Novel targets for interleukin 18 binding protein

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Abstract

Background—Interleukin 18 (IL18) is related to the IL1 family by structure, receptors, signalling molecules, and function. IL18 induces gene expression and synthesis of tumour necrosis factor (TNF), IL1, Fas ligand, several chemokines, and vascular adhesion molecules. Similar to IL1β, IL18 is synthesised as a biologically inactive precursor molecule lacking a signal peptide. The IL18 precursor requires cleavage into an active, mature molecule by the intracellular cysteine protease, IL1β converting enzyme (ICE, or caspase-1). Inhibitors of ICE activity limit the biological activity of IL18 in animals and may be useful in reducing the activity of IL18 in human disease. However, a constitutively secreted IL18 binding protein (IL18BP) exists which functions as a natural inhibitor of IL18 activity. IL18BP binds IL18 with a high affinity (Kd of 400 pM) and, at equimolar ratios, inhibits 50–70% of IL18; at twofold molar excess, IL18BP neutralises nearly all IL18 activity.

Method—IL18 was investigated for its role in human myocardial function. An ischaemia/reperfusion (I/R) model of suprafused human atrial myocardium was used to assess myocardial contractile force.

Results—The addition of IL18BP to the perfusate during and after I/R resulted in improved post-I/R contractile function from 35% of control to 76% with IL18BP. Also, IL18BP treatment preserved intracellular tissue creatine kinase levels (by 420%). Because active IL18 requires cleavage of its precursor form by ICE, inhibition of ICE attenuated the depression in contractile force after I/R (from 35% of control compared with 75.8% in treated atrial muscle, p<0.01).

Conclusion—Myocardial ischaemia is a target for IL18BP and use of IL18BP may thereby reduce ischaemia-induced myocardial dysfunction.

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Interleukin (IL) 18 was first described as an interferon (IFN) γ-inducing factor[1] which circulated during endotoxaemia in mice preconditioned with an infection of *Propionibacterium acnes*. Because of its ability to induce IFNγ, IL18 is by default a member of the T cell helper type I (Th1)-inducing family of cytokines (IFNγ, IL2, IL12, IL15). However, because antibodies to IL18 also reduced the hepatotoxicity of endotoxaemia, IL18 was considered to possess other biological properties beyond that of inducing IFNγ. It has become clear that IL18 is a proinflammatory cytokine and that its mechanism of action can be independent of its ability to induce IFNγ.

IL18 is related to IL1β more than any other cytokine. The similarities between IL1β and IL18 exist at several levels. Firstly, the IL18 precursor form (proIL18), like proIL1β, does not contain a signal peptide, and proIL18 requires cleavage to an active cytokine by the IL1β converting enzyme (ICE, caspase-1). IL1β and IL18 are structurally related because both cytokines are primarily all β-pleated sheet folded molecules.[2] This structural relationship is significant because there are very few all-β sheet molecules among the different cytokines.

At the receptor level, the activity of IL18 is through a heterodimeric complex, the IL18 receptor (IL18R) complex. The IL18R binding chain is termed IL18Rα. IL18Rα is a member of the IL1 receptor family,[3] previously identified as the IL1R related protein (IL1Rrp).[4] A signalling chain (IL18Rβ), also termed accessory protein-like (AcPL),[5] is related to the IL1R accessory protein.[6] Although similar to the IL1 receptor accessory protein in that the IL18Rβ does not itself bind its ligand in solution, the IL18Rβ chain is part of the IL18 receptor complex. After binding of IL18 to the IL18Rα, the IL18R AcPL binds to form a high affinity heterodimeric complex with the ligand. The high affinity IL18R complex recruits the IL1 receptor activating kinase (IRAK), resulting in phosphorylation of nuclear factor κB (NFκB)-inducing kinase (NIK) with subsequent translocation of NFκB to the nucleus.[7] Initially identified as part of the IL1R signalling events,[8] IRAK is recruited to the IL1R complex after exposure to IL1. In cells possessing both the IL18Rα and β chains, nuclear translocation of NFκB is seen after incubation with IL18, and this property helps to explain the pleotropic nature of IL18. In IL18 deficient mice, production of IFNγ and cytotoxic T cells is markedly diminished despite ample amounts of IL12.[9] A similar finding exists in mice deficient in ICE.[10 11] The role of IL12 in IFNγ production therefore seems to require IL18.

