How much is too much? Interleukin-6 and its signalling in atherosclerosis

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Summary
The importance of inflammation as a driver of pathology is no longer confined to autoimmune and infectious diseases. In line with convincing experimental data as well as abundant clinical findings the current view of atherosclerosis points to inflammation as a critical regulator of atherosclerotic plaque formation and progression leading to the fatal clinical endpoints myocardial infarction, stroke or sudden cardiac death. The underlying mechanisms have been a matter of intense research during the last decades. In this regard, the interleukin-6 (IL-6) cytokines and their signalling events have been shown to contribute to both, atherosclerotic plaque development and plaque destabilisation via a variety of mechanisms. These involve the release of other pro-inflammatory cytokines, oxidation of lipoproteins by phospholipases, stimulation of acute phase protein secretion, the release of prothrombotic mediators, and the activation of matrix metalloproteinases. Moreover, the formation of reactive oxygen species generated by vascular enzyme systems may play a critical role in the regulation of IL-6 indicating a cross talk between vasoactive substances i.e. angiotensin II or adrenaline and pro-inflammatory cytokines such as IL-6. In this review we will summarise and discuss the underlying molecular and cellular mechanisms how IL-6 as an early and central regulator of inflammation contributes to atherosclerosis and how this knowledge can be integrated into the clinical context.

Keywords
Cytokines, atherosclerosis, inflammation, signal transduction

Introduction
The importance of inflammation as a driver of pathology is no longer confined to autoimmune and infectious diseases. In line with convincing experimental data as well as abundant clinical findings the current view of atherosclerosis points to inflammation as a critical regulator of atherosclerotic plaque formation and progression leading to the fatal clinical endpoints myocardial infarction (MI), stroke or sudden cardiac death (1). Insight from histological, morphological and state-of-the-art imaging (i.e. MRI, angioscopy and intracoronary thermosensor analysis) studies demonstrated that these clinical events may be triggered by an extensive inflammatory reaction at the site of the plaque – leading to instability and plaque rupture – as well as vascular remodeling processes, both of which may finally result in a symptomatic narrowing of the vessel lumen (2–5).

The underlying mechanisms have been a matter of intense research during the last decades. In this regard, the interleukin-6 (IL-6) cytokines and their signalling events have been shown to contribute to both, atherosclerotic plaque development and plaque destabilisation via a variety of mechanisms. These involve the release of other pro-inflammatory cytokines, oxidation of lipoproteins by phospholipases, stimulation of acute phase protein (APP) secretion, the release of prothrombotic mediators, and the activation of matrix metalloproteinases (MMPs) (6). Moreover, the formation of reactive oxygen species (ROS) generated by vascular enzyme systems may play a critical role in the regulation of IL-6 indicating a cross talk between vasoactive substances i.e. angiotensin (ANG) II or adrenaline and pro-inflammatory cytokines such as IL-6 (7, 8). In this review we will summarise and discuss the underlying molecular and cellular mechanisms how IL-6 as an early and central regulator of inflammation contributes to atherosclerosis and how this knowledge can be integrated into the clinical context.

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Diversity of the IL-6/gp130 signalling

IL-6 is the eponym of a whole cytokine family which comprises molecules such as IL-11, IL-27, leukemia inhibitory factor (LIF), oncostatin M (OSM) and several other more (9). All these members of the IL-6 cytokine family require gp130 as a co-receptor to exert their biological functions. In this regard, the diversity of biological effects of the lead-cytokine IL-6 is somewhat surprising, since the specific IL-6 receptor (IL-6R) is only expressed on defined cell types like hepatocytes, monocytes and inactive T- and B-lymphocytes. Binding of IL-6 to the IL-6R on the cell surface leads to the recruitment and complexation of two gp130 molecules and subsequently to the activation of certain intracellular signal transduction pathways (10). This so-called classical IL-6 signalling plays a pivotal role in early immune responses and in the induction of APPs in hepatocytes. Besides the membrane-bound IL-6R, a soluble form (sIL-6R) can be generated by proteolytic cleavage by ADAM17 (11, 12) or alternative splicing (13). Accordingly, IL-6 can bind to sIL-6R to form an IL6/sIL-6R complex which subsequently binds to cell surface gp130 in order to initiate intracellular signalling cascades. Of interest, this also happens in cells which do not express an endogenous IL-6R. Consequently, cells which release the sIL-6R render all cells via ubiquitary expressed gp130 responsive towards IL-6. This mechanism has been termed IL-6 transsignalling and is thought to play a key role in the pathophysiology of chronic inflammatory disorders and different forms of cancer (14).

