Increased Production of CXCL16 in Experimental and Clinical Heart Failure
A Possible Role in Extracellular Matrix Remodeling

Christen Peder Dahl, MD; Cathrine Husberg, PhD; Lars Gullestad, MD, PhD; Anne Wæhre, MD; Jan Kristian Damås, MD, PhD; Leif Erik Vinge, MD, PhD; Alexandra V. Finsen, MD, PhD; Thor Ueland, PhD; Geir Florholmen, PhD; Svend Aakhus, MD, PhD; Bente Halvorsen, PhD; Pål Aukrust, MD, PhD; Erik Øie, MD, PhD; Arne Yndestad, PhD; Geir Christensen, MD, PhD

Background—Inflammation has been implicated in the pathogenesis of heart failure (HF), but knowledge about the production and role of inflammatory actors remains incomplete. On the basis of its role in vascular inflammation, vascular proliferation, and matrix degradation, we hypothesized a role for the chemokine CXCL16 in the pathogenesis of myocardial remodeling and development of HF.

Methods and Results—Our main findings were (1) patients with chronic HF (n=188) had increased plasma levels of CXCL16, which correlated with disease severity. (2) Left ventricular tissue from patients with end-stage HF (n=8) showed enhanced CXCL16 levels compared with nonfailing left ventricular (n=6) as assessed by Western blotting. (3) In mice with postmyocardial infarction HF, expression of CXCL16, as assessed by real-time RT-PCR, was increased in the infarcted and the noninfarcted areas of left ventricular 3 and 7 days after coronary ligation, indicating early onset of CXCL16 production. Furthermore, mice exposed to aortic banding had enhanced CXCL16 expression in left ventricular, indicating that CXCL16 expression is not related to ischemia alone. (4) In vitro, CXCL16 promoted proliferation and impaired collagen synthesis in myocardial fibroblasts, and in cardiomyocytes and myocardial fibroblasts, CXCL16 increased matrix metalloproteinase activity, primarily reflecting increased matrix metalloproteinase-2 levels. (5) By using specific inhibitors, we showed that the effect of CXCL16 on fibroblasts involved activation of Jun N-terminal kinase.

Conclusion—We show enhanced myocardial CXCL16 expression in experimental and clinical HF. The effect of CXCL16 on cardiomyocytes and fibroblasts suggests a role for CXCL16 in matrix remodeling and ultimately in the development of HF. (Circ Heart Fail. 2009;2:624-632.)

Key words: heart failure ■ inflammation ■ metalloproteinases remodeling ■ CXCL16

Chronic heart failure (HF) is a progressive disorder in which dysregulation of several physiological systems contributes to its pathogenesis. HF is, despite state-of-the-art treatment, characterized by high morbidity and mortality, indicating that important pathogenic mechanisms remain active and unmodified by the present treatment modalities.1 It has been suggested that persistent inflammation could represent one such unmodified mechanism, contributing to the myocardial remodeling process characterizing chronic HF.2,3 However, although both experimental and some clinical studies indicate a pathogenic role for inflammation in the development of myocardial failure, the role of cytokine dysregulation in human HF and the identification and characterization of the different inflammatory actors need to be further elucidated.

Clinical Perspective on p 632

CXCL16 is an exceptional chemokine because it is expressed as a multidomain molecule consisting of a chemokine that is linked to a mucin-like stalk followed by a transmembrane segment and a short cytoplasmic tail, similar only to CX3CL1.4 As a transmembrane molecule on the surface of...
cells, CXCL16 can interact with its receptor CXCR6, promoting tissue homing and activity of various leukocyte subsets. Within the vasculature, CXCL16 has been located on endothelial cells, smooth muscle cells (SMCs), and macrophages, and upregulation has been found under inflammatory conditions such as atherosclerosis, consistent with a role in vascular inflammation. However, growing evidence from in vitro studies suggests that the function of CXCL16 is not exclusively limited to leukocyte recruitment. Thus, although the chemokine domain of CXCL16 can act as a classical chemoattractant for various leukocyte subsets, recent studies suggest that soluble CXCL16, which is shed from the cell surface by the metalloproteinase ADAM10, may induce proliferation of vascular SMCs and matrix degradation.

Cell proliferation and alterations in extracellular matrix are central aspects in the myocardial remodeling process characterizing chronic HF. Currently, there are no data on the production and possible effects of CXCL16 within the myocardium, but on the basis of its combined role in vascular inflammation, vascular proliferation, and matrix degradation, we hypothesized that CXCL16 could be involved in the pathogenesis of myocardial remodeling and development of HF. In this study, our hypothesis was examined by different approaches, including studies in clinical and experimental HF and in vitro experiments using cardiomyocytes and myocardial fibroblasts.

**Methods**

**HF Patients**

One hundred eighty-eight patients with stable HF for >6 months in New York Heart Association (NYHA) functional class II–IV were consecutively included in the study (Table). Blood samples (platelet-poor EDTA plasma) were collected and stored as previously described. Details are given in the online-only Data Supplement.

**Mouse Models of Experimental HF**

To study the production of CXCL16 in the myocardium during HF development, we used mouse models with HF after aortic banding and myocardial infarction (MI). The mouse models allow measurements of CXCL16 gene expression and protein content in the myocardium at various time points during early stages of HF development. Moreover, by using these models, we were able to relate CXCL16 production more reliably to the HF etiologies