Cardiovascular Biology of Interleukin-6

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Abstract: Interleukin-6 (IL-6) is a multifunctional pro-inflammatory cytokine that is tightly regulated and expressed at low levels in healthy individuals. Increased IL-6 expression has been associated with a variety of diseases, including inflammatory conditions such as atherosclerosis and cardiovascular disease (obesity, myocardial infarction and type II diabetes). Cytokines including IL-6 and tumour necrosis factor alpha as well as acute phase proteins such as C-reactive protein (CRP) and fibrinogen are key biochemical risk factors for the development of these disease conditions. IL-6 is the key cytokine responsible for the stimulus of synthesis and secretion of CRP. IL-6 activates cell surface signalling via the assembly of IL-6, the IL-6 receptor (IL-6R) and the signalling receptor gp130. Assembly of the (hexameric) signalling complex of IL-6, IL-6R and gp130 occurs in a sequential manner and therefore this signalling complex lends itself to several potential sites for drug targeting. This review discusses some of the mechanisms of IL-6 signalling on various aspects of cardiovascular biology as well as some recent developments in drug targeting of this complex.

Key Words: Interleukin-6, inflammation, cardiovascular disease, endothelium, C-reactive protein, insulin resistance.

INTRODUCTION

Deaths due to cardiovascular disease worldwide still remain as the leading cause of mortality in the developing world. It is estimated that 15.3 million people die from cardiovascular disease every year (or 30% of all total deaths). In addition, it has been estimated that the prevalence of cardiovascular disease will increase rapidly in developing countries [1]. Cardiovascular disease is closely linked with risk factors such as elevated total cholesterol, insulin resistance, diabetes and obesity, and these risk factors are also increasing in developing countries [2-4].

Interestingly it has emerged that the traditional risk factors only account for approximately half of the incidence in cardiovascular disease [5]. It is now believed that chronic low grade inflammation may play a role in the development of cardiovascular disease as well as insulin resistance, diabetes and obesity [6, 7]. Cytokines including interleukin-6 (IL-6), and tumour necrosis factor alpha (TNFα) as well as acute phase proteins such as C-reactive protein (CRP) and fibrinogen are key biochemical risk factors for the development of these disease conditions [8]. IL-6 is the key cytokine responsible for the stimulus of synthesis and secretion of CRP. Both IL-6 and CRP are known to be correlated with a number of markers for insulin resistance syndrome and endothelial dysfunction [8]. This review will concentrate on what is known about IL-6 in vascular biology and the potential role of IL-6 as a candidate for intervention.

Interleukin-6 (IL-6) is a multifunctional pro-inflammatory cytokine that is tightly regulated and expressed at low levels in healthy individuals. During infection, trauma or other stress, IL-6 is known to be expressed at much higher concentrations and has been implicated in the pathogenesis of several chronic disease conditions including cardiovascular disease, atherosclerosis and obesity [9, 10]. IL-6 (21-kDa) is produced by a variety of cells including fibroblasts, endothelial cells, mononuclear phagocytes, neutrophils, hepatocytes, lymphocytes (T and B) and neural tissue - neurons, astrocytes and glial cells. IL-6 is known to be one of a group of cytokines which share similar biological functions. Other members of the IL-6 family include IL-11, leukaemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neutrotrophic factor (CNTF), cardiotrophin-1 (CT-1), and novel neurotrophin-1/B cell stimulatory factor-3 (NNT-1/BSF-3) [11, 12]. A unique feature of the IL-6 family of cytokines is that they share a common receptor subunit which may account in part, for the overlapping biological activity.

IL-6 SIGNAL TRANSDUCTION

The actions of IL-6 are known to be signalled through the IL-6 receptor (IL-6R). The receptor consists of two distinct glycoproteins, a membrane bound alpha ligand specific subunit (80kDa) and a 130kDa signal transducing beta subunit (gp130). The alpha subunit is present predominantly in hepatocytes and monocytes, whereas gp130 is present in almost all tissues [13, 14]. In addition there is also a soluble form of the IL-6 alpha subunit (sIL-6R; 50 kDa), which lacks the membrane tether and anchor domain [15]. This form is biologically active and found naturally in low concentrations in human urine and serum of healthy individuals.
and increased levels of sIL-6R have been found in patients suffering from a number of clinical conditions indicating that this could be indicative of a disease response [14]. It is unclear whether sIL-6R is a result of proteolytic shedding of the membrane bound receptor [17] or an alternative spliced mRNA [18]. This accounts for a paracrine function of IL-6 where sIL-6R can elicit gp130 signalling in cells that do not express IL-6R. It is this receptor which is primarily responsible for the multiplicity of actions of IL-6. When IL-6 is bound to either the bound or soluble form of the alpha receptor subunit, the complex has a high affinity for the gp130 subunit [19]. The binding between the IL-6R complex with gp130 subunit creates a hexameric complex [20] (Fig. (1A) and Fig. (1B)). This in turn activates IL-6 actions through activating a Janus kinase/signal transducer and activator of transcription signalling (JAK/STAT) pathway to change the expression of specific genes [21] (Fig. (1C)). Briefly, once the IL-6R complex binds to gp130, there is a