Constitutive production from monocyte/macrophage

The production of IL18 in freshly obtained human peripheral blood mononuclear cells (PBMC) and in splenic macrophages from non-preconditioned mice has been studied.[12] Reverse transcriptase polymerase chain reaction and western blot analysis were used to compare the production of IL18 with that of IL1β in the same preparations.

There is no constitutive gene expression for IL1β in freshly obtained human PBMC from healthy donors using over 40 cycles of PCR.[13 14]

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Surprisingly, the same PBMC expressed constitutive mRNA for IL18. This was also seen using western blot analysis for proIL18 in lysates from the same PBMC which contained no proIL1β in the same cells. Constitutive IL18 gene expression and the presence of proIL18 protein were also observed in freshly obtained murine splenocytes.[12] The promoter regions for IL1β and IL18 gene expression have been studied and may provide an insight into these observations. The promoter for IL18 is TATA-less and IL18 promoter activity upstream of exon 2 acts constitutively.[15] Therefore, it is not unexpected that IL18 mRNA is constitutively expressed even in whole blood freshly obtained from healthy donors where no artefact is introduced. The additional finding that the 3' untranslated region of human IL18 lacks the AUUUA destabilisation sequence[15] is also consistent with these observations. This would allow for more sustained levels of the polyadenylated species and translation into protein. Daily injections of IL12 into mice result in high circulating levels of IFNγ, which is completely prevented by prior treatment with anti-IL18 antibodies, or absent in mice deficient in ICE.[11] In this model, IL12 may activate ICE. Osteoclasts also produce IL18,[16] and regulation of bone density may be a property of IL18, as it is for IL1β.

In addition to monocyte/macrophages, the epidermal cells from mouse skin produce IL18 constitutively.[17] The keratinocyte is the major source of the IL18. Moreover, contact sensitisers increased IL18 synthesis, whereas skin irritants did not. A murine keratinocyte cell line (PAM 212) also produced IL18 constitutively.[17] IL18 has also been found constitutively expressed in colonic specimens and isolated mucosal cell populations from patients with Crohn's disease.[18] Similar to IL1, IL18 is found in the rat adrenal cortex and pituitary gland.[19] The adrenal IL18 was increased by cold stress, whereas the pituitary IL18 was not. Two transcripts of adrenal IL18 were sequenced: an abundant form was clearly the rat homologue of mouse IL18 and shared 91% amino acid homology. The other transcript was less abundant. It had a 59 base pair deletion and the corresponding 19 amino acids at the 3' end of the molecule.[19] Possibly, this truncated rat IL18 molecule may act as a receptor antagonist.

IL18 induces cytokines and chemokines
There is no dearth of evidence for the role of chemokines in promoting inflammation. For example, the neutrophil-activating chemokine IL8 is a primary agonist in several pathological processes of the lung. IL18 stimulates the production of IL18 from human NK cells as well as other CXC and CC chemokines.[20] In the HIV-1 infected macrophage cell line U1, which is derived from the histiocytic lymphoma cell U937, IL18 induces IL8.[21] In addition, the myelomonocytic leukaemia cell line KG-1 also releases IL8 when stimulated with IL18.[22] The ability of IL18 to induce CC and CXC chemokines places IL18 in a strategic role in inflammation. When human PBMC were used,

