

REVIEW

IL-6 in diabetes and cardiovascular complications

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IL-6 is a pleiotropic cytokine that participates in normal functions of the immune system, haematopoiesis, metabolism, as well as in the pathogenesis of metabolic and cardiovascular diseases. Both pro- and anti-inflammatory roles of IL-6 have been described, which are distinguished by different cascades of signalling transduction, namely classic and trans-signalling. The present review summarizes the basic principles of IL-6 signalling and discusses its roles in diabetes and associated cardiovascular complications, with emphasis on the different outcomes mediated by the two modes of IL-6 signalling and the value of developing therapeutic strategies to specifically target the deleterious trans-signalling of IL-6.

Abbreviations

ADAM10, disintegrin and metalloprotease-10; ADAM17, disintegrin and metalloprotease-17; GLP-1, glucagon-like peptide-1; gp130, glycoprotein 130; IL-6R, IL-6 α -receptor; sgp130, soluble glycoprotein 130; sIL-6R, soluble IL-6 α -receptor; TACE, TNF- α converting enzyme; T2DM, type 2 diabetes mellitus; VSMC, vascular smooth muscle cell

Introduction

Investigations into the versatile biological activities of IL-6 expanded two and half decades ago. The function of IL-6 was first considered to be exerted in the acute phase of the inflammatory response but this cytokine was later found to be active in haematopoiesis, production of monoclonal antibodies by B-cells, development of cytotoxic T-cell and the transition from innate to acquired immunity (Van Snick, 1990). The role of IL-6 is not restricted to the immune system, as it is also involved in neuronal differentiation and regeneration, liver regeneration and regulation of metabolic process. Although IL-6 is mostly regarded as a pro-inflammatory cytokine that promotes inflammation under various pathological conditions, its anti-inflammatory and regenerative properties have been increasingly recognized (Scheller *et al.*, 2011). On the contrary, a significant elevation of plasma IL-6 level has been characterized as a marker for metabolic disorder and cardiovascular disease. However, the exact *in vivo* pathophysiological significance of IL-6 remains unsolved. This article reviews the complexity of IL-6 action in the pathogenesis and devel-

opment of type 2 diabetes mellitus (T2DM) and associated cardiovascular complications, hoping to shed light on the potential therapeutic strategies targeting IL-6 signalling in combating metabolic vascular diseases.

Classic signalling of IL-6

IL-6 is normally not expressed constitutively, but its expression is extensively induced by a spectrum of stimuli such as viral and bacterial infection, pro-inflammatory cytokines (TNF- α and IL-1), angiotensin II, oxidative stress and physical exercise. The circulating concentration of IL-6 in humans ranges from approximately 1 pg·mL⁻¹ in healthy individuals, with a several-fold surge in chronic inflammation, a hundred-fold after physical exercise, to fatal levels of greater than 1 μ g·mL⁻¹ in sepsis (Hack *et al.*, 1989; Waage *et al.*, 1989; Ostrowski *et al.*, 1998; Ruotsalainen *et al.*, 2010).

The classic IL-6 signalling is initiated by the binding of IL-6 to the membrane-bound IL-6 α -receptor (IL-6R), which exhibits a low-binding affinity and does not possess an