Fig. (1). Schematic diagram of the cellular process of the signal transducing subunit of IL-6 signal transduction. (A, B) demonstrate the binding of IL-6 with its specific receptor subunit with the gp130 signalling transducing subunit. (C) is a diagram of the intracellular processes of the activated gp130 subunit and the possible mechanisms of how genes (such as acute phase protein, α2-macroglobulin) may be regulated. Abbreviations: Janus kinase (JAK), signal transducer and activator of transcription (STAT), suppressor of cytokine signalling (SOCS), tyrosine kinase (Tyrk).
rearrangement of the receptor with a phosphorylation of the associated JAKs. There is then recruitment of specific STATs which are tyrosine phosphorylated by the JAKs, released and migrate to the nucleus to alter the synthesis of specific genes such as the up-regulation of acute phase protein (α2-macroglobulin). This process can be positively regulated by a serine phosphorylation of STATs through mitogen-activated protein (MAP) kinase [21] or negatively regulated by suppressors of cytokine signalling (SOCS) which inhibit the activity of the JAKs [22, 23] (Fig. (1C).

The IL-6 gene does not contain any common polymorphisms within coding regions and therefore attention has focused on promoter polymorphisms [24] and in particular, the common IL-6 -174 G>C polymorphism [25, 26]. Indeed, the IL-6 -174 G>C polymorphism has been shown to be associated with inflammation [25] and increased arterial stiffness and pulse pressure, as demonstrated in the recent “Rotterdam Study” [26]. The IL-6R gene maps to an important candidate locus for type 2 diabetes on chromosome 1q21 [27] and the D358A polymorphism was shown to be associated with type 2 diabetes in Danish whites [28], however there are no studies demonstrating a cardiovascular disease association with polymorphisms in the IL-6R gene.

IL-6, IL-6R AND GP130 STRUCTURE

IL-6 belongs to the 4-helix bundle structural family of small signalling proteins which include most known cytokines, while the extra cellular domains of IL-6R and gp130 are highly modular and composed of multiple immunoglobulin-like sub-domains (each of about 100 residues) connected in a linear way to a membrane spanning region. In gp130 this membrane section connects the extracellular domain to a cytoplasmic Janus Kinase globular domain, while the IL-6R transmembrane section connects a small domain of unknown function that might only act as a membrane anchor or be involved in the cytosolic part of the hexameric signalling complex. Soluble IL-6R is an alternatively spliced version or proteolytically shed version of IL-6R [29] that lacks this transmembrane anchor. Thus cells expressing both gp130 and IL-6R can activate the Janus kinase pathway while cells that do not express IL-6R will depend on sIL-6R to activate this pathway. The extra cellular domain of IL-6R consists of three immunoglobulin like domains, the N-terminal domain D1, the most distal from the membrane has no known function and is dispensable for ligand recognition signal initiations and is probably involved in receptor internalisation and protein stability and receptor aggregation on the cell surface [30-33]. The following two domains (D2 and D3) form a cytokine binding domain (CBD) which has been observed in all of the receptors in the class I cytokine family. The CBD consists of two fibronectin type III domains (a class of Ig domains) that have a characteristic proline rich “hinge” region and a conserved WSXWS sequence motif [34]. This domain is structurally conserved in all cytokine receptors and the outer elbow formed by the connection of the two immunoglobulin domains (D2 and D3) is the binding site of the cognate cytokine - in this case IL-6. The C-terminal end of IL-6R then acts as the membrane anchor which tethers the receptor probably by an extended peptide chain linking D3 to the membrane. There is some evidence that IL-6R might dimerise on the cell surface [35]. The beta receptor gp130 which has six immunoglobulin like domains consists of, like IL-6R, an N-terminal domain D1 followed by a CBD (domains D2 and D3) and then a stalk consisting of a further three immunoglobulin-like domains (D4 – D6) followed by the cytoplasmic kinase domain linked by a transmembrane section.