Intracellular dimerization of two gp130 proteins represents the initial step for all further downstream signalling pathways. This brings two members of the JAK family (JAK1,2,3 or TYK2) into close proximity allowing trans-phosphorylation. Subsequently, activated JAKs phosphorylate additional targets, including the gp130 receptor and other signalling components. Phosphorylation of the four distal tyrosines of gp130 is necessary for the activation of STAT, in the case of IL-6 mainly STAT3 and STAT1. STAT proteins act as latent transcription factors that linger in the cytoplasm until activated. JAK-dependent phosphorylation of STATs leads to their dimerization and nuclear translocation in order to bind to the promoter region of target genes (15, 16).

IL-6/gp130/STAT activation could be switched off by different mechanisms to prevent long-lasting activation and to damp up overshooting inflammatory processes. The most famous one is mediated by the suppressors of cytokine signalling (SOCS) protein family. Following activation, STATs stimulate SOCS gene transcription leading to SOCS protein expression which bind to gp130 or to phosphorylated JAKs to switch off the entire pathway (17).

Alternatively, JAK2-dependent phosphorylation of the second most membrane-proximal tyrosine residues of gp130 leads to the recruitment of the tyrosine phosphatase SHP-2 which links

Figure 1: Schematic cascades of the classical and the IL-6 transsignalling.
to the Ras/MAPK signalling pathway (15, 16). Finally, JAK-dependent phosphorylation of the tyrosine kinase Tec leads to the phosphorylation of PI-3 kinase subunits such as p85 and links gp130 to the PI-3/Akt signalling pathway (18, 19) (Fig. 1).

Multiple IL-6/gp130-dependent signalling pathways regulate the expression of target genes such as c-myc, junB, egr-1 and bcl-2 and could elicit both pro- and anti-inflammatory effects, depending on the environmental circumstances. The high diversity of the IL-6/gp130 signalling pathway may explain the pleiotropic nature of IL-6 that not only affects the immune system, but also acts in many physiological and pathophysiological processes in various organs.

**Diversity of the IL-6 effects**

**Cellular sources and responses**

IL-6 is a pleiotropic cytokine produced by numerous cell types and acting on an even more diverse population of cells and tissues (Fig. 2). In a healthy and quiescent organism IL-6 is expressed at low levels kept in check by a complex network that comprises glucocorticoids, catecholamines and secondary sex steroids. In response to infection, trauma and other stress conditions IL-6 gene expression especially in monocytes and macrophages is rapidly induced by viruses and bacterial endotoxins as well as inflammation-associated cytokines such as IL-1, tumour necrosis factor-α (TNF-α), platelet derived growth factor (PDGF) and interferons. The IL-6 promoter acts as a sophisticated biosensor for environmental stress and contains in its highly conserved control region most of the relevant binding motifs for transcription factors associated with inflammatory or proliferative states e.g. nuclear factor kappaB (NF-kB), C/EBPβ (CCAAT-enhancer-binding protein), activator protein-1 (AP-1) and corticosteroids (20–22). Furthermore, IL-6 is produced in the adipose tissue in response to adipocytokines linking obesity to the state of chronic low-level inflammation as a potential trigger for cardiovascular and metabolic diseases (23–25). On the other hand IL-6 is also rapidly secreted from the working skeletal muscle thereby ensuring the short-term energy supply of the muscle cells and contributing to the anti-inflammatory properties of temporary IL-6 secretion (26, 27).