intrinsic kinase activity (receptor nomenclature follows Alexander *et al.*, 2013). This interaction of IL-6 with IL-6R recruits another membrane component, the glycoprotein 130 (gp130) to form a high-affinity and signalling-competent assembly of the three molecules (illustrated in Figure 1, left) (Boulanger *et al.*, 2003). IL-6R is expressed only by a few types of cells, mainly hepatocytes, pancreatic islet cells, macrophages, neutrophils and certain subtypes of T-cells (Doganci *et al.*, 2005; Ellingsgaard *et al.*, 2008; Waetzig and Rose-John, 2012). The glycoprotein gp130 is a ubiquitously expressed signal transducer shared by all members of the IL-6 family, including IL-11 leukaemia inhibitory factor, ciliary neurotrophic factor, oncostatin-M and cardiotrophin-1 (Taga and Kishimoto, 1997). Formation of the IL-6/IL-6R/gp130 complex induces phosphorylation of JAKs in the cytoplasmic domains of gp130, and subsequent phosphorylation of docking sites for the transcription factor STAT. STAT3 and, to a lesser extent, STAT1, are recruited to gp130, phosphorylated and, in turn, translocated to the nucleus and then bind to DNA of target genes including acute phase proteins in liver, 'suppressor of cytokine signalling' proteins that negatively regulate JAK/STAT signalling, and a variety of other genes (illustrated in Figure 1, middle) (Heinrich *et al.*, 1998). In addition to JAK/STAT activation, the SRC homology domain 2-containing tyrosine phosphatase 2 (SHP-2) is recruited to its binding site on gp130 and phosphorylated to initiate the MAPK cascade (Heinrich *et al.*, 2003; McFarland-Mancini *et al.*, 2010). Moreover, IL-6 can activate the PI3K/Akt pathway in hepatocytes, cardiomyocyte and certain cancer cell lines, where it confers protective effects against cell apoptosis (Hideshima *et al.*, 2001; Zhang *et al.*, 2003; Smart *et al.*, 2006; Chou *et al.*, 2013).

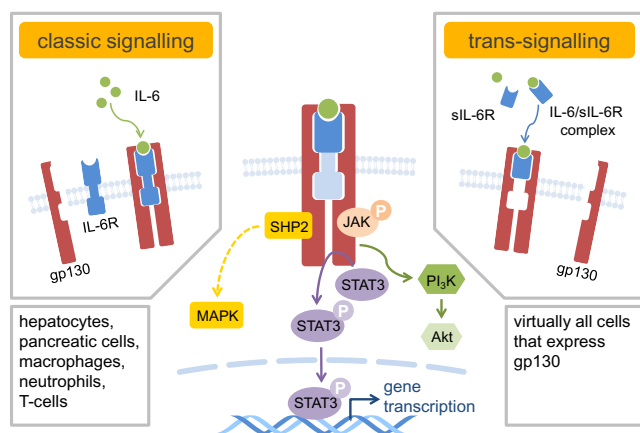


Figure 1

The intracellular IL-6 signal transduction is initiated by dimerization of gp130 through binding of IL-6/IL-6R (classic signalling, left panel) or IL-6/sIL-6R (trans-signalling, right panel) to gp130. Phosphorylation of tyrosine residues in gp130 upon dimerization leads to activation of three gp130 signalling pathways: JAK/STAT pathway (middle), SHP2/MAPK pathway (left) and PI₃K/Akt pathway (right). SHP2, SRC homology domain 2-containing tyrosine phosphatase 2; PI3K, phosphatidylinositol 3-kinase.

Trans-signalling of IL-6

IL-6R also exists in a soluble form (sIL-6R) lacking the trans-membrane and cytoplasmic domains, produced by either shedding of membrane-bound IL-6R or alternative splicing of IL-6 mRNA, with hepatocytes and immune cells as major sources (Peters *et al.*, 1998). IL-6 binds to sIL-6R with affinity similar to that of the membrane-bound IL-6R and initiates subsequent signalling via engagement with gp130 (illustrated in Figure 1, right) (Rose-John and Heinrich, 1994). Therefore, the IL-6/sIL-6R complex acts as an agonist of gp130-mediated IL-6 signalling. This process, called trans-signalling, expands the spectrum of potential targets of IL-6 to virtually any cell type, because of the ubiquitous expression of gp130.

A further complication is that gp130 also naturally occurs in a soluble form. This can associate with the IL-6/sIL-6R complex and leads to inhibition of IL-6 trans-signalling, without affecting the classic signalling mode (Narazaki *et al.*, 1993; Garbers *et al.*, 2011b). Soluble gp130 (sgp130) is generated by alternative splicing and is detectable in the circulation of healthy individuals at 100–400 ng·mL⁻¹, buffering the systemic response to circulating IL-6 (Narazaki *et al.*, 1993; Jostock *et al.*, 2001).