The three dimensional structure of IL-6 [36], the extracellular domain of IL-6 α-receptor (IL-6Rα) [37] and the first three domains of gp130 have been determined by X-ray crystallography [38]. The structure of a hexameric complex [19] which consists of IL-6, the second and third domain of IL-6R and the first three domains of gp130 has also been determined and is depicted in Fig. (2). The structural assembly of the functional IL-6/IL-6Rα/gp130 hexamer signalling complex occurs in a stepwise fashion. Assembly of the hexameric complex occurs sequentially as follows: IL-6 is first engaged by IL-6R at nano-molar binding and then presented to gp130 to facilitate a transition into the high-affinity (pico- molar), signalling-competent hexamer [39]. Neither IL-6 nor IL-6R alone have measurable affinity for gp130, however the additional surfaces provided by the (“site II”) interfaces, enhance the overall binding affinity, ultimately forming the signalling hexamer [19] as shown in Fig. (2B). Therefore, there are potentially several levels at which to target the binding to either IL-6, IL-6R or gp130. Blocking the interfacial regions, one could suggest, may potentially prevent the formation of either the trimeric complex or the resulting dimer (ie the hexameric signalling complex). Indeed, there have been attempts using structure-based drug design on the basis of these constructed models using computer-aided methods [37, 40, 41]. For example, peptide antagonists directed at IL-6R could antagonise the function of human IL-6 [40]. In that study, the peptide antagonist bound to the same key residues on IL-6R as does IL-6 (ie Leu108, Gly163, Gly164, Phe168, Phe229, Arg231, Glu278, Phe279 and Gln281). Thus, the binding of the peptide antagonist was able to compete with binding of IL-6 to the IL-6R. Low molecular mass IL-6 signalling antagonists such as madindoline A were found to suppress dimerisation of the trimeric complexes, resulting in inhibition of IL-6 signalling activity [42]. The determination of the three dimensional structures of the signalling complex of IL-6, IL-6Rα and gp130 should allow specifically targeted drug design at several binding interfaces in the signalling complex.

IL-6 AND CARDIOVASCULAR DISEASES

Diseases of the cardiovascular system constitute the leading cause of death in humans, and atherosclerosis has been recognised as one of the primary causes of such diseases. Whilst this is a complex and multifaceted process involving several cell types and mechanisms, the key underlying factors are inflammatory and innate immune mechanisms involving monocytes, receptor systems and cytokines. For example, activation of the inflammatory cytokine cascade including TNFα, IL-1 and IL-6 can challenge the homeostasis of the vascular wall, leading to accumulation of cells and fatty deposits. Indeed, the role of cytokines in atherogenesis has been confirmed by studies involving cytokine-deficient animals.

It is also known that IL-6 exerts pleiotropic effect on cells from different lineages. IL-6 enhances lipid turnover,
stimulating lipolysis and oxidation of fat, and may also trigger inhibition of other pro-inflammatory cytokines indirectly. For example, the anti-inflammatory effects of regular exercise have been reported to be mediated via the inhibition of pro-inflammatory cytokine TNFα by anti-inflammatory cytokines such as IL-1ra (interleukin-1 receptor antagonist) and IL-10 which are produced in response to IL-6 released by skeletal muscle fibres during exercise [43]. IL-6 plays an important role in peripheral arterial disease (PAD), and is one of the most studied cytokines in this condition. It has been shown that type-2 diabetes patients (who carry a two fold increase in risk of PAD compared to non-diabetics) have significantly elevated serum levels of IL-6 and other pro-inflammatory cytokines and mediators such as C-reactive protein, fibrinogen, MMP and adhesion molecules [44]. It has also been suggested that the IL-6 -174 G>C polymorphism influences the release of IL-6, and influences PAD development in type 2 diabetes [44].

Activation of the cardiac IL-6 system has also been found in advanced heart failure [45]. While elevated plasma IL-6 is known to be associated with an impaired prognosis of this condition, these investigators also found that intracardiac expression of the IL-6/IL-6Rα system, determined by using heart tissue from 20 patients at the time of transplantation, is increased in patients with advanced heart failure. Both left ventricular dysfunction (LVD) and congestive heart failure (CHF) show characteristic increases in IL-6 and brain natriuretic peptide (BNP). For some time IL-6 has been considered to be the best marker of disease severity in the heart [46]. However, a more recent head to head comparison of BNP and IL-6 as markers of clinical and experimental heart failure has showed that although the failing left ventricle is a potential source of IL-6, the expression of IL-6 is far exceeded by the expression of BNP [47]. In this study, the N-terminal fragment of the BNP-precursor NT-proBNP was independently correlated with LV ejection fraction, LV mass and MI history in addition to disease symptoms of the failing heart whereas IL-6 was only correlated with a history of myocardial infarction.

Haugen et al. [48] recently reported that chronic treatment (12 weeks) of spontaneously hypertensive rats with the TNFα antagonist etanercept resulted in favourable cardiac remodelling, positive inotropy and a further upregulation of IL-6. TNFα (which is absent in the normal myocardium) is a well characterized inflammatory mediator in chronic heart failure. It is known that a complex network of inflammatory mediators including IL-6, IL-1α and interferon-γ are associated with heart failure, and this study proved that targeting a single constituent of the inflammatory cascade is not an effective treatment strategy in heart failure due to hypertension.

The extent of involvement of IL-6 in several cardiovascular disease settings are discussed in detail below.