IL-6 was originally identified as a factor that induces the synthesis of immunoglobulins (Ig) in activated B-cells but has now been found to exhibit a wide range of biological functions apart from the B-lymphocyte system (9). IL-6 induces differentiation of myeloid leukaemic cell lines into macrophages (28), megakaryocyte maturation (29), neural differentiation of PC12 cells (cell line derived from a rat pheochromocytoma) (30), and development of osteoclasts (31, 32). IL-6 acts as a growth factor for myeloma/plasmacytoma (33, 34), keratinocytes (35, 36), mesangial cells (37), renal cell carcinoma (38), as well as Kaposi sarcoma (39), and promotes the growth of haematopoietic stem cells (40, 41). In addition, IL-6 is also able to inhibit the growth of...
myeloid leukemic cell lines and certain carcinoma cell lines (42, 43).

During acute inflammation IL-6 expression is induced via NF-κB and triggers the hepatic acute phase reaction (APR) (44). Through the activation of endothelial cells with subsequently increased expression of adhesion molecules and secretion of chemotactic factors, IL-6 elicits the recruitment of neutrophils into the affected tissue (19, 45). Resident fibroblasts, part of the inflammatory milieu, produce MMPs upon IL-6 stimulation in order to degrade the extracellular matrix (46, 47). Furthermore, IL-6 orchestrates the temporal switch in the pattern of leukocyte recruitment from a predominately neutrophilic to mostly monocytic population thereby promoting the transition from the initial innate immune response to a more sustained, adaptive immune response (48).

Beside the differentiation of B-lymphocytes into immunoglobulin-producing plasma cells and the potential to induce their malignant transformation into myeloma cells (49), IL-6 also promotes migration and proliferation of T-lymphocytes (50–52). It is responsible for the induction of cytotoxic T-lymphocyte differentiation (53) and comprises a key regulatory signal in the development of Th17-cells while concomitantly blocking the differentiation of CD4+ cells into T-regulatory cells (54). In this way, IL-6 participates in the coordination of the innate – from which it comes initially – and the adaptive immune system.

Beyond its effects on resident endothelial cells IL-6 was proven to stimulate proliferation and migration of circulating endothelial progenitor cells (55) thereby underlining the pro-angiogenic character of this cytokine. Additionally, IL-6 is involved in the migration of smooth muscle cells (SMCs) by mediating the effects of vascular endothelial growth factor (VEGF) and TNF-α (7, 56, 57). Moreover, it can also stimulate the proliferation of SMCs in a PDGF-dependent as well as PDGF-independent manner (58–60).

Of note, recent studies challenged the general held view that IL-6 is solely able to mediate the detrimental effects of acute and chronic inflammatory diseases. Particularly, the short-term secretion of IL-6 by the skeletal muscle in response to exercise is supposed to dam up inflammation through the induction of IL-10, soluble TNF-α-receptors and IL-1 receptor antagonists (26).

The renin-angiotensin system

The role of the renin-angiotensin-system (RAS) as an important factor in blood pressure regulation was first suggested over a century ago, when renin was isolated from the kidney. During the following years, Goldblatt et al. demonstrated the induction of hypertension by renal artery occlusion and some time later on, renin was found to be responsible for this observation (61). ANG II is a potent vasoconstrictor and triggers the release of aldosterone, which leads to sodium and water retention in the kidney (62). Thus, acute ANG II release prevents fatal hypotension. Chronic activation of the RAS may result in inadequately and constantly enhanced blood pressure as well as volume overload of the vasculature, leading to pathological mechanical vascular wall stress (63).

The activation of the tissue-specific RAS as well as increases in the systemic RAS enhances the vascular production of ROS (64, 65). Of interest, clinical and experimental evidence indicate, that an increased ROS production plays a critical role in the development of hypertension (66). Seemingly, ANG II influences blood pressure via various mechanisms. Beyond blood pressure regulation, ANG II effects inflammation, remodelling and thrombosis within the vessel wall by induction of pro-inflammatory cytokines like IL-6 or TNF-α (67–69), chemokines and growth factors in SMCs (70–72). This is further aggravated by exhibiting profibrogenic actions through the induction of MMPs and PAI-1 (73–76).

