Interleukin-18 mediates interleukin-1-induced cardiac dysfunction

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Heart failure (HF) is a chronic and deadly disease that impairs the quality of life of millions of people in the developed as well as underdeveloped world (19, 28). This condition requires lifelong treatment; however, for most patients, treatment reduces severity but fails to stop worsening of the disease. In the past years, an inflammatory component has been recognized to contribute significantly to HF, associated with its progression and correlating with its severity (6, 21). The inflammatory state in HF is characterized by elevated levels of cytokines, chemokines, enzymes, and inflammatory mediators such as nitric oxide (NO) and ROS (6, 21, 23). Understandably, the complexity-contributing mediators render the determination of which signals directly contribute to the pathological process to progressive HF and which are secondary products of the disease difficult.

IL-1 is an inflammatory cytokine produced in response to local or systemic stimuli (14, 16). There are two genes coding for IL-1, IL-1α and IL-1β, and both trigger the same receptor and have near-identical properties (14, 16). Whereas IL-1α is constitutively present in near all tissues, IL-1β is first synthesized as an inactive precursor and requires the intracellular enzyme caspase-1 for cleavage and secretion of the active cytokine (14, 16). IL-1β is associated with the development of coronary heart disease, myocardial remodeling, and HF, as evidenced by various preclinical models and human trials (14, 16, 21, 45). IL-1β is a known cardiodepressant factor, reducing cardiomyocyte contractility in vitro and in vivo (13, 24, 44, 45). Elevated levels of circulating IL-1β are present in patients with HF, which is thought to contribute to exercise intolerance (44).

Several mechanisms by which IL-1 suppresses heart function have been studied (10, 13, 24, 27, 44, 45). IL-1β depresses the function of isolated human atrial trabeculae in a dose-dependent manner, an effect mediated by sphingosine and NO (10). In experimental studies in vivo, the contractile function falls a few hours after exposure to the cytokine, a time course compatible with the production of a second mediator (10, 13, 24, 27, 44, 45). This second mediator could be IL-18, which has increased expression in the hearts of patients with HF (26). In fact, IL-18 is induced by IL-1 (34), and, like IL-1, IL-18 has been shown to mediate myocardial hypertrophy, dysfunction, and fibrosis after chronic administration in the mouse (35, 47). In contrast to IL-1, IL-18 fails to induce the systemic inflammatory events seen with IL-1, such as fever and neutropenia (25, 36, 39, 48). The similarities and differences between IL-18 and IL-1 and, in particular, the overlapping cardiac phenotype and evidence of increased IL-18 expression in HF, led us to hypothesize that IL-18-induced contractile dysfunction could be mediated by IL-18.

METHODS

Animal and treatments. All animal experiments were conducted under the guidance of the guidelines on the humane use and care of laboratory animals for biomedical research published by the National Institutes of Health (Pub. No. 85-23, Revised 1996). The Institutional Animal Care and Use Committee of Virginia Commonwealth University approved this study. Ten-week-old male outbred Institute of Cancer Research (CD-1) mice supplied by Harlan Laboratories (Frederick, MD) were randomly assigned to different treatment groups. Genetically modified mice lacking the gene for IL-18 (IL-18 knockout KO) mice; B6.129P2-H18Rtm1AkiJ) or the gene for the IL-18 receptor (IL-18R) α-chain (IL-18R KO mice; B6.129P2-H18Rtm1AkiJ) as well as wild-type (WT) C57BL/6J control mice were supplied by Jackson Laboratories (Bar Harbor, ME).
Mice were treated with a single intraperitoneal injection of murine IL-1β (3 mg/kg, Millipore, Billerica, MA), murine IL-18 (0.25, 2.5, 25, and 125 μg/kg, MBL, Woburn, MA), or vehicle [NaCl (0.9%, 0.2 ml), Baxter, Deerfield, IL] administered alone or in combination with recombinant human IL-1 receptor (IL-1R) antagonist [IL-1Ra (10 mg/kg ip), anakinra, Kinetics, SOBI, Stockholm, Sweden] given to block IL-1R, IL-18-binding protein (IL-18BP; clinical grade at 1 and 10 mg/kg) (32), or IL-18-blocking antibody [IL-18AB (5 mg/kg, ip), Rockland Immunocchemicals, Gilbertsville, PA] used to block IL-18. Z-WEHD-FMK, a caspase-1 inhibitor [Z-Trp-Glu(OMe)-His-Asp(OMe)-FMK (3 mg/kg), 0.1 ml ip, R&D Systems, Minneapolis, MN] was also given to inhibit caspase-1 and the conversion of pro-IL-18 to IL-18. NaCl (0.9%, 0.2 ml) was used as a vehicle for recombinant human IL-18BP or IL-18AB, DMSO (0.1 ml) was used as a vehicle for Z-WEHD-FMK, and a rabbit antibody against human Fc (5 mg/kg, Thermo Scientific, West Palm Beach, FL) was used as a control for IL-18AB. In consideration of the preferential signaling of IL-18 through p38 MAPK (48), we used two different p38 MAPK inhibitors, SB-202190 (2 mg/kg in 0.1 ml ip, Sigma-Aldrich, St. Louis, MO) and SD-169 (30 mg/kg in 0.1 ml ip, Tocris Bioscience, Minneapolis, MN) to determine the effects of such inhibitors on IL-1-induced cardiac dysfunction.