ATHEROSCLEROSIS

There is increasing evidence linking low grade inflammation with the development of atherosclerosis and cardiovas-
cular disease and IL-6 has been implicated in this association [49]. Briefly, the process of atherosclerosis begins with aggravation of the endothelial cells of the arterial wall, due to lipid accumulation and oxidation. In response there is an increase in the expression of cellular adhesion molecules with an activation of inflammatory cells such as macrophages and lymphocytes which secrete different cytokines and growth factors resulting in the accumulation of foam cells. The vascular smooth muscle cells react to the accumulation of foam cells by migration and proliferation some of which enters the extracellular matrix and contributing to both the progression of the lesions and the fibrous cap of atherosclerotic plaques [49, 50]. A recent study which evaluated associations of long term circulating IL-6 levels with CHD risk in two population based cohort studies along with a systematic review has suggested a strong association akin to those described for major established risk factors whilst the exact causality remain unresolved [51].

IL-6 has been implicated in the development of atherogenesis, specifically initiating the cascade of events leading to atherosclerosis. IL-6 mediates the synthesis and secretion of CRP, in turn inducing the secretion of cellular adhesion molecules and tissue factors [52]. The classic belief that CRP is produced exclusively by hepatocytes has been challenged by recent findings regarding the extrahepatic production of CRP in different cells, including atherosclerotic lesions [53]. CRP is probably the best clinical utility presently available to assess the degree of inflammation. Elevated levels of CRP lead to a prediction of future cardiovascular risk in healthy individuals. However, there is also uncertainty as to whether CRP can protect humans from developing atherosclerosis [54]. It has also been observed that the major acute phase response of CRP triggered by MI may contribute acutely to the severity and outcome of the ischemic injury. IL-6 and related pro-inflammatory cytokines are likely to play a key role in these events.

The specific role of IL-6 on vasculature in the development and progression of atherosclerosis is unclear. However during basal conditions, IL-6 acts on a wide range of tissues influencing cell growth and differentiation including angiogenesis, re-vascularisation, and healing in vivo and in situ. Cellular adhesion molecules regulate the adhesion of white blood cells to the vessel wall whilst tissue factors promote coagulation [55]. Vascularogenesis, the first wave of vessel formation, is a phase of endothelial cell differentiation and proliferation, with the formation of primitive vessels. This process is especially dependent on vascular endothelial growth factor (VEGF) [56, 57]. IL-6 significantly induced the proliferation of brain microvessel endothelial cells in vitro in a dose dependent manner [58]. Moreover, the presence of IL-6 significantly enhanced the development of capillary tube formation by brain microvessel endothelial cells in response to VEGF [58] and IL-6 is also known to induce the expression of VEGF [59]. In vascular smooth muscle cells (VSMC) within atherosclerotic plaques there is an increased expression of VEGF and IL-6 expression is increased in VSMC exposed to VEGF [60]. Interestingly, treatment with an anti-IL-6 antibody attenuated the VEGF migration of VSMC [60]. In addition it also appears that exposure of VSMC with IL-6 significantly enhances the cell’s response to angiotensin II, due to an increase in the expression of the angiotensin II type 1 receptor [61]. Ang II exposure to VSMC stimulates the production of reactive oxygen species (ROS) through NADPH oxidase and is enhanced after pre-treatment with IL-6 [61].

IL-6 concentration correlates with arterial blood pressure in healthy adults [62, 63] and is increased in patients suffering from cardiovascular disease and atherosclerosis [64]. In addition IL-6 is associated with dyslipidemia and is related to the thickness of the arterial wall [65]. In ApoE knockout mice, which spontaneously develop atherosclerotic lesions, there was a significantly greater increase in synthesis and secretion of IL-6 which was positively correlated with the degree and number of atherosclerotic plaques [66]. Interestingly the development of atherosclerotic plaques was significantly increased in IL-6 and ApoE double knockout mice when compared with either IL-6 knockout, ApoE knockout or wild type mice [67]. These data suggest that IL-6 potentially promotes the progression of atherosclerosis. IL-6 is also involved in the induction of matrix metalloproteinases (MMP) and therefore plays an important role in the instability of the vulnerable plaque. Suzuki et al. [68] recently demonstrated higher MMP-1, MMP-13 and IL-6 levels in blood aspirated from culprit coronary arteries compared to the peripheral levels in patients of early stage AMI.

The role of IL-6 in the vasculature is complex. It has been reported that superfusion of the cremaster muscle with IL-6 results in vasoconstriction [69] or vasodilation [70] in the male rat. The differing outcomes may be due to the possibility that IL-6 does not directly act on the vasculature to cause a change in the diameter of the vessel. Data from our laboratory suggests that IL-6 may act at sites other than on the vasculature as addition of IL-6 into a myograph preparation did not significantly alter the tension of the vessels (Abeywardena et al., unpublished observations). It is also possible that a change in the diameter seen in superfused preparations is a secondary effect of IL-6 exposure such as an increase in prostaglandins. This is possible as exposure of rat aortic rings to IL-6 significantly enhanced the production of prostaglandin synthesis [71]. However IL-6 exposure has also been shown to decrease the cumulative dose response curve to phenylephrine in aortic rings [71, 72]. It is clear from these data that there is still much controversy over the specific role of IL-6 on the vasculature, but it seems likely that the role of IL-6 on the vasculature is more likely to be due to a more systemic role of IL-6 rather than specifically acting at the vessel level.