IL18 induced IL8 gene expression and synthesis.[20] When IL1 receptor antagonist was used to block IL1 activity in these cells, IL18-induced IL8 production was reduced by 40%. When tumour necrosis factor (TNF) activity was inhibited, IL18-induced IL8 production was reduced by 80%, suggesting that the primary action of IL18 was through a TNF dependent pathway. The source of TNF was the CD3+ and the natural killer cells. The induction of TNF can be seen at the level of gene expression within two hours after exposure to IL18. IL18 also stimulates the synthesis of macrophage chemoattractant protein 1 in these cultures.[20]

Using neutralising anti-IL18 antibodies, Netea *et al* compared the myeloperoxidase activity in lungs and livers of mice after endotoxaemia.[23] ICE deficient mice were completely resistant to lethal endotoxaemia induced by lipopolysaccharide (LPS) derived from either *Escherichia coli* or *Salmonella typhimurium*. IFNγ deficient mice were not resistant to *S typhimurium* LPS, suggesting an IFNγ-independent role for IL18. Anti-IL18 protected mice against a lethal injection of either LPS and also reduced neutrophil accumulation in liver and lungs. The increased survival was accompanied by decreased levels of chemokine, macrophage inflammatory protein 2.[23]

IL18 up regulates expression of Fas ligand
IL18 enhances Fas ligand expression and induces apoptosis in Fas-expressing human myelomonocytic KG-1 cells.[22] KG-1 cells are monocyte-like and also produce the intercellular adhesion molecule-1 (ICAM-1). IL18 increases ICAM-1 expression in these macrophagic cells.[24] These cells also express constitutively Fas antigen (CD95) and, after exposure to IL18, KG-1 cells became apoptotic. Fas ligand is up regulated in T cells and NK cells after incubation with IL18.[25] In the liver, cytotoxic T cells are thought to contribute to the destruction of hepatic cells, particularly when infected by viruses. Cytotoxic T cells and NK cells isolated from the liver and then stimulated with IL18 exhibit enhanced killing of Jurkat T cells.[26] The IL18-induced enhanced killing by liver derived cytotoxic T lymphocytes was through a perforin dependent pathway and not CD95.[26] Liver derived NK cells also exhibited enhanced killing of Jurkat cells after exposure to IL18, but the mechanism was thought to be independent of TNF. However, failure to detect TNF in cell culture supernatants is not an argument that any cellular change is TNF independent because membrane forms of TNF are biologically active but often hardly measurable in supernatant. In fact, the ability of IL18 to induce HIV-1 in U1 cells[21] and IL8 in PBMC[20] is TNF dependent. In both studies, the dependence on TNF was shown by reducing the IL18 effect with the soluble TNF p55 receptor. Therefore, it is likely that IL18-induced killing by liver NK cells may still be due to induction of membrane TNF.

In cockroach allergen-induced airway response, IL18 levels were observed within the lungs.[27] When IL18 was instilled into the

airway at the time of allergen challenge, a significant increase in peribronchial eosinophil accumulation was seen in both allergic and non-allergic mice. After IL18 instillation, there was a significant increase in eotaxin, but not other eosinophil chemotactic factors, in bronchoalveolar lavage fluid.[27] The role of eotaxin was confirmed using eotaxin −/− mice, which demonstrated significantly less eosinophil accumulation than control mice.

IL18 binding protein

Methods established for the purification and characterisation of ligand binding cytokine receptors in human urine[28–31] were used to purify a 38 kDa IL18 binding protein to homogeneity from urine using ligand affinity chromatography, and the N-terminal amino acids were determined.[32] The amino acid sequence of the IL18 binding protein (IL18BP) is not that of a known cytokine receptor presently listed in the data banks. Although this binding protein probably represents the extracellular domain of the ligand binding receptor chain, to date a transmembrane and cytosolic component to the IL18BP has not been found. Even genomic clones do not contain sequences that would translate into a transmembrane domain for insertion into the cell membrane.[32] Therefore, IL18BP is a constitutively produced inhibitor of IL18. However, from its structure and function, it has probably been at one time in evolution, a cell associated membrane receptor.