Other groups of mice were injected intraperitoneally with plasma (200 μl) collected from patients with acute decompenesated HF (ADHF). ADHF patients were selected based on hospitalization with a diagnosis of ADHF in the previous 24 h, a documented LV ejection fraction of <40%, and high blood levels of high-sensitivity C-reactive protein (>2 mg/l), which works as a surrogate marker of IL-1β activity. Collected plasma was stored at −80°C, and part of it was already used in a previous study (44) aimed at the determination of the effectiveness of IL-1Ra therapy in the treatment of HF patients. Plasma from healthy patients was used as a control. The study design and protocol received approval from the Institutional Review Board of Virginia Commonwealth University, and all patients provided written informed consent.

In a subset of experiments, ADHF plasma was preincubated for 30 min with IL-18BP (50 μg/ml of ADHF plasma) at 37°C to neutralize the activity of any human IL-18 present in the plasma before the injection to the mice; this concentration was calculated to be the one present in the extracellular fluid after injection of 300 μg recombinant human IL-18BP in an animal of 30 g mass (equal to 10 mg/kg) and having a 20% (vol/wt) of extracellular fluid (6 ml).

Doppler echocardiography. LV fractional shortening (LVFS) was measured using M-mode echocardiography in all the animals under light anesthesia (50 –70 mg/kg pentobarbital). Echocardiography was performed with the Vevo 770 Imaging System (Visual Sonic, Toronto, Ontario, Canada) and a 30-MHz probe as previously described (2, 41). LVFS was calculated as the ratio of (LV end-diastolic diameter – LV end-systolic diameter)/LV end-diastolic diameter.

For IL-18-treated mice, echocardiography was performed at baseline and repeated at 1 h, 4 h, 24 h, 7 days, and 14 days. For IL-1β- and ADHF plasma-treated mice, echocardiography was performed at baseline and after 4 h. Results are expressed as percent changes in LVFS at each time point compared with baseline.

LV catheterization. LV pressures were measured invasively to assess the effects of IL-1β. Mice were deeply sedated (50–70 mg/kg pentobarbital), and a pressure probe catheter (Millar Instruments, Houston, TX) was retrogradely inserted in the LV through the left carotid artery to measure LV end-diastolic pressure. LV peak systolic pressure, +dP/dt, and –dP/dt. Measurements were recorded and analyzed with Labchart Pro software (Millar Instruments).

ELISA. Blood was collected from mice through a heart puncture at the time of death. Blood was incubated in tubes with silica act clot activator (BD, Franklin Lakes, NJ) for 15 min and then centrifuged at 2,000 rpm at 4°C for 10 min to obtain serum. Alternatively, blood was obtained in heparin tubes (BD) and then centrifuged at 2,000 rpm at 4°C for 10 min for plasma. Samples were stored at −80°C and subsequently analyzed with a specific ELISA for murine IL-18 (serum, MBL) and for mouse IL-6 (plasma, R&D Systems) accordingly to the supplier’s instructions to assess the induction of IL-18 and IL-6 in plasma after IL-1β challenge. Absorbance was read with a Bio-Tek plate reader (model μQuant, Bio-Tek, Winooski, VT) at 450 nm. Data are expressed in picograms per milliliter.

Statistics. Statistical analysis was completed using SPSS 19.0 software (IBM, Armonk, NY). Values are presented as means ± SE. Student’s t-test or ANOVA test followed by a Bonferroni-corrected Student’s t-test was used to compare data between two or more groups, respectively. Calculations were completed using the SPSS 15.0 package for Windows (SPSS, Chicago, IL).