It is becoming apparent that IL-6 may play a role in the regulation of arterial blood pressure during times of stress. Lee et al. [73] found that IL-6 knockout mice had a significantly higher increase in mean arterial blood pressure in response to a psychosocial stress than wild type mice when exposed to the same stress [73]. This is supported by another recent study which demonstrated that an infusion of IL-6 into pregnant rats resulted in a significant increase in arterial blood pressure which did not occur when non-pregnant rats were infused with IL-6 [74]. These data suggest that IL-6 does not play a role in the regulation of arterial blood pressure under basal conditions, but that it does regulate blood pressure when the system is compromised such as during stress.
The mechanisms underlying IL-6 regulation of arterial blood pressure are unclear, however, there appears to be an important relationship between the sympathetic nervous system and IL-6 production. A subcutaneous injection of adrenaline into rats caused a significant increase in plasma IL-6 concentrations, 2h after the injection, whilst an intravenous (iv) infusion of adrenaline also significantly increased heart rate and plasma IL-6 concentrations [75]. In addition, IL-6 knockout mice had significantly higher basal noradrenaline concentrations when compared with wild type controls. However the basal response to an Ang II infusion was similar between the IL-6 knockout and wild type mice. These data suggest that the sympathetic nervous system stimulates the production of IL-6 in the periphery. Recent reports indicate cardiovascular autonomic dysfunction, which is responsible for high mortality in type-II diabetics, is influenced by IL-6. Shinohara et al. [76] reported that cardiac autonomic function – as assessed by baroreflex sensitivity, heart rate variability, plasma noradrenaline concentrations and I125-metaiodobezylguanidine scintigraphy- was depressed by elevated IL-6 levels and obesity in type-II diabetic patients. Further studies to validate the predictive value of elevated serum IL-6 in this condition may be warranted.

VASOSPASM AND MYOCARDIAL INFARCTION

The risk of strokes and vascular complications after a first ischemic stroke has been found to be associated with systemic inflammatory mechanisms. Cytokines such as IL-6 and TNF alpha, VCAM1, ICAM1 and metalloproteinases have been implicated and some may be useful the diagnosis of ischemic stroke [77].

The mechanisms underlying the pathogenesis of subarachnoid haemorrhage (SAH) are unknown. It has been thought however, that inflammatory cytokines are involved in the development of post-hemorrhagic vasospasm [78]. Cytokines including IL-6 are known to be elevated in the cerebrospinal fluid (CSF) of SAH patients [79, 80] and IL-6 appears to be a reliable early marker for vasospasm after SAH [81]. The ability of a novel pro-inflammatory cytokine inhibitor (CNI-1493) to protect against the occurrence of experimental vasospasm has recently been demonstrated in the rat femoral artery vasospasm model [78]. That study concluded that inhibition of pro-inflammatory cytokines, in particular IL-6, may be an effective strategy for the treatment of vasospasm after SAH.

Cytokines are known to be synthesised by microglia and astrocytes [82]. In addition, injection of IL-6 significantly decreased the diameter of basilar artery in the dog [83] possibly through the activation of prostaglandins. An intracerebral injection has been shown to increase the expression of the enzyme COX-2 [84] as well as elevating prostaglandins, PGE2 and 6-keto PGF1α [83]. In addition when cultured rat cerebral endothelial cells are treated with IL-6 there is an acute increase in PGE2 secretion [85]. These data suggest that vasospasm induced by IL-6 may involve an activation of eicosanoid cascade.

IL-6 also plays a crucial role in the healing process of injured brains. The brains of IL-6 deficient mice were significantly slower to recover from an injury when compared with wild-type controls [86]. IL-6 deficient animals also demonstrated leaky vessels compared to controls. However, these investigators did not study potential reversal of these defects due to IL-6 deficiency by infusing the transgenic mice with IL-6. Nevertheless these data suggest that IL-6 plays a role in the development of vasculature which may be an important mechanism for the repair of damaged vasculature seen in atherosclerosis or brain injury.

Compared to its role in post-hemorrhagic vasospasm in the cerebral circulation, the possible involvement of IL-6 in coronary vasospasm is less well described. It is also noteworthy that coronary vasospasm whilst not frequently observed in Caucasians is a common occurrence in certain Asian populations [87-89] where coronary vasospastic angina pectoris is known to occur in the absence of hemodynamically significant coronary artery disease (CAD) [90]. In such patients treatment with anti-spastic agents (eg., calcium antagonists, isosorbide dinitrate) has been accompanied with reductions in high sensitivity CRP suggesting that inflammation in early atherosclerosis could promote coronary vasospasm. A recent study by Hung et al. (2006) [91] has further shown that IL-6 levels were independently associated with the diagnosis of coronary vasospastic angina pectoris in the absence of advanced CAD supporting the hypothesis that coronary vasospasm at least in part represent an inflammatory condition. In addition, Li et al. [92] recently reported that patients with variant angina display higher white blood cell and monocyte cell counts. Similarly, plasma IL-6 and CRP levels in that study were higher in variant angina patients compared with those patients with stable CAD. These authors also suggested that a more chronic and severe inflammation may underlie the pathogenesis of variant angina. These findings may at least in part also account for the relatively high coronary events reported in certain populations where vascular inflammation, rather than high plasma cholesterol level, has been thought to be central to the cardiovascular disease development [93].