Similar to neutralising antibodies to IL18,[10] the IL18BP prevents LPS-induced IFNγ production.[32] Also, similar to anti-IL18 antibodies, IL18BP does not affect IFNγ production after stimulation with mitogens such as concanavalin A. However, unlike antibodies to IL18, the IL18BP does not exhibit species specificity, and hence human IL18BP neutralises both human and murine IL18.[32] With a single immunoglobulin domain, IL18BP resembles the extracellular segment of cytokine receptors with immunoglobulin-like structures; however, IL18BP is a novel protein distinct from the IL1 and IL18 receptor families. Located in chromosome 11q3 at the inverted position of the nuclear mitotic apparatus protein-1, the human IL18BP gene encodes for at least four distinct isoforms (IL18BPa, b, c, and d), each derived from a single mRNA by splice variants. They differ primarily in their carboxyl termini, whereas at the N-terminal one third to two thirds of the amino acids are identical.

A BIAcore sensor chip with immobilised human IL18 bound IL18BP. The mean dissociation constant for human IL18BPa was 0.399 ± 0.034 nM. The binding of increasing amounts of IL18BPa to immobilised human mature IL18 reveals a rapid on-rate and a markedly slow off-rate.[33]

IL18BP circulates in healthy humans at concentrations of 2.15 (0.15) ng/ml (range 0.5–7).[34] In general, the molar excess of IL18BP to IL18 is in the order of 20–30-fold greater. Given the high affinity of IL18BP for neutralisation of IL18 (400 pM), it seems that IL18BP

is an effective inhibitor of immune and inflammatory disease associated with increased production of IL18.

Cytokines in the heart

It must be emphasised that measuring cytokines in human disease and making direct correlations between any particular cytokine and any particular disease is not proof that the cytokine has a causal role in the disease process. This is also the case with assessing the role of cytokines in the pathogenesis of an atherosclerotic lesion of the coronary artery, an acute ischaemic event associated with myocardial infarction, progression of myocardopathies, or loss of myocardial function in congestive heart failure. Nevertheless, a highly significant correlation of the level of a particular cytokine or cytokines with severity of disease contributes to an understanding of the role of cytokines in that disease process. Two experimental approaches do provide absolute evidence of a causal role of any particular cytokine in the pathogenesis of a particular disease. Firstly, in animal models of the disease, administration of highly specific cytokine antagonists reduces the severity of the disease. In heart disease, several animal models exist which have application to human disease. Animal models include gene deletion studies or transgenic mice overexpressing specific genes. Secondly, specific blockade or neutralisation of some cytokines—namely IL1, IL18, or TNF, is now possible in patients.

In studies on the effects of cytokines on myocardial function in septic shock, the data have been primarily physiological and derived from experimental models of septic shock. In those animal models, intravenous infusion of IL1 or TNF, but particularly the combination of IL1 and TNF, exhibited a profound ability to suppress myocardial function.[35] The local production of cytokines from the myocardium itself (as would take place in ischaemia) is more likely to affect myocardial function at concentrations that are clinically relevant. For example, in human atrial muscle strips, suspended in an oxygenated balanced salt buffer and electrically paced, contractile function as measured by developed force is dramatically reduced by the presence of <10 pg/ml of exogenous TNFα in the bath.[36] IL1β induces a similar reduction in developed force at similar low concentrations. Therefore, the human heart appears to be exquisitely sensitive to the direct effects of IL1 and TNF, and in both cases these cytokines depress function.