RESULTS

**IL-18 blockade prevents the systolic dysfunction induced by HF plasma.** Plasma derived from patients with ADHF induces acute cardiac dysfunction through an IL-1-dependent mechanism (44). In the present study, we hypothesized that biologically active IL-1β present in the plasma of patients with ADHF could cause cardiac dysfunction through the induction of IL-18 in the mouse. IL-18BP is a natural occurring protein that binds and neutralizes IL-18 (32). Clinical grade recombinant human IL-18BP (40), which binds both human and murine IL-18, was injected intraperitoneally 30 min before the administration of plasma from patients with ADHF. As shown in Fig. 1, IL-18BP prevented the reduction in LVFS compared with mice without IL-18BP pretreatment (P < 0.001), suggesting that the dysfunction induced by the plasma of patients with ADHF is mediated by IL-18 produced in the mouse (Fig. 1). Plasma from healthy patients (Fig. 1), vehicle, and IL-18BP alone had no effect on LVFS.

To test whether the effects observed with IL-18BP might be due to blockade of IL-18 present in ADHF plasma rather than blockade of IL-18 produced by the mouse, ADHF plasma was first mixed with IL-18BP at a concentration expected to neutralize all human IL-18 in the plasma sample but not IL-18 produced by the mouse. Whereas pretreatment of the mouse...
Table 1. Effects of IL-1β on LV dimensions and function at 4 h

<table>
<thead>
<tr>
<th></th>
<th>LVEDD, mm</th>
<th>LVESD, mm</th>
<th>LVFS, %</th>
<th>HR, beats/min</th>
<th>LVPSP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>+dP/dt, mmHg/ms</th>
<th>−dP/dt, mmHg/ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3.95 ± 0.09</td>
<td>2.7 ± 0.07</td>
<td>31.8 ± 0.9</td>
<td>354 ± 20</td>
<td>64.8 ± 2.3</td>
<td>3.5 ± 0.3</td>
<td>4,358 ± 93</td>
<td>−3,538 ± 267</td>
</tr>
<tr>
<td>Recombinant murine IL-1β</td>
<td>3.98 ± 0.05</td>
<td>3.2 ± 0.06*</td>
<td>22.6 ± 1.6*</td>
<td>505 ± 22*</td>
<td>65.3 ± 2.3</td>
<td>7.9 ± 2.3*</td>
<td>3,667 ± 167*</td>
<td>−2,367 ± 384*</td>
</tr>
</tbody>
</table>

Values are means ± SE. LV, left ventricular; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; LVFS, LV fractional shortening; HR, heart rate; LVPSP, LV peak systolic pressure; LVEDP, LV end-diastolic pressure. *P < 0.05.

To confirm the central role of IL-18 in IL-1-induced cardiac dysfunction, 30 min before the administration of IL-1β, mice received one of two different doses of IL-18BP (1 or 10 mg/kg). Pretreatment with high-dose IL-18BP prevented the fall in LVFS induced by IL-1β (Fig. 3B). To confirm the data with IL-18BP, we next pretreated WT mice with a neutralizing antibody to mouse IL-18. In vivo neutralization of murine IL-18 fully prevented the decrease in LVFS compared with the control nontargeted antibody (Fig. 3C). In these experiments, administration of either IL-18BP or anti-IL-18 had no effect on LVFS in the absence of IL-1β administration.

As the inactive precursor of IL-18 is present in mouse tissues, the release of biologically active IL-18 requires the processing of precursor IL-18 by caspase-1 (36). Thus, the increase in active IL-18 after IL-1 presupposes IL-18 cleavage (activation) by caspase-1. We therefore tested whether a caspase-1 inhibitor prevents the systolic dysfunction induced by IL-1. As shown in Fig. 3D, pretreatment of mice with the caspase-1 inhibitor prevented the reduction in LVFS induced by IL-1β, whereas the caspase-1 inhibitor alone had no effect. In addition, we treated mice with two different inhibitors of p38 MAPK, SB-202190 and SD-169. Both inhibitors prevented the systolic dysfunction induced by IL-1β (Fig. 3D), consistent with the notion that IL-18 preferentially activates p38 MAPK signaling (48).

IL-18 induces an acute reversible cardiac dysfunction. Although repeated daily administration of IL-18 in mice results in myocardial dysfunction (35, 47), the acute effects of a single administration of IL-18 have not been previously described. We treated mice with a single dose of IL-18 (25 μg/kg) and measured LVFS over time. As shown in Fig. 4A, IL-18 induced a reduction in LVFS as early as 1 h, which persisted for up to 7 days. There was a complete recovery by 14 days. We next studied the effects of increasing the amount of single doses of IL-18 (0.25, 2.5, 25, and 125 μg/kg). After 24 h, systolic dysfunction was present for doses of 25 and 125 μg/kg, whereas the lower doses minimally altered LVFS (Fig. 4B). Since the 25- and 125-μg doses induced the same level of dysfunction, the 25-μg dose achieved the maximal effect.