Similarly to the situation after haemorrhage, significantly elevated circulating plasma levels of IL-6 and IL-6R (α and β) have been observed in patients with acute myocardial infarction (AMI). In contrast, neither IL-6 nor IL-6R showed any increase in patients with angina pectoris [9]. The clinical relevance and localisation in the ischaemic myocardium of IL-6 in AMI patients has been examined histochemically [94]. The expression of IL-6 in the myocardium in AMI found to be associated with the mechanism of cardiac hypertrophy. Interestingly though, there was no difference in the myocardial infarction size, ventricular remodelling or mortality rate in IL-6 knockout mice when compared with wild type controls [95]. This study however demonstrated that whilst there was an activation of the JAK/STAT pathway, with an upregulation of angiotensin II, this may have been possible due to other IL-6 related cytokines being activated [95]. Isoproterenol-induced myocardial necrosis in Wistar rats was accompanied with an early up-regulation of IL-6 expression within 1-3 hours following single subcutaneous administration of 0.5 mg isoproterenol [96]. This suggests that IL-6 may be involved in priming the downstream targets and pathways central to the development of tissue necrosis/apoptosis such as the MAPK and NF-kappaB signalling pathways.
It has been demonstrated that the degree of inflammation predicts short-term coronary deaths [97]. Supporting evidence has emerged in a more recent investigation where a lower inflammatory burden - low interleukin-6 and decreased neutrophil count – at the time of an AMI have been found to associate with long-term (>8 years) survival [98]. A structural variant of the IL-6 receptor subunit gp130 has been found to be associated with decreased risk of myocardial infarction in a hypertensive population, independent of other known risk factors [99]. More specifically the Gly148Arg polymorphism of the gp130/IL-6ST subunit of the IL-6R was associated with the observed lower risk offering a genetic explanation for the high individual variation in vulnerability to cardiovascular disease.

CONGESTIVE HEART FAILURE

IL-6 has been implicated in congestive heart failure. Plasma IL-6 concentrations are elevated in patients suffering from CHF [46] and are higher in patients in the latter stages of the disease when compared with patients in the first stages of the disease [46, 100]. In vitro, IL-6 is synthesised and secreted by endothelial and vascular smooth muscle cells [101, 102]. The source of circulating plasma IL-6 levels, in CHF however, has been shown to be the lungs in both rats [103] and humans [100]. In addition the intracardiac expression of IL-6 mRNA is inversely correlated with degree of heart failure [45]. This is in contrast to patients with acute coronary syndrome in which there was an elevated transcardiac IL-6 gradient suggesting the most likely source of IL-6 in these patients were coronary plaques and/or myocardial cells [104]. These data suggest that IL-6 plays a role in peripheral systems in congestive heart failure patients rather than having a direct effect on the heart.

IL-6 and IL-6 related cytokines, eg CT-1 and LIF, play a crucial role in the development of the myocardium. Mice deficient of the signalling receptor subunit (gp130) have significantly abnormal hearts in utero. The hearts have abnormally thin ventricular walls, down to one cell layer of thickness [105]. In contrast mice over expressing both IL-6 and IL-6R had significantly thickened ventricular walls and hence hypertrophy of the ventricular myocardium [106]. Interestingly, mice lacking specific cardiac gp130 have normal cardiac growth but when challenged with mechanical stress, such as pressure overload, these animals had an increased rate of apoptosis resulting in heart failure and death, whilst normal mice exposed to the same mechanical stress compensated with cardiac hypertrophy of the left ventricle and survived the experiment [107]. In human atrial tissue, from patients undergoing surgery for coronary artery disease, it was found that cardiac myocytes and fibroblasts spontaneously synthesise and secrete IL-6 and the IL-6 related cytokines, LIF and IL-11 [108]. In addition gp130 also has been observed in fibroblasts and cardiac myocytes from human atrial slices [109]. Taken together these data suggest that IL-6 and IL-6 related cytokines, through the activation of gp130 play an important role in the development and maintenance of the myocardium and protect the myocardium from apoptotic cell death.