The first evidence that IL1 and TNF directly suppress myocardial function came from studies on human serum of patients with acute septic shock marked reversible myocardial depression.[37] Using immunoabsorption, removal of both TNFα and IL1β (but not either alone) from these sera resulted in elimination of serum myocardial depressant activity. IL2, IL4, IL6, IL8, IL10, or IFNγ failed to cause significant cardiac myocyte depression over a wide range of concentrations. Individually, TNFα and IL1β each depressed peak velocity and

myocyte shortening in vitro, but the combination of TNFα and IL1β depressed myocardial cell contractility at substantially lower concentrations, consistent with a synergistic effect.[37] The role of nitric oxide (NO) and cyclic guanyl monophosphate (cGMP) in depression of myocardial contractility induced by either IL1β, TNFα, or the combination of these cytokines, was investigated.[38] Inhibitors of NO synthase such as N-methyl-L-arginine (L-NMA) and methylene blue for inhibition of guanylate cyclase prevented cytokine induced myocardial suppression; an excess of L-arginine with L-NMA restored the effect. In addition, TNFα, IL1β, or TNFα plus IL1β induced NO formation in myocyte cultures; moreover, serum samples from patients with acute septic shock also induced NO production.

In considering the direct effects of IL1, IL18, or TNF on myocardial function, one should keep in mind several pathogenic situations—namely, (*a*) the local production of IL1, IL18, and TNF from resident myocardial macrophages; (*b*) the ability of ischaemia to induce the release of IL1, IL18, or TNF from these macrophages; (*c*) the level of IL1, IL18, and TNF receptor expression on myocytes; and (*d*) relevant post-receptor events after IL1, IL18, or TNF in triggering of their respective receptors on myocytes.

IL18 in the heart

During ischaemia, as well as reperfusion, numerous endogenous mediators, such as small molecule second messengers, are produced which affect myocardial function. Within minutes of an ischaemic episode, myocardial contractile force diminishes and the overall recovery of contractile force is largely dependent on the duration of the ischaemic period.[39] For example, during an ischaemic event, Ca^{2+} homeostasis is perturbed, oxygen derived free radicals are generated, and nitric oxide (NO) synthesis and release takes place. In addition, there is also local production of cytokines, particularly TNFα and IL1β.[40] In the intact heart, these cytokines contribute to ischaemia-induced myocardial dysfunction by inducing gene expression for inducible NO synthase (iNOS), cyclo-oxygenase-2 (COX-2), and phospholipase A_2, as well as vascular adhesion molecules and several chemokines. As a result, there is immediate depression of myocardial contractile force mediated by small molecule messengers followed by cytokine mediated neutrophil infiltration, which further damages heart muscle. Animal hearts studied in the absence of blood or blood products elaborate TNFα[41] and IL1β during an ischaemic challenge. Cardiomyocytes also lose contractile force owing to the action of these endogenous cytokines.[42]

Most of the experimental data concerning TNFα and IL1β mediated myocardial dysfunction are derived from animal studies. However, human myocardial tissues obtained from patients undergoing elective cardiopulmonary bypass procedures have been studied under controlled, ex vivo conditions.[43 44] In this experimental model, human atrial trabeculae are suspended in a blood-free, physiologically oxygenated buffer bath and then exposed to an episode of simulated ischaemia. During this time, contractile force decreases dramatically; when the tissue is re-exposed to oxygen, the contractile force returns but is diminished (60–70% reduction), and evidence of myocardial damage is seen by release of creatine kinase.[43 44] When TNF bioactivity is specifically neutralised during ischaemia/reperfusion (I/R), a greater return of contractile force is seen, suggesting that endogenous myocardial TNF activity contributes to the contractile dysfunction induced by the ischaemic event.[45]

We asked whether the cytokine IL18 contributes to human ischaemia-induced myocardial dysfunction. As discussed above, IL18 is a proinflammatory cytokine structurally and functionally related to IL1β.[2 46 47] Both IL1β and IL18 are initially synthesised as inactive precursors requiring ICE for cleavage to mature, biologically active molecules.[48 49] Although IL1β and IL18 have distinct cell surface receptors, the receptor chains for each cytokine are members of the same receptor superfamily,[3 5] and signal transduction is similar.[7] To assess a role for endogenous IL18 in the heart, a specific, natural inhibitor of IL18 activity, IL18BP, was added to the suprafusing bath during I/R.