The anti-inflammatory and regenerative activities of IL-6 are generally mediated by the classic signalling mode, whereas pro-inflammatory responses in many pathological conditions involve trans-signalling (Rabe *et al.*, 2008; Rose-John, 2012). Tocilizumab, a humanized IL-6R-specific mouse antibody that blocks both membrane-bound and soluble IL-6R, is the only drug approved, so far, that targets IL-6 signalling and has been shown to be effective in treating rheumatoid arthritis, a condition where increased IL-6 levels are correlated with disease progression (Ash and Emery, 2012). The major side effects include the increased risk of infections, mild increase in lipid, liver malfunction and body weight gain (Tanaka *et al.*, 2012). Given the dys-regulation of IL-6 production in the pathogenesis of autoimmune, chronic inflammatory and even malignant diseases, blocking IL-6R or antagonizing IL-6 is therefore considered to be a rational therapeutic strategy. In addition to the proven efficacy and safety in treating rheumatoid arthritis, tocilizumab and several anti-IL-6R antibodies or IL-6 antagonists still waiting for approval are in clinical trials for the treatment of Castleman's disease, ankylosing spondylitis, diabetes mellitus and are being assessed for their benefits in reducing cardiovascular risks in patients with rheumatoid arthritis (Tanaka *et al.*, 2012; Waetzig and Rose-John, 2012).

During the past decade, there has been increasing interest in understanding the IL-6 trans-signalling pathway because this pathway could be a target for therapeutic intervention to inhibit inflammation. Recent animal studies showed that selective inhibition of IL-6 trans-signalling by sgp130Fc, a recombinant version of sgp130, did ameliorate IL-6-driven inflammation in various diseases, with the same or even higher therapeutic efficacy and better side effect profile than global blockade of IL-6 signalling (Waetzig and Rose-John, 2012). In addition, high levels of the IL-6/sIL-6R complex in synovial fluid has been reported in patients with rheumatoid arthritis, which is responsible for joint destruction (Kotake *et al.*, 1996), indicating the potentially important contribution of the inhibition of IL-6 trans-signalling to the clinical

efficacy of tocilizumab. Due to the success of tocilizumab and the promise of selective inhibition of trans-signalling, studies are now increasingly being redirected to develop drugs that specifically inhibit IL-6 trans-signalling, without affecting the classic signalling pathway.

IL-6 in obesity and diabetes

Low-grade chronic inflammation in obesity, reflected by a two- to threefold increase in the systemic level of cytokines including IL-6, appears to precede and is a risk factor of the subsequent development of insulin resistance and T2DM (Spranger *et al.*, 2003; Wang *et al.*, 2013; Lowe *et al.*, 2014). T2DM is a metabolic disorder characterized by hyperglycaemia due to the failure of pancreatic beta-cells to compensate for peripheral insulin resistance (Pradhan *et al.*, 2001). Consequently, hyperglycaemia, dyslipidaemia and chronic inflammation jointly precipitate cardiovascular complications including atherosclerosis, coronary artery disease, stroke and peripheral arterial disease, which are the major causes of morbidity and mortality in T2DM (Haffner *et al.*, 1998; Grundy *et al.*, 1999). IL-6 has been identified as an independent predictor of T2DM and associated cardiovascular events (Spranger *et al.*, 2003; Lowe *et al.*, 2014). Adipocytes and macrophages residing in adipose tissue are the major sources for the elevated plasma IL-6 concentration up to 2–3 pg·mL⁻¹ in patients with obesity and T2DM (Pradhan

et al., 2001; Spranger *et al.*, 2003). Nevertheless, the existing evidence is not enough to establish a causal association between IL-6 levels and the progression to metabolic and cardiovascular disorders. Due to its pleiotropic actions in various tissues and organs, the exact role of IL-6 in the pathogenesis of diabetes must be examined carefully in a cell- and tissue-specific manner, but allowing for the possibility of crosstalk between affected tissues and organs.