The induction of the pro-inflammatory cytokine IL-6 is regulated by several pathways. In the case of ANG II-dependent IL-6 induction and release we and other could demonstrate, that this process mainly depends on the activation of the non-phagocyte NAD(P)H-oxidase – the major source of ROS within the vascular wall – since blockade of this system abolishes IL-6 transcription and release (8, 69, 77). In this scenario, it was shown that the ANG II-mediated release of IL-6 induces ANG II Type 1 (AT1) receptor expression, leading to increased oxidative stress which further augments the IL-6 production (78). This causes a vicious circle driven by ANG II and further promoted by IL-6 with detrimental vascular effects like increased vasoconstriction, enhanced oxidative stress, inflammatory processes and endothelial dysfunction.

The involvement of IL-6 in ANG II-induced endothelial dysfunction, vascular hypertrophy (79), and blood pressure (80) was previously demonstrated in mice deficient for IL-6. More mechanistically, Coles et al. extended the observation of the ANG II-induced IL-6 dependent hypertension by using an inhibitor of the IL-6 transsignalling, and observed that blockade of the IL-6 transsignalling reduced ANG II-induced blood pressure elevation in wild-type mice. By contrast, the ANG II-induced IL-6-dependent cardiac and aortic hypertrophy was unaffected by blocking the IL-6 transsignalling (80). Thus, differential inhibition of IL-6 transsignalling or classical IL-6 signalling seems to be a potential target for the therapy of hypertension or cardiac hypertrophy respectively.

The acute-phase reaction

The first reaction of the body to immunological stress is the innate, non-specific APR. The APR is a prominent systemic, liver derived reaction of the organism to tissue damage or infection. This first line of defence is important as it provides the body with the time to activate the adaptive immune response.

At the site of infection or injury, a number of responses of the tissue as well as local inflammatory cells are initiated, leading to the release of pro-inflammatory cytokines (e.g. IL-6, IL-1, IL-8 and TNF-α) that are involved in the regulation of local inflammatory reactions, but also, upon release into the circulation, exert systemic effects via the APR (81).

Cytokines such as TNF-α, IL-1 or IL-6 play a key role in the hepatic APR (82), whereas IL-6 is the major mediator for the hepatocytic secretion of most of the APPs (83, 84). Upon binding to the membrane-bound IL-6 receptor on hepatocytes and dimerisation of gp130 intracellular signalling cascades lead to the de novo synthesis of APPs within hours. This includes in human the major factors C-reactive protein (CRP) and serum amyloid A (SAA), which are increased up to 100-fold during in-
flammation, but also angiotensinogen, fibrinogen, haptoglobin and complement components (85).

On the one hand CRP and SAA are strong and consistent markers associated with cardiovascular events, i.e. MI, stroke, peripheral artery disease, and sudden cardiac death in healthy patients as well as in patients with acute coronary syndrome (86, 87). On the other hand both APPs have also intrinsic biological properties, such as activating the complement cascade, mediating phagocytosis, and regulating the inflammatory response (88). Moreover, they are involved in vascular disease initiation and progression and persistence of the APR over a longer period might have negative clinical consequences (89). CRP for instance promotes adhesion and chemotaxis of monocytes via increased production of monocyte chemotactic protein-1 (MCP-1) and expression of various cell adhesion molecules in endothelial cells (90, 91). Recently, we could demonstrate that the hepatic APR is crucially involved in atherosclerotic plaque development and macrophage recruitment within the atherosclerotic lesion of atherosclerosis-prone ApoE-deficient mice. To translate these findings into clinical relevance, we tested in two independent populations whether genetic variations in the human homologue of gp130 (IL6ST) influence atherosclerosis. In line with our experimental data from the animal study we could obtain significant evidence for an association of a genetic variation within the human IL6ST gene and atherosclerosis (92).