Neutralisation of endogenous IL18 with IL18BP prevents the loss in post-ischaemic developed force

Control human atrial trabeculae were suprafused under normoxic conditions throughout the experiment. Under these conditions, the developed force in the control trabeculae is reduced (10%). Trabeculae subjected to 20 minutes of ischaemia exhibit a rapid decline in contractile function; upon reperfusion, contractile force returns to approximately 25% of the control developed force. In contrast, trabeculae exposed to ischaemia, but in the presence of IL18BP, returned to 55% of the control developed force.[50]

These results suggest that I/R leads to release of biologically active IL18 after processing the endogenous precursor IL18 by ICE. Therefore, IL18 was measured in freshly obtained atrial tissue. Basal IL18 was present in trabeculae obtained before the insertion of the pump-oxygenator cannula into the right atrium. After 90 minutes of equilibration, 30 minutes of ischaemia, and 45 minutes of reoxygenation, trabeculae were homogenised and IL18 levels determined. A 4.5-fold increase in IL18 in the post-I/R tissue was found.[50]

Steady state mRNA levels for IL18 and IL18BP were also determined in these tissues. We observed basal gene expression for IL18 and IL18BP in the freshly obtained, pre-ischaemic atrial homogenates. Similar to the increase in IL18 protein, I/R induced a further increase in steady state IL18 mRNA levels (4.7-fold increase). Gene expression for IL18BP was also present in freshly obtained atrial tissue and increased only modestly (1.3-fold) after I/R.

Atrial tissue was obtained just before insertion of the pump-oxygenator cannula and immediately snap frozen. IL18 was localised in macrophages and endothelial cells before any operation-related ischaemia took place and also was present in the absence of contact with any foreign surfaces. The localisation of IL18 in resident macrophages and endothelial cells is consistent with previous studies of constitutive preformed precursor IL18 in freshly obtained human peripheral monocytes from healthy subjects.[12] Therefore, we conclude that preformed precursor IL18 exists in the myocardium of patients scheduled for coronary artery bypass for ischaemic heart disease.

ICE inhibition also reduces post-ischaemic developed force

Because IL18BP effectively attenuated ischaemia-induced myocardial dysfunction, we suggested that inhibition of the conversion of preformed precursor IL18 to mature IL18 would also attenuate ischaemia-induced myocardial dysfunction. Therefore, the specific ICE inhibitor YVAD was added to the suprafusion bath before the onset of ischaemia. ICE inhibition by the addition of YVAD was continued throughout the ischaemic period and during reperfusion. Inhibition of ICE resulted in attenuation of ischaemia-induced myocardial dysfunction as shown by the improvement in contractile function from 35% of control in I/R to 60% at 10 μg/ml and 75.8% at 20 μg/ml.[50] These results confirm that biologically active IL18 in human myocardium is the result of cleavage of preformed precursor IL18 by ICE. In addition, these results suggest that myocardial ischaemia may activate latent ICE.

Implications of IL18 blockade in ischaemic heart disease

The generation of oxygen derived free radicals, nitric oxide, calcium overload, or decreased responsiveness of the myofilaments to calcium may each contribute to post-I/R contractile dysfunction.[39] In addition to these immediate acting mediators, the relationship of cytokines to post-I/R myocellular dysfunction remains unclear. The data in the present studies suggest that IL18 and IL1β are processed and released from their endogenous precursor forms in human heart tissue during ischaemic injury and function to suppress contractile force. Furthermore, the processing of the precursors appears to be ICE dependent, and latent ICE is probably activated by ischaemia. Previously, neutralisation of endogenous TNFα was shown to protect human trabeculae from ischaemia-induced dysfunction.[44] At present, it is likely that a combination of IL18, IL1β, and TNFα accounts for the ischaemia-induced dysfunction.