To exclude the possibility that IL-18 induced cardiac dysfunction was mediated by induction of IL-1, we pretreated...
mice with anakinra, which, however, failed to prevent the IL-18-induced depression in LVFS (Fig. 4C), indicating that IL-18-induced cardiac dysfunction is not IL-1 mediated. 

**IL-18 blockade does not reduce IL-6 levels after IL-1.** IL-6 is an IL-1-inducible cytokine that mediates many of the systemic effects of IL-1. IL-1β significantly induced IL-6 plasma levels 4 h after challenge in WT mice as well as in IL-18 KO mice and IL-18R KO mice (Fig. 5A). Similarly, IL-18 blockade with IL-18BP or p38 MAPK inhibition (SD-169) had no effect on IL-6 levels after IL-1β (Fig. 5B), suggesting that, contrary to LV dysfunction, the induction of IL-6 by IL-1β is not IL-18 mediated.

**DISCUSSION**

Inflammation has a pathophysiological role in the establishment and progression of HF (21). Here, we confirm that the plasma of HF patients exerts a cardiodepressant action on the mouse heart (44) and show, for the first time, that this effect is not only dependent on IL-1 plasma activity in patients with HF but also mediated by the production of active IL-18 in the mouse.

IL-1 is a known cardiodepressant factor able to reduce cardiomyocyte contractility in vivo, ex vivo, and in vitro (13, 24, 27, 44, 45), and, accordingly, we show that exogenous IL-1β induces LV systolic and diastolic dysfunction in the mouse. IL-1 blocking strategies have been used to reduce myocardial damage and preserve heart function in several animal models of ischemic and nonischemic HF (2, 5, 7, 42, 43, 49), and IL-1 blockers are now under clinical investigation in patients with ischemic and nonischemic heart diseases (1, 3, 4, 38, 44, 45).

In the present study, we show that the cardiodepressant effects of IL-1 are mediated by IL-18, another member of the IL-1 family. IL-1 is an apical inflammatory cytokine that induces the activation of several kinases and the transcription of many genes. Experimental studies (10, 13, 24, 27, 44, 45) in vitro and in vivo have reported an effect only some time after the cytokine exposure, a phenomenon compatible with the production of a secondary mediator. The identification of a second messenger of IL-1-induced cardiac dysfunction in vivo is of the utmost importance as it may lead to the development of a targeted therapy to abolish the cardiodepressant effects of IL-1 while potentially not interfering with other IL-1 functions.

Consistent with our findings, lipopolysaccharide (LPS), which signals through Toll-like receptor 4 and myeloid differentiation factor 88 (a converging point for Toll-like receptor 4, 38, 44, 45).
and IL-1Ra failed to prevent the systolic dysfunction of IL-18 (37). We show here, however, that IL-18-induced systolic dysfunction in IL-18R KO mice 4 h after IL-1β administration.

**A**

![Graph showing plasma IL-6 levels after IL-1β administration](http://ajpheart.physiology.org/)

**B**

![Graph showing plasma IL-6 levels after IL-18R KO mice](http://ajpheart.physiology.org/)

Fig. 5. Blockade of IL-18 signaling does not prevent the IL-6 increase after IL-1β administration. **A**: plasma levels of IL-6 in C57Bl6 WT, IL-18 KO, and IL-18R KO mice 4 h after IL-1β (3 μg/kg) injection. n = 6 mice/group. *P < 0.01 vs. vehicle. **B**: plasma levels of IL-6 in CD-1 WT mice treated with IL-18BP and the p38 inhibitor SD-169. n = 4–6 mice/group. *P = 0.01 vs. vehicle; #P < 0.05 vs. vehicle.

and IL-1R signaling), also induces IL-18, which appears to mediate LPS-induced cardiac dysfunction in the mouse (37).

IL-1 has a positive feedback on IL-1, thus inducing a self-perpetuating signal (15, 20), but IL-1 also induces IL-18 release in vitro (34). Here, we show that IL-1β increases circulating levels of active IL-18 in mouse plasma. The IL-18 precursors are constitutively expressed in several cell types (34) but increases during inflammation, cellular stress, and tissue injury. The release of mature IL-18, however, requires the proteolytic cleavage of the inactive propeptide and active transport to outside the cell (36). Cleavage by caspase-1 is the principal mechanism for the processing of mature IL-18 and IL-1β production (36). In the present study, we show that inhibition of caspase-1 in vivo prevents the systolic dysfunction after the administration of IL-1β, which implies that a product of caspase-1, IL-1β, or IL-18 mediates the contractile dysfunction. IL-18, however, may also induce IL-1β and further amplify the inflammatory response. Indeed, neutralization of IL-18 after LPS challenge reduced IL-1β expression (37). We show here, however, that IL-18-induced systolic dysfunction is not mediated by IL-1, as pretreatment with IL-1Ra failed to prevent the systolic dysfunction of IL-18 injected into mice. An open question remains as whether the sustained IL-18-mediated systolic dysfunction is due to endogenous production of IL-18 or to a long-lasting effect downstream of IL-18R signaling.

