholesterol efflux is important for the efficient disposal of cytotoxic free cholesterol through reverse cholesterol transport. Interestingly, it was suggested recently that anti-inflammatory M2 macrophages but not pro-inflammatory M1 macrophages rapidly accumulate oxLDL (58). Because IL-10 is a known promoter of M2 macrophage polarization as mentioned above (55, 70, 71), it is likely that IL-10’s role in lipid accumulation is predominantly in M2 macrophages. Overall, there is a wealth of data to suggest a comprehensive anti-atherogenic role of IL-10 in macrophages, including its role in lipid homeostasis along with a more traditional role of IL-10 in inhibiting inflammatory molecules (e.g. TNF-α, iCAM-1, and MMP9) and reducing apoptosis (24, 25). A cartoon demonstrating the multi-faceted anti-atherogenic role of IL-10 in macrophages is shown in Figure 1.

Human studies and in vivo animal models

The role of IL-10 in atherosclerosis has been investigated using different animal models as listed in Table 1.

It was first described in 1996 that IL-10 was present in human atherosclerotic lesions and that ox-LDL induced IL-10 release from monocytes in vitro (15). Interestingly, the inhibition of IL-12 by IL-10 observed in this study suggests that the balance between IL-12 and IL-10 production likely contributes to the level of immune-mediated tissue injury in atherosclerosis. Activated monocytes produce IL-12 and IL-10 that regulate the Th1 and Th2 responses, respectively. IL-12 can act as a T cell growth factor that selectively induces the Th1 cytokine pattern. One of the roles of IL-10 is to inhibit the local production of IL-12 which may potentiate the chronic inflammatory Th1 cell and macrophage responses leading to tissue injury in atherosclerosis (15). The issue of athero-regulation by both IL-12 and IL-10 was further complicated by IL-12 being expressed at an earlier stage of atherosclerosis than IL-10 in apoE-/- mice (81). This suggests that IL-12 and IL-10 may have distinct roles in regulating the immune response during different stages of the disease.

The expression and potential effects of IL-10 in advanced human atherosclerotic lesions was reported in 1999 (13). Immunostaining from this study indicated that the main source of IL-10 in advanced human atherosclerotic plaques is the macrophage. The local anti-inflammatory response of IL-10 and its inhibitory effects on excessive cell death in the plaque was indirectly shown by the observation that IL-10 expression was associated with low levels of inducible nitric oxide synthase (iNOS) expression and cell death.

IL-10-deficient and IL-10-overexpressing murine models on either apoE-/- or LDLR-/- background have greatly advanced our understanding of how IL-10 might modulate atherogenesis. In 1999, two groups independently showed athero-protective properties of IL-10 (72, 73). Many other groups since then have utilised various animal models and IL-10 delivery systems to understand how IL-10 affects atherosclerosis. The first study involving athero-
genic diet-fed IL-10-deficient mice showed increased lipid accumulation, T-cell infiltration, IFN-γ expression, as well as decreased collagen in the lesion compared with wild-type mice (72). Intramuscular electroporations of IL-10 plasmid DNA resulted in a 60% reduction in lesion size in IL-10-deficient mice. Another group found that diet-induced atherosclerotic lesions were larger in IL-10−/− mice than in control mice (73). They also observed that transgenic murine IL-10 expression driven exclusively in T cells by human IL-2 promoter significantly attenuated atherosclerosis development (73).

In 2001, von der Thusen et al. reported that increased plasma concentrations of IL-10 as a result of adenoviral gene transfer in LDLR−/− mice led to reduced atheroerotic lesion size by inhibiting the production of TNF-α (25, 74). The mechanism involves inhibition of anti-inflammatory TNF-α production by IL-10 (25, 74). Concordantly, Pinderski et al. demonstrated that overexpression of IL-10 by activated T lymphocytes attenuated lesion formation by driving the shift to a Th2 phenotype with decreased IFN-γ production (by peripheral blood lymphocytes, splenocytes, and circulating monocytes) (75). Alteration of macrophage function was exhibited by markedly decreased apoptosis in macrophage foam cells within the lesions of IL-10 transgenic mice (75).

The athero-protective results obtained with IL-10-deficient mice on the C57BL/6J background (72, 73) were confirmed in LDLR−/− mice on the C57BL/6J background (72, 73). They also observed that transgenic murine IL-10 expression driven exclusively in T cells by human IL-2 promoter significantly attenuated atherosclerosis development (73).

In an attempt to utilise IL-10 as a therapeutic agent, several techniques have been used by different groups to deliver the IL-10 gene in vivo. One study showed that intramuscular gene transfer of IL-10 cDNA reduces atherosclerotic lesion formation in apoE−/− mice.