IL-6 classic signalling regulates metabolism

IL-6, considered as a pro-inflammatory cytokine and a predictor of T2DM, was originally considered to mediate adverse metabolic effects, contributing to insulin resistance and deteriorating glucose homeostasis (Bastard *et al.*, 2000). However, when a large body of earlier studies is reviewed, the role of IL-6 in insulin resistance still remains controversial. Hepatocytes, skeletal muscle cells and adipocytes, the main cell types involved in the regulation of peripheral insulin sensitivity and glucose homeostasis, respond differently to IL-6. The ability of IL-6 to reduce insulin sensitivity in hepatocytes by interfering with insulin signalling is supported by strong experimental evidence (illustrated in Figure 2, left), whereas the results on adipocytes and skeletal muscle cells are not always consistent (Kristiansen and Mandrup-Poulsen, 2005). The higher responsiveness of hepatocytes, compared with

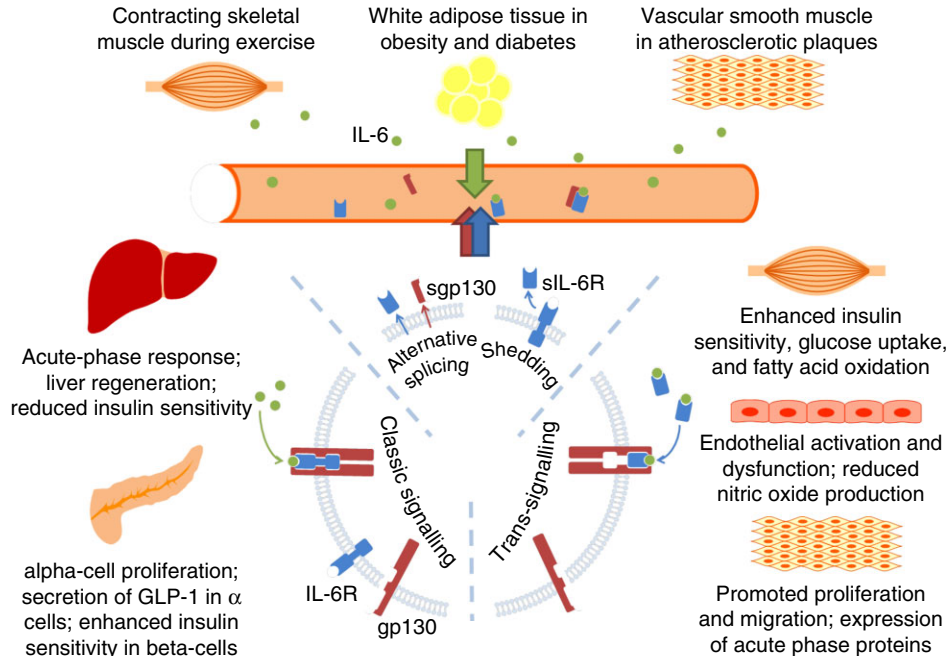


Figure 2

The pleiotropic role of IL-6 is demonstrated by its differential actions on various tissues and organs, which partly depends upon whether the classic signalling or trans-signalling is involved. The diagram illustrates features relevant to metabolic regulation and disorders, and cardiovascular complications. There are three sources of elevated plasma IL-6 – adipose tissue in diabetes and obesity, contracting skeletal muscle during exercise and vascular smooth muscle cells in atherosclerotic plaques (upper panel). Given the presence of membrane-bound IL-6R in hepatocytes and pancreatic islet cells, IL-6 is most likely to elicit its effects in liver and pancreas through classic signalling (left); whereas in skeletal muscle and the vascular wall, IL-6 acts through trans-signalling, because these cells lack expression of membrane-bound IL-6R (right).

other cell types, may be related to the presence of membrane-bound IL-6R. It should be noted that these studies are mainly performed *in vitro* on cell lines and for a short term, and with use of the supraphysiological concentrations of IL-6 that are much higher than those involved in low-grade chronic inflammation. Also, the experimental conditions may not mimic closely the chronic pathophysiological situations, where IL-6 acts in synergy with other cytokines and mediates crosstalk between different cell types and tissues, during inflammatory reactions. Moreover, in those early studies the level of sIL-6R and sgp130 is not assessed in targeted pathological models, nor is IL-6 trans-signalling taken into consideration, which is especially critical to investigate cells lacking membrane-bound IL-6R.