IL-6 in experimental atherosclerosis

Atherosclerosis is an inflammatory disease, and studies in the last decades have clearly shown that atherogenesis comprises more than the passive accumulation of lipids within the vascular wall. This process is initiated by an endothelial dysfunction driven by factors like elevated and modified lipoproteins, free radicals, smoking, diabetes mellitus or hypertension. The inflammatory response of the endothelium disturbs its physiological integrity and permits the influx of low-density lipoproteins (LDL) and monocytes as well as T-lymphocytes. LDL is not only one major cause of endothelial injury but upon oxidative or enzymatic modification also promotes the inflammatory reaction in the evolving atherosclerotic plaque. Modified LDL aggravates the vascular injury by further attracting inflammatory cells into the lesion and stimulates the release of pro-inflammatory cytokines. Prolonged inflammation stimulates migration and proliferation of SMCs which together with the accumulating macrophages and lymphocytes further release cytokines, chemokines, growth factors and degrading enzymes. This leads finally to a perpetuated inflammatory response and a destructive remodeling of the vessel structure with the formation of complex atherosclerotic lesions (93–95).

Since the concept of atherosclerosis as a chronic inflammatory disease was widely accepted, many researchers focused on the identification of potential mediators which initiate and maintain this vascular disease (94). In this context, IL-6 as one of the most prominent pro-inflammatory cytokines represented an interesting target and its involvement in athrogenic processes has been extensively studied.

As described above, IL-6 – either generated locally by cells of the atherosclerotic lesion or released into the circulation e.g. by the adipose tissue (96–99) – can exert several detrimental effects that augment atherogenesis. IL-6 promotes endothelial dysfunctions, SMC proliferation and migration as well as recruitment and activation of inflammatory cells, thereby perpetuating vascular inflammation. Additionally, it was previously demonstrated that IL-6 affects locally the expression of the scavenger receptors SR-A and CD36 – involved in the uptake of modified LDL – and thus promotes the formation of macrophage-derived foam cells, as a hallmark of early and advanced atherosclerotic lesion formation (78, 100–102).

Despite the conclusive in-vitro and in-vivo findings clearly indicating a pro-atherogenic role of IL-6, results of experimental atherosclerosis studies remained rather controversial so far. Treatment with recombinant IL-6 exacerbated atherosclerosis of atherosclerosis-prone ApoE-deficient mice which was accompanied by increased levels of other pro-inflammatory cytokines and APPs (103). This is in line with our own experiments studying atherosclerotic plaque formation in ApoE-deficient mice without hepatic gp130 expression. These mice lack completely the IL-6-mediated APR and hence display a greatly diminished atherosclerosis (92). Controversially, we and others could also identify an atheroprotective role of IL-6 since a systemic IL-6 deficiency in mice did not inhibit plaque formation as primarily expected but lead to an even more pronounced atherosclerosis (104, 105). Obviously, an overall lifetime IL-6-depletion did not protect the organism to suffer from this inflammatory vascular disease. Although initially confusing, these observations provide an even more profound insight into the physiological and pathophysiological actions of IL-6. One has to take into consideration that IL-6 is not only a major pro-inflammatory cytokine but also an important anti-inflammatory mediator which is required for the control of inflammatory responses (106).

In this context it is also important to take the diverse metabolic effects of IL-6 into account. Although elevated IL-6 plasma levels in humans are associated with obesity and insulin resistance, IL-6 knockout mice display controversially mature-onset obesity accompanied by hypertriglyceridemia and glucose intolerance, too (107–109). Supporting evidence comes from experiments demonstrating that short-term IL-6 application stimulates mobilisation and utilisation of fatty acids and increases the glucose metabolism in skeletal muscle (110). Furthermore, treatment with anti-IL-6 receptor antibodies in patients with Castleman disease or rheumatoid arthritis led to increased cholesterol and triglyceride levels (111–113). On the other hand, IL-6 administration mimicking the levels present in obesity have been shown to induce hyperlipidemia, hyperglycemia and insulin resistance emphasising the different metabolic effects of IL-6 dependent on dose and duration (114).