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mice (76). IL-10 gene transfer quelled the Th1 response by inhibiting IL-12 and IFN-γ expression in transgenic mice (76). These results were confirmed in another study in which adeno-associated virus vector-mediated IL-10 gene transfer via intramuscular injection inhibited atherosclerosis in apoE<sup>−/−</sup> mice (77) by lowering MCP-1 expression in both the vascular wall of the ascending aorta and serum. Similar results were observed in apoE<sup>−/−</sup> mice transplanted with bone marrow cells transduced by IL-10/lentivirus (80). In agreement with these results, a systemic delivery of adeno-associated virus type 2-hIL-10 inhibited atherogenesis in LDLR<sup>−/−</sup> mice by combating inflammation and oxidative stress (78). Similar effects of IL-10 overexpression on neointima formation were seen in the hypercholesterolaemic apoE<sup>3-Leiden</sup> mice as well (82).

Anti-atherosclerotic properties of IL-10 were further displayed in high fat diet-fed LDLR<sup>−/−</sup> mice in which IL-10 was overexpressed in macrophages by utilising a macrophage-specific retroviral vector that allows long-term in vivo expression of IL-10 in macrophages through transplantation of retrovirally transduced bone marrow cells (BMCs) (25). The IL-10 expressed by macrophages in the plaques derived from transduced BMCs inhibited atherosclerosis in these mice, at least in part by reducing inflammation and apoptosis in IL-10-overexpressing macrophages. These results are consistent with previous findings (24) and provided evidence that IL-10 production in macrophages is protective against atherosclerosis. Their results also highlight a novel therapeutic technique against atherosclerosis using an effective stem cell transduction system that allows prolonged production of IL-10 from macrophages.

It is worth emphasising that most strategies mentioned above had systemic effects on multiple cells including T cells, monocytes and endothelium resulting from overexpression of IL-10 in circulation. For example, overexpression of IL-10 in activated T lymphocytes inhibited monocyte activation and led to a shift to either Th2 phenotype (75) or Th1 phenotype (83). As a cytokine with diverse effects on most haematopoietic cell types, IL-10 can inhibit the activation and effector function of T cells, monocytes, and macrophages (84). In addition, IL-10 can regulate the growth and/or differentiation of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells (84). In particular, several studies support the concept that IL-10 exerts inhibitory effects on vascular smooth muscle cells (VSMC). There is evidence that athero-protective role of IL-10 is mediated in part by regulating vessel wall remodelling through inhibition of VSMC proliferation following vascular injury (85). IL-10 may play an essential role in the maintenance of normal vasculature, as IL-10 inhibits VSMC activation (86) and IL-10 deficiency results in vascular damage and remodelling (87). Interestingly, deficiency in CCR5 has been shown to protect against neointima formation by up-regulating IL-10 in the neointimal VSMC in atherosclerosis-prone mice (88).

Collectively, these studies indicate alterations in circulating IL-10 levels can influence the function of other immune cells which may in turn influence atherosclerosis. In the study by Han et al. there was no detectable IL-10 in circulating plasma at any time point during the atherogenic diet feeding whereas IL-10 was readily detected in IL-10-overexpressing macrophages in atherosclerotic lesions (25). This suggests that IL-10 was expressed in differentiated macrophages but not in circulating monocytes. Therefore, their technique of overexpressing IL-10 only in differentiated macrophages is useful to evaluate the unique role of locally-produced IL-10 in atherogenesis, and clearly shows that IL-10 acting in the vessel wall can decrease the development of atherosclerosis despite ongoing hyperlipidaemia.

Although most studies so far support the protective mechanism by IL-10, the exact role of IL-10 in attenuating atherosclerosis remains controversial, and is dependent on the animal model in question. For example, a recent study using a rabbit model showed that prolonged and stable expression of IL-10 in rabbit carotid arteries achieved with a helper-dependent adenoviral vector had neither an atheroprotective effect nor any effect on adhesion molecules or any other atherogenic cytokines (79). This study suggests that gene therapy involving IL-10 delivery may bring about different results in different species.

**Therapeutic considerations**

In light of the findings that systemic and intral visually delivery of IL-10 can be anti-atherogenic, it is tempting to speculate that IL-10 treatment may have the potential to be a novel therapeutic agent against atherosclerosis in the future. IL-10 expression after intramuscular DNA electroporation or other techniques leads to a persistent expression of this protective cytokine in circulation and in local lesion (25, 75, 89). It is likely that systemic delivery of IL-10 will result in suppression of immune response and increase the opportunity of infection, particularly involving intracellular pathogens such as *Chlamydia* and *Listeria* monocytogenes (18). Compared with systemic delivery of IL-10, local expression of IL-10 in atherosclerotic lesions may have much less impact on the general immune response. On the other hand, a robust local expression driven by retrovirus or adenovirus makes it difficult to regulate IL-10 expression in a temporally and spatially controllable manner as desired. Accordingly, the safety and effectiveness of exogenous IL-10 administration utilizing these techniques will need to be evaluated in the future before they are adopted in human patients for the treatment of atherosclerosis.

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**Conflicts of interest**

None declared.
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