In the present context, it is important to note that IL-6-deficient mice develop mature-onset obesity, hepatic inflammation and systemic insulin resistance (Wallenius *et al.*, 2002; Matthews *et al.*, 2010). In line with these observations, increased cholesterol and triglyceride levels, accompanied by weight gain, were observed in patients with rheumatoid arthritis treated by tocilizumab, in addition to immunosuppression, as the main unwanted effect of global IL-6 inhibition (Richez *et al.*, 2012; Md Yusof and Emery, 2013; Strang *et al.*, 2013). Moreover, IL-6 released from contracting skeletal muscle during exercise can increase circulating levels to 100 pg·mL⁻¹ and this response is much greater than those of other cytokines involved (Pedersen, 2000; Steensberg *et al.*, 2000). The undoubted benefit of exercise on prevention and amelioration of obesity, diabetes and cardiovascular diseases points to a beneficial role of circulating IL-6 as an endocrine factor in regulating metabolism (Febbraio *et al.*, 2004). Now, the elevation of IL-6 has been increasingly recognized to activate pathways that facilitate energy turnover and enhance insulin sensitivity.

Several recent studies emphasizing the physiological relevance of IL-6 *in vivo* and chronic effects of moderate elevations of plasma IL-6 have contributed to our awareness of the paradox regarding both benefits and harms of IL-6. In humans, IL-6 increased fatty acid oxidation and glucose transporter 4 (GLUT4) translocation to plasma membrane, hence promoting glucose uptake in skeletal muscle via AMP-activated protein kinase (Carey *et al.* (2006); illustrated in Figure 2, right). Holmes *et al.* (2008) carried out a study to mimic chronic elevation of IL-6 in rats via either continuous IL-6 infusion or twice-daily intermittent injection and found that treatment for 14 days with IL-6, by either method, increased basal insulin sensitivity, improved glucose tolerance and enhanced fat oxidation through up-regulation of PPAR α and uncoupled protein-2 in skeletal muscle, which accounts for over 90% of the insulin-stimulated glucose uptake into cells. Ellingsgaard *et al.* (2008) reported that membrane-bound IL-6R was expressed in alpha-cells of pancreatic islets (secreting glucagon), and also in beta-cells (secreting insulin), albeit to a lesser extent. The same research group has recently demonstrated that elevation of plasma IL-6, released from either contracting skeletal muscle or white adipose tissues, stimulates the secretion of glucagon-like peptide-1 (GLP-1) from intestinal L cells and pancreatic alpha-cells via the classic signalling pathway to induce insulin secretion in the beta-cells, resulting in improved glycaemic control (illustrated in Figure 2, left) (Ellingsgaard

et al., 2011). Furthermore, IL-6 knockout reduced the level of pancreatic GLP-1 in mice; neutralization of IL-6 in wild-type mice fed with high-fat diet and, in *db/db* mice, impaired this obesity-induced alpha-cell adaptation and further disrupted glucose homeostasis. In addition, raised circulating IL-6 promoted pancreatic alpha-cell proliferation in response to a high-fat diet (Ellingsgaard *et al.*, 2008). Although increased alpha-cell mass is normally expected to cause an increase in glucagon production, elevated IL-6 increased PC1/3 expression in alpha-cells, leading to a shift in peptide production from glucagon towards GLP-1, thus promoting functional beta-cell compensation to maintain proper insulin secretion and glucose homeostasis (Ellingsgaard *et al.*, 2008; 2011). It is of interest to note that even in obese and diabetic mice with chronic and sustained elevation of plasma IL-6, acute administration of IL-6 still exerted similar beneficial effects on GLP-1 production and improved insulin sensitivity and glucose homeostasis (Ellingsgaard *et al.*, 2011). An adipose tissue and skeletal muscle endocrine-islet axis may exist, allowing for metabolic adaptation to the increased insulin demand in obesity and the improved beta-cell function in response to physical exercise.