A number of endocrine mediators involved in the pathogenesis of CHF have also been shown to stimulate IL-6 synthesis and secretion. These include other cytokines (TNFα and IL-1), angiotensin II and adrenergic stimulation [110]. In vitro studies have shown that angiotensin II enhances IL-6 mRNA expression in cardiac myocytes and fibroblasts, and stimulates IL-6 production in VSMC [110-112]. Noradrenaline and adrenaline up regulate IL-6 synthesis in cultured myocytes [111]. Treatment of patients with moderate CHF, with candesartan cilexetil, an angiotensin II type 1 receptor antagonist, not only significantly improved the compensatory mechanism in CHF patients but also significantly reduced plasma IL-6 concentrations [113]. In addition a high dose of enalapril, an angiotensin converting enzyme inhibitor, significantly improved heart compensation, i.e. a decrease in cardiac hypertrophy, and reduced plasma IL-6 concentrations [114]. The reasons for this association is unclear, however it has been shown that plasma noradrenaline and adrenaline levels are significantly higher in patients with CHF and were positively correlated with plasma IL-6 and that there is a significant negative relationship between the use of beta-adrenergic blockers and plasma IL-6 concentrations [46, 100]. It is therefore suggested that IL-6 production is associated with the activation of the sympathetic nervous system [46, 100, 103, 115] in congestive heart failure.

METABOLISM, OBESITY, INSULIN RESISTANCE AND TYPE-II DIABETES

IL-6 is increasingly believed to play a role in the regulation of metabolism. IL-6 is expressed in both skeletal muscle and adipose tissue. Approximately 25-30% of circulating IL-6 levels is believed to be secreted from adipose tissue [116] and supports the current view that obesity in most people is associated with a low grade inflammation of the white adipose tissue (WAT) which can lead to insulin resistance, impaired glucose tolerance and diabetes [117]. Recent findings also suggest that in addition to adipocytes, macrophages infiltrated in to WAT as a potential source of pro-inflammatory cytokines. It is noteworthy that weight loss has shown to be associated with reduced macrophage infiltration of WAT paralleled with an improved profile of the expression of pro-inflammatory genes (reviewed in [117]).

Plasma IL-6 concentrations are increased with increasing exercise [118], which are a result of an increased expression of IL-6 mRNA in both skeletal muscle [119, 120] and adipose tissue [121, 122]. In addition, infusion of IL-6 increases plasma fatty acid concentrations, fat oxidation [123] and the expression of IL-6 mRNA in skeletal muscle [119] while also increasing hormones such as cortisol and adrenaline [123, 124]. Interleukin-6 has also been shown to increase insulin-stimulated glucose disposal in humans and IL-6 treatment of myotubes in vitro has been shown to increase fatty acid oxidation, basal and insulin-stimulated glucose uptake and translocation of Glut4 to the plasma membrane. These cellular metabolic effects of IL-6 are thought to be mediated via the activation of AMP-activated protein kinase (AMPK) since the metabolic effects of IL-6 were absent in myotubes infected with dominant negative AMPK α-subunit [43, 125].

It has also been demonstrated that a low dose infusion of IL-6 significantly decreased plasma insulin concentrations further implying that IL-6 has a role as a modulator of fat
metabolism [123]. Interleukin-6 has been shown to mediate anti-inflammatory actions by suppressing the release of TNFα and promoting the production of anti-inflammatory cytokines highlighting its pleiotropic nature. These observations have formed the basis for recent claims that IL-6 and other muscle-derived cytokines (myokines) could play a role in defending type-II diabetes [43].

IL-6 however has also been associated with disease states such as obesity, insulin resistance and type II diabetes mellitus [126]. Indeed there is a positive association of plasma IL-6 concentrations with fasting insulin and fasting insulin resistance index in healthy individuals [62]. Intriguingly, IL-6 pretreatment significantly blunted the insulin response during a hyperinsulinemic-euglycemic clamp in conscious mice [127]. This is supported by in vitro data which demonstrated that exposure to IL-6 inhibits insulin receptor signal transduction and insulin action in hepatocytes [128]. Studies using experimental animals have shown that IL-6 plays a role in body composition. IL-6 knockout mice developed mature-onset obesity, at 6 months of age which was partially reversed with IL-6 replacement [129] although this is controversial, as Di Gregorio and colleagues found that there was no difference in the body composition between IL-6 deficient and wild-type mice [130]. Ryan et al. [131] showed that PPARα and PPARγ agonist (fenofibrate and pioglitazone, respectively) treatment of obese, glucose tolerant men, reduced several pro-inflammatory cytokines, including IL-6.

A recent study by De Lorenzo et al. [132] identified a new syndrome called the “normal-weight obese syndrome”. That study showed that women with ‘normal’ weight and body mass index but whose fat mass was >30% of their total body weight had higher levels of circulating IL-6 and therefore they could be classified into an early proinflammatory state. Taken together these data support a role of IL-6 in the development of insulin resistance and obesity and it is likely that IL-6 (amongst other proinflammatory cytokines) could potentially be regarded as prognostic indicators of obesity and CVD.