Oxygen metabolites present after ischaemia depress myocardial contractile function in several animal models, both in vitro and in vivo.[39] The source of the oxygen radicals is unclear, though xanthine oxidase may be an important mediator of oxy radical production.[51] Oxy radicals may interact with cellular proteins, lipids, calcium, and myofilaments to induce contractile depression. In addition to xanthine oxidase, TNFα is an inducer of oxygen metabolites. Recent data indicate that IL18 primes human neutrophils for superanion production.

Ischaemia is a direct stress signal to the myocyte and as a result, gene expression of stress related molecules is increased. For example, after 15 minutes of ischaemia in rodent hearts perfused with Kreb's buffer, TNFα gene expression is up regulated.[40] However, the sudden and marked reduction in atrial trabecular function in the present study is apparent within minutes and it is unlikely that cytokines account for the early dysfunction. During reperfusion, however, the failure to return completely to functionality appears to be cytokine mediated because specific cytokine blockade or neutralisation restores functionality to a greater degree than ischaemic controls. Depressed function during reperfusion may be due to oxygen radical-induced loss of myocyte integrity, increased production of NO, or altered calcium flux. Therefore, do IL1β or IL18, or both, trigger the above changes? The addition of IL1β to oxygenated human trabeculae suppresses function,[36] and it is known that IL1β induces NOS in cardiac myocytes.[52] However, it is not known if IL18 acts similarly.

Nitric oxide is a myocardial depressant. However, the effect of of NO after ischaemia is controversial. This controversy stems from the different tissue levels of NO present depending on which pathway of NO synthesis is activated. Lower levels of NO resulting from synthesis through the constitutive NOS pathway seem to protect the myocardium,[53] whereas the NO produced from inducible nitric oxide synthase (iNOS), which is significantly higher, leads to myocardial injury.[54] After a moderate ischaemic insult, induction of iNOS occurs in the rat myocardium, followed by increased NO production.[55] This NO subsequently leads to myocardial contractile depression. Using the same trabeculae model as the present study, Cain *et al* showed that specific inhibition of nitric oxide synthase attenuated TNFα- and IL1β-induced human myocardial dysfunction.[36] As previously discussed, endogenous TNFα accounts for some of the post-ischaemic myocardial dysfunction. There are numerous hypotheses as to how TNFα mediates ischaemia-induced myocardial dysfunction. Finkel *et al*, showed that TNFα induced contractile dysfunction in isolated hamster papillary muscle.[54] This effect was abolished with inhibition of NO synthase. Nitric oxide has been shown to have a role in TNFα-induced myocardial dysfunction through desensitisation of the myofilaments to calcium.[52] In addition, TNFα may also lead to phosphorylation of troponin, which further desensitises the myofilaments to calcium.

Calcium is a vital mediator of myocardial contractile function. Changes in intracellular Ca^{2+}, cellular calcium overload, and modulation of the myofilaments' response to Ca^{2+} each affect contractile force. Most investigation has focused on the role of calcium as the effector of

myocardial contractile dysfunction. The relationship between myocardial calcium changes and myocellular contractile dysfunction has been well described.[39] After an I/R injury, the myofilaments' responsiveness to calcium decreases and is thought to account for most of the decrease in contractile function after ischaemia. In addition to calcium overload, an ischaemic insult leads to the production and activation of intracellular calcium dependent proteases. Upon activation, these proteases begin intracellular myofilament proteolysis, leading to post-ischaemic contractile dysfunction. Given the protection afforded by the anticytokine interventions in the present study, it is likely that IL1β or IL18, or both, alter intracellular calcium homeostasis during and after ischaemia.