In addition, the anti-inflammatory and immunosuppressive role of IL-6 in both local and systemic inflammatory responses has been widely accepted (Kristiansen and Mandrup-Poulsen, 2005). During exercise, muscle-derived IL-6 is likely to inhibit the expression of TNF- α and IL-1 β , hence offering protection against the risk of insulin resistance induced by these two pro-inflammatory cytokines (Pedersen, 2007). Moreover, IL-6 is elevated in concert with a number of anti-inflammatory cytokines such as IL-10, and may regulate the level of pro-inflammatory cytokines (Xing *et al.*, 1998). The association between IL-6 and progression to T2DM may merely reflect an attempt to counteract the chronic inflammation triggered by other inflammatory mediators.

IL-6 trans-signalling mediates inflammation leading to cardiovascular complications

Plasma concentration of the sIL-6R in healthy individuals ranges between 50 and 80 ng·mL⁻¹, and increases during viral infection (Honda *et al.*, 1992), although another study reported a decrease of plasma sIL-6R in bacterial infection (Frieling *et al.*, 1995). Elevation of sIL-6R and sgp130 is also associated with metabolic syndrome and endothelial cell inflammation (Weiss *et al.*, 2013). Although little is known about how sIL-6R is being altered under pathological conditions, the potential importance of sIL-6R-mediated trans-signalling should not be neglected. It is believed that the availability of sIL-6R, rather than that of IL-6, determines the switch-on of the deleterious impact of IL-6 and the extent to which the inflammatory response progresses. However, the plasma concentration of sIL-6R does not necessarily represent the whole extent of IL-6 trans-signalling. The local ratio of IL-6 and sIL-6R and the ratio of IL-6R to gp130 are important in determining the outcome of IL-6 signalling. Further investigation into the dynamics of sIL-6R and IL-6 trans-signalling will lead to better understanding of the balance between

anti-inflammatory and pro-inflammatory roles of IL-6 under pathophysiological conditions.

Administration of tocilizumab to patients with rheumatoid arthritis benefits endothelial function and attenuates aortic stiffness (Protogerou *et al.*, 2011). As endothelial cells and smooth muscle cells of the vascular wall lack membrane-bound IL-6Rs, the consistently reported, deleterious, action of IL-6 in vasculature is most likely to be mediated through binding to sIL-6R and subsequent activation of gp130 signalling, namely trans-signalling.

Regardless of whether IL-6 is elevated in response to metabolic adaptation or infectious stress, its presence in the circulation will affect the cells first encountered, i.e., the vascular endothelium. IL-6 suppresses insulin-stimulated NO production in endothelial cells through enhancing production of TNF- α (illustrated in Figure 2, right). This effect appears to be relatively specific as IL-6 does not increase TNF- α levels in skeletal muscle and adipose tissue (Yuen *et al.*, 2009). Moreover, IL-6 inhibits endothelium-dependent NO-mediated relaxations in systemic blood vessels of pregnant rats (Orshal and Khalil, 2004) and impairs the vasodilator effect of insulin through activation of JNK and ERK1/2 (Andreozzi *et al.*, 2007). Given the lack of membrane-bound IL-6R on both endothelial cells and smooth muscle cells, IL-6 trans-signalling is most likely to mediate these detrimental effects. Therefore, therapeutic strategies targeting IL-6 trans-signalling might be able to specifically block the pro-inflammatory effect of IL-6 while retaining its beneficial action on regulating metabolism, with the promise of ameliorating inflammation and improving metabolic adaptation in diabetes and related cardiovascular complications.

On the contrary, the soluble form of gp130, sgp130, acts as an endogenous inhibitor, specifically against IL-6 trans-signalling, but not classic signalling or other IL-6 family members (Schuett *et al.*, 2012). Patients with coronary artery disease have significantly lower plasma levels of endogenous sgp130, suggesting that a compromised counterbalancing of IL-6 trans-signalling may contribute to atherogenesis in humans (Schuett *et al.*, 2012). However, the change of sgp130 level under different pathological conditions has not been thoroughly investigated. Notably, the inhibition of IL-6 trans-signalling by sgp130Fc produces beneficial effects in several mouse models of human diseases with elevated inflammation (Rose-John, 2012), especially in atherosclerosis, a major cardiovascular complication associated with obesity and diabetes.