**IL-6 ANTAGONISTS**

Interestingly not only is IL-6 known to be elevated in disease states but studies have also implicated an increase in circulating soluble IL-6R (sIL-6R) levels in a number of diseases. These include rheumatoid arthritis [133], inflammatory bowel disease [134] and asthma [135]. Since IL-6 and its receptor are increased in many different diseases it is not surprising that there has been a drive in the development for antagonists of the IL-6Rs and antibodies raised against both IL-6 and its soluble receptor. Inhibition of the interaction of IL-6 and I-L6R is the most obvious target for the development of a small molecule/ antibody antagonist to IL-6 signalling. Since gp130 signalling is initiated via several cytokines and their cognate alpha chain receptors (IL-6R, IL-11R, CNTF-R and CLF-1) and other beta-receptors (LIF-R, OSMR, GPL and Wsx-1), blocking the IL-6/IL-6R interaction would exclusively disrupt IL-6 signalling via gp130 whilst leaving all other signalling pathway intact. However disruption of protein-protein interactions by small molecule inhibitors is extremely difficult to achieve due in part to the nature of protein-protein interfaces. Unlike enzyme cavities, the relative featureless topology of the interacting surfaces of IL-6 and IL-6R makes structure based drug design based methods difficult. CSIRO conducted an extensive search for small molecule inhibitor leads through *in silico* screening of the IL-6R crystal system (Branson & Varghese unpublished data) and assaying potential leads by the response of cannulated rat blood vessels *ex vivo* or in intact animals (Abeywardena et al., unpublished data). However no leads were found to justify further studies in this area. To date there have been few studies reporting on the development of a IL-6R antagonist [136, 137]. There have been positive results reported from Phase I and II clinical trials for the use of an anti-IL-6R antibody for the treatment of rheumatoid arthritis as well as with Phase III randomised clinical trials [138-140]. We are unaware of studies involving either an IL-6 antibody or antagonist treatment on the improvement of cardiovascular disease or related disorders and this may be due to the complex role that IL-6 plays in the development and progression of atherosclerosis, cardiovascular disease, obesity and insulin resistance.

Screening methods for the detection of new agonists and antagonists of the binding events between gp130, IL-6 and IL-6R have been limited. For example Montero-Julian [141] described an ELISA method for the measurement of binding between IL-6 and IL-6R. The assay allowed reproducible measurements of soluble IL-6R in serum or plasma. More recently, Scheller et al. [142] developed an assay that could be used for high throughput screening of peptide and chemical compound libraries that act as antagonists (or agonists) of binding between gp130, IL-6 and sIL-6R. Although competitive binding was shown for high molecular weight variants of gp130 and IL-6, the lower molecular weight (non-peptide) compound that displayed antagonist properties was the non-specific agent, suramin, which is known to act at many signalling pathways.

Derivatives of bufogenin isolated from the skin of the Chinese toad, *Bufo bufo gargarizans* Cantor (“Ch’an Su”), were evaluated for interleukin-6 (IL-6) antagonistic activity due to their growth-inhibitory activities on IL-6-dependent MH-60 cells [143]. Among the low molecular weight, naturally derived compounds, 20S,21-epoxy-resibufogenin formate was the most potent inhibitor of IL-6 dependent cell growth of MH-60 cells [143]. Furthermore, in receptor binding assays, both 20S,21-epoxy-resibufogenin-3-formate and 20S,21-epoxy-resibufogenin-3-acetate dose dependently suppressed IL-6 activity [136, 144]. For potential human application, it is probable that low molecular weight antagonists would be more desirable compared with higher molecular weight compounds.

**SUMMARY**

The data reviewed to date demonstrate that IL-6 plays an integral role in the development of several inflammatory conditions such as obesity, insulin resistance, atherosclerosis and cardiovascular disease. This has lead to IL-6 being a key target for therapeutic intervention. IL-6 activates cell surface signalling via the assembly of IL-6, the IL-6 receptor (IL-6R) and the signalling receptor gp130. IL-6 affects several key physiological processes any of which may contribute to the development of cardiovascular disease (Fig. 3). IL-6 is the key cytokine responsible for the production of CRP, and
also involved in the stimulation of matrix-degrading enzymes such as MMP responsible for the rupture of vulnerable plaques therefore contributing to the precipitation of acute coronary syndromes. A non-conservative polymorphism of the IL-6 signal transducer gp130 has recently been reported to be associated with decreased risk of myocardial infarction whilst the long-term survival after AMI is significantly improved with low systemic level of IL-6, at the time of infarction. Increased serum IL-6 is associated with depressed cardiovascular autonomic dysfunction and the important role of IL-6 play in CHF and the development of post-hemorrhagic vasospasm are well recognised. In addition to exerting effects on cardiovascular events, IL-6 also modulates metabolism which includes lipolysis, fat oxidation, glucose disposal as well as mediating anti-inflammatory effects. This diverse profile of biological actions confirms the pleiotropic nature of IL-6. Further study is required to specifically work out the mechanisms by which IL-6 can be a predictive marker of cardiovascular disease, influence arterial blood pressure, and protect cells against apoptosis whilst also having a role in normal metabolism.

REFERENCES
References 146-148 are related articles recently published.


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