Although mature IL1β has been shown to suppress function directly when added to human atrial trabeculae,[36] it has not been shown whether endogenous IL1β in the heart participates in ischaemia-induced dysfunction. In the present study, inhibition of IL1β activity by IL1 receptor blockade suggests that biologically active endogenous IL1β is present in the heart after ischaemia. Furthermore, the formation of active IL1β in the ischaemic heart is ICE dependent. The data are consistent with the concept that synthesis of the precursor for IL1β, and activation of ICE, take place during I/R.

The studies support the concept that human atrial myocardium is highly sensitive to IL18 and IL1β and that the combination of these two cytokines seems synergistically to depress myocardial function. We have previously shown that the presence of exogenous IL1β or TNFα decreases contractile force in human trabeculae in the absence of ischaemia.[36] In addition, the presence of these two cytokines together has a synergistic effect on the depression of myocardial contractility. Furthermore, we have preliminary data to suggest that exogenous IL18, under normoxic conditions, also depresses myocardial contractile function.

The ability of ICE inhibition to reduce post-ischaemic dysfunction suggests that the processing of precursor IL1β and IL18 is necessary for cytokine mediated myocardial suppression. The immunohistochemical studies showed that IL18 is preformed in the resident macrophages and endothelial cells of atrial tissues from patients with ischaemic heart disease, but it is not clear if precursor IL1β is also preformed. However, IL1β mRNA is rapidly increased in rat hearts within 15 minutes after an ischaemic insult,[40] and therefore it is likely that there is also increased precursor IL1β synthesis in atrial trabeculae during ischaemia. Ischaemia itself may be an activator of latent ICE activity in heart tissue. Several investigators have reported that ICE inhibition during myocardial I/R injury in animals reduces apoptotic cell death. The criteria used for determining cell death were DNA fragmentation and cleavage of poly ADP-ribose polymerase.[56–58] Importantly, the present studies expand these observations by demonstration that ICE inhibition preserves functionality

within the immediate post-I/R injury. ICE inhibition also preserves cell viability because creatine kinase levels remained high in post-ischaemic tissues treated with an ICE inhibitor.

IL1β and TNFα have also been implicated in the pathogenesis of human myocardial suppression in sepsis.[37 59] The mechanism(s) by which IL1β and TNFα induce contractile dysfunction has also been linked to NO and changes in cellular calcium handling.[59] In addition, inhibition of the sphingomyelin signalling pathway abrogated TNFα/IL1β-induced myocardial contractile dysfunction.[36] Although the present study does not deal with the role of NO in IL18 mediated, ischaemia-induced dysfunction, TNFα depresses the myocardium in a NO dependent pathway.[44] Blockade of IL1 receptors revealed a role for endogenous IL1β in the I/R injury, a finding that was not unexpected given the large amount of animal data. That endogenous IL18 also plays a part in the injury was unexpected but based on the fact that IL18BP only neutralises mature IL18.[32 33] Because ICE inhibition prevents the cleavage of both precursor IL1β and IL18, it would not be surprising if IL18 and IL1β acted synergistically in suppressing myocardial function. In fact, specific TNFα, IL1β, and IL18 blockade each attenuate, but do not entirely reverse, I/R-induced myocardial dysfunction, suggesting that the three cytokines act together to suppress myocardial function.

The ability to modulate or interrupt cytokine signalling has been demonstrated in numerous disease states in both animal models and clinically in humans—for example, rheumatoid arthritis and Crohn's disease. IL1β and TNFα have both been associated with acute myocardial dysfunction and hence are logical targets in patients. However, IL18, a new member of the IL1 super family, has to date not been associated with myocardial dysfunction but should be considered a target for ischaemic treatment based on the present studies. IL1β and IL18 share numerous properties, including cleavage of the inactive precursor forms to the active forms by ICE (caspase-1). Given the myocardium's response to proinflammatory cytokines and the ability to interrupt the inflammatory process we suggest that the inhibition of caspase-1 protects ischaemia-induced human myocardial dysfunction through the inhibition of IL1β and IL18 processing.

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