As the consequence of an imbalanced lipid metabolism and chronic inflammation in the arterial wall, atherosclerosis is manifested as atherosclerotic plaques and luminal narrowing of arteries, increasing risks for stroke and coronary artery disease. The atherogenesis is initiated by endothelial dysfunction and structural alterations at those sites in the vasculature susceptible to disturbed laminar flow (Chiu and Chien, 2011), and subsequent infiltration of intimal immune cells (Weber and Noels, 2011). Vascular smooth muscle cell (VSMC) proliferation and migration from media to intima plays a critical role in neointima formation after vascular injury and subsequent development of atherosclerotic plaques (Hansson *et al.*, 2006). The IL-6 level is elevated locally in atherosclerotic plaques (Schieffer *et al.*, 2000), with VSMCs as the major source of IL-6 production in plaques

(Chiu *et al.*, 2007; Loppnow *et al.*, 2011). IL-6 acts in an autocrine manner via trans-signalling to further accelerate inflammatory responses in VSMCs, including expression of acute phase proteins, proliferation and migration, which are critical in early atherosclerosis (illustrated in Figure 2, right) (Ikeda *et al.*, 1993; Wang *et al.*, 2007; Chava *et al.*, 2009). Also, IL-6 promotes endothelial activation by inducing expression of chemoattractant proteins and adhesion molecules that recruits immune cells into sub-intimal space (illustrated in Figure 2, right) (Marin *et al.*, 2001; Hansson, 2005). Moreover, IL-6 activates macrophages to migrate and differentiate, which potentially accelerates atherogenesis (Bacon *et al.*, 1990).

The deleterious effect of IL-6, probably through trans-signalling, makes sgp130Fc a promising candidate for the treatment of atherosclerosis. In hypercholesterolaemic Ldlr^{-/-} mice, sgp130Fc treatment reduces atherosclerosis, without affecting body weight or the serum lipid profile (Schuett *et al.*, 2012). Also, endothelial activation and intimal smooth muscle cell infiltration were decreased in sgp130Fc-treated mice, resulting in a marked reduction of monocyte recruitment and subsequent progression of atherosclerotic plaques (Schuett *et al.*, 2012). These findings illustrate the critical involvement of IL-6 trans-signalling in atherosclerosis and it warrants further investigation of sgp130Fc as a novel therapeutic agent for the treatment of atherosclerosis and related diseases.

Notably, IL-6 is positively associated with coronary artery disease (Saremi *et al.*, 2009; Tehrani *et al.*, 2013). Two recently published studies in *The Lancet* further suggest a causal pro-inflammatory role of IL-6 in coronary artery disease based upon genetic investigations. A single nucleotide polymorphism, Asp358Ala, of IL-6R affects its proteolytic cleavage, leading to a reduced level of its membrane-bound form in hepatocytes, monocytes or macrophages, and concomitant elevation of its soluble form in circulation (Collaboration IRGCERF *et al.*, 2012; Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium *et al.*, 2012). Reduction of membrane-bound IL-6R attenuates the classic pathway that induces production of acute phase proteins such as C-reactive protein and fibrinogen, correlating to a reduction of the risk of coronary artery disease (Collaboration IRGCERF *et al.*, 2012; Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium *et al.*, 2012). However, these studies are limited by their lack of examination of the trans-signalling, because elevated sIL-6R, as a side-effect, is likely to enhance the extent of trans-signalling and may also alter other components of IL-6 signalling, such as sgp130. In this context, the beneficial effect provided by the attenuation of classic signalling to reduce production of acute phase proteins may override the deleterious effects of trans-signalling in the development of atherosclerotic plaques. However, the possibility that sgp130 levels increase in response to the elevated sIL-6R and counterbalance sIL-6R-mediated trans-signalling cannot be ruled out. Moreover, the Asp358Ala mutant results in a similar reduction in circulating concentrations of inflammatory biomarkers, such as C-reactive protein and fibrinogen, as the effect of tocilizumab treatment, whereas the alteration of blood lipids to a pro-atherogenic profile in tocilizumab-treated patients was not seen in carriers of the Asp358Ala mutation (Interleukin-6

Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium *et al.*, 2012). This again indicates a critical role of IL-6 in metabolic regulation and the necessity to distinguish the two downstream signalling pathways and the consequent effects on metabolic regulation and cardiovascular risks in response to IL-6. These new results add additional weight to the consideration of selectively inhibiting the pro-inflammatory pathway of IL-6 as an attractive strategy to reduce cardiovascular risks.

Regulation of balance between classic and trans-signalling

Instead of simply inhibiting trans-signalling, the tuning of balance between classic and trans-signalling might be more beneficial under conditions of obesity and diabetes. For instance, two membrane-bound proteases, disintegrin and metalloprotease (ADAM)-10 and ADAM-17, are responsible for sIL-6R shedding in humans (Garbers *et al.*, 2011a; Waetzig and Rose-John, 2012), and the expression of ADAM17 was raised in arterial tissue from patients with atherosclerosis (Canault *et al.*, 2006). TNF- α converting enzyme (TACE), the counterpart of human ADAM17 in mice, is elevated in high-fat diet-induced obese mice (Voros *et al.*, 2003), whereas heterozygous *Tace*^{+/−} mice are protected from diet-induced obesity (Serino *et al.*, 2007). Treatment with the TACE inhibitor improves insulin sensitivity, corrects hyperglycaemia and inhibits vascular inflammation (Federici *et al.*, 2005). Therefore, ADAM over-activity probably shifts the IL-6 signalling balance towards trans-signalling, thus contributing to vascular inflammation and diabetes.

Perspectives

With more results on the pro-inflammatory action of IL-6 trans-signalling available and the state-of-the-art evaluation of classic signalling in metabolic regulation, it is possible to better understand the complexity of both the physiological and pathological properties of IL-6. However, how some key components such as endogenous sIL-6R and sgp130 respond to inflammation is still unclear. In addition, insights into the upstream regulation of the balance between classic and trans-signalling pathways will be extremely valuable. Also, several important issues with regard to the delicate risk-benefit balance between these two divergent pathways downstream of the common point of gp130 activation, as well as the regulatory mechanism, are yet to be resolved. Additional complexity arises from the extensive crosstalk between cells and tissues secreting IL-6 upon stimulation and effectors that respond differently, due to the presence or absence of classic signalling. Moreover, the biological outcome of IL-6 activation also depends upon its interaction with the many other cytokines concomitantly stimulated in response to inflammation, oxidative stress or physical exercise.

From a pharmacological point of view, the success of tocilizumab in alleviating inflammation in rheumatoid arthritis has supported the promise of targeting IL-6 in obesity and T2DM where chronic inflammation contributes

to the disease progression. As IL-6 signalling transduction through either membrane-bound or soluble IL-6R ends in the activation of gp130, the pharmacological strategy to discriminate between classic and trans-signalling must have a target beyond the common point. As growing evidence supports the notion that inhibiting trans-signalling is as effective as or even better than global inhibition in many IL-6-driven diseases, the design of selective inhibitors of IL-6 trans-signalling through sIL-6R which do not interfere with the function of membrane-bound IL-6R is a logical and promising approach to new treatments. Fortunately, nature has provided us a solution with the existence of the endogenous sgp130 that sterically prevents the association of the IL-6/sIL-6R complex with membrane-bound gp130 and hence specifically inhibits trans-signalling. Recombinant proteins that mimic the property of sgp130 have been developed and are in clinical trials for the treatment of inflammatory diseases. In summary, blocking the unwanted IL-6 trans-signalling, while maintaining the physiological classic signalling, holds out the promise of developing selective trans-signalling inhibitors in order to alleviate diabetes-related cardiovascular complications.

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Conflict of interest

The authors declare that no conflict of interest exists.

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