Although IL-18 induces myocardial hypertrophy, fibrosis, and dysfunction (35, 47), as IL-1 does (9, 45), the two cytokines do not overlap their downstream effects. In fact, we found that lack or inhibition of the IL-18 pathway does not prevent the IL-6 increase due to IL-1 treatment. In agreement with our results, mouse embryonic fibroblasts derived from IL-18R KO mice exposed to IL-1 had augmented elevation of IL-6 compared with WT mice (31). In the same experimental setting, lack of IL-18 signaling did not prevent the increase in the acute-phase response molecules macrophage inflammatory protein-1α and PGE2 (31), confirming that IL-18 is not involved in the acute-phase response (39). Indeed, IL-18 minimally activates the cyclooxygenase-2 pathway and is not involved in the induction of fever and neutropenia, as seen with IL-1 (25, 39). Thus, IL-18 and IL-1 share many but not all effects.

IL-18 shows preferential signaling through p38 MAPK (39). Accordingly, we show that inhibitors of p38 MAPK protected against IL-1-induced dysfunction and that neither IL-18 blockade nor p38 MAPK inhibition prevented the increase in serum levels of IL-6, a key secondary proinflammatory mediator that is often used as a surrogate for IL-1 activity and correlates well with systemic inflammatory signs, such as fever (30). The signaling steps downstream of IL-18R or p38 MAPK that directly (within the cardiomyocyte) or indirectly (through an effect on a different and intermediate cell type) induce the development of the systolic dysfunction are unknown and were not explored in the present study.

Adverse effects of exogenous IL-18 have been demonstrated with chronic administrations (35, 47), and we found acute systolic dysfunction with a single dose of IL-18. IL-18 blocking strategies in animal models of heart diseases have, on the other hand, presented some conflicting results, with some showing impairment in the development of compensatory hypertrophy in a mouse model of pressure overload due to transverse aortic constriction (11), whereas others showed beneficial effects of IL-18 blockade in ischemia-reperfusion models (46).

IL-18 blockers are available and have been tested in phase I and II clinical trials (18, 40). However, no data are available concerning the effectiveness of these blockers in patients with heart diseases.

Other cytokines, i.e., TNF-α, may also regulate the expression of IL-18 (37). TNF-α KO mice have reduced IL-18 expression in the heart after LPS stimulation (37). IL-18 may therefore represent a common signal downstream of different pathways; therefore, blockade of IL-18 may have even greater effects since IL-1 and TNF-α have been shown to have synergistic effects on contractile function (10, 24). IL-1 blockers are currently under investigation in clinical trials for HF (44). TNF-α blockers, on the other hand, have failed as therapeutic tools for the treatment of HF (12). This study increases the understanding of the mechanisms by which systemic IL-1 modulates cardiac function through the induction of IL-18 and provides a rationale for a novel and more targeted intervention with IL-18 blockers in HF.

In terms of the preserved systolic function and IL-6 response after IL-1β, IL-18 and IL-18R KO mice were equally protected. However, a slight increase in heart rate was observed in IL-18R KO mice, highlighting a potential difference between the two KO mice. Differences have been previously described, and a second ligand for the IL-18R has been proposed and
identified (8, 22, 31). This suggests that IL-18 KO mice may have a different control on the peripheral resistance.

The lack of characterization of other constituents in the plasma of patients with ADHF used in this study is a limitation. Patients with HF have high levels of circulating IL-18 (26, 29); however, circulating levels of IL-18, as is the case for other cytokines, do not always correlate with disease activity. This is particularly challenging for circulating IL-18, as the cytokine is mostly bound to IL-18BP and is therefore inactive (17, 33).

Furthermore, although HF is a chronic condition, our data were collected using an acute model of systolic dysfunction. The data suggest that IL-18 is a target for intervention; however, it is difficult to predict whether IL-18 inhibition will be effective in the treatment of HF, and thus further studies are needed.

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


IL-18 MEDIATES SYSTOLIC DYSFUNCTION


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