Taken together, current studies indicate that IL-6 levels in a physiological range are necessary to keep inflammatory responses in check as well as for the regulation of glucose and lipid metabolism. Hence, a full disruption of all IL-6 properties, affecting both the classical signalling via membrane-bound IL-6 receptor and transsignalling via the soluble IL-6 receptor might not represent a reasonable approach for the therapy of cardiovascular diseases. In contrast, first promising in-vivo experiments of our own group investigating the impact of a long-term IL-6 transsignalling inhibition in mice revealed no unfavorable meta-
bolic effects. Thus, it is tempting to speculate that similar to the pathophysiology of many other chronic inflammatory diseases the detrimental vascular effects of IL-6 are mainly mediated through the IL-6 transsignalling (115). Therefore, a selective interference with the IL-6 transsignalling could overcome the adverse effects observed in IL-6 knockout mice as well as under the treatment with anti-IL-6 receptor antibodies while preserving the capacity to attenuate the deleterious pro-inflammatory effects of IL-6.

IL-6 as target for future treatment regimes: Potential clinical implication

The clinical endpoints of atherosclerosis myocardial infarction, sudden death or stroke remain one of the leading causes of death in the Western world. Therefore, it seems of the utmost interest to be able to identify persons at risk for future cardiovascular events. In this regard, elevated levels of IL-6 have been shown to be associated with an increased risk for myocardial infarction in healthy male subjects (116). In addition, IL-6 may predict not only mortality in patients with unstable coronary artery disease (CAD) but also identify candidates which benefit from early invasive treatment strategy (117, 118). Of note, elevated pre-operative IL-6 levels are also a marker for early graft occlusions in patients undergoing bypass surgery (119). Furthermore, some studies revealed a role for IL-6 in the risk assessment of patient populations, which have been proven difficult to identify and treat successfully when suffering from CAD, e.g. old people and women. However, in a society with a growing population of elderly and old people which are often excluded from clinical studies based on co-morbidities but represent a large part of our patients today, IL-6 may be a more accurate predictor of future cardiovascular events and overall mortality in the elderly than other risk assessments, e.g. The Framingham or PROCAM risk score (120, 121). Thus, one might consider the addition of IL-6 to these established risk scores when dealing with older patients.

Beside the elderly population, IL-6, as demonstrated in the Women’s Health Initiative Observational Study may also represent an attractive target for the identification of women at risk for cardiovascular events especially when women are subjected to hormone replacement therapy (122). The definition of successful drug therapies in atherosclerosis-based diseases also remains unclear as blood pressure reduction or LDL-cholesterol lowering alone may not be sufficient to assess the individual state of inflammation. However, this kind of information could lead to a more individualised therapy and thereby result in improved outcome. Although reduction of IL-6 can be received by RAS-inhibitors, such as AT1-receptor antagonists and angiotensin converting enzyme (ACE)-inhibitors as well as with statins the functional relevance of these findings is unclear (123–125). Blockade of the IL-6 receptor as currently under investigation in the treatment of other chronic inflammatory diseases, such as rheumatoid arthritis, could be a first step towards clarifying the impact of this cytokine on the clinical manifestations of atherosclerosis (126). However, due to a probably unfavourable effect of this approach on lipoprotein fractions, usage of an anti IL-6 receptor antibody in patient with coronary artery disease has to be very carefully evaluated.

Conclusion

Taken together, IL-6 as a major player in the inflammatory scenario is not only produced during infection and trauma but has also a crucial relevance in the course of atherosclerosis. Upon induction by vasoactive peptides, ROS and other cytokines, IL-6 is expressed and released from a variety of cells including monocytes and macrophages as well as resident cells of the affected vasculature. IL-6 drives this chronic inflammatory process systemically via the APR and locally in the atherosclerotic vessel wall. In this context it is of particular interest that, similar to other chronic inflammatory diseases like rheumatoid arthritis or Crohn’s disease, the detrimental pro-inflammatory effects might be predominantly mediated by the IL-6 transsignalling (115, 127). The importance of this notion is further strengthened by the finding that a systemic IL-6 deficiency leads to adverse effects in the regulation of inflammation and lipid metabolism with an even accelerated atherosclerosis. This points to the anti-inflammatory mode of action of IL-6 and its critical requirement in controlling the extent of the local and systemic inflammatory response as well as its role in maintaining the metabolic homeostasis. Therefore, a specific inhibition of the IL-6 transsignalling rather than a general IL-6-inhibition seems to be a promising innovative therapeutic approach to fight against atherosclerosis as the major cardiovascular disease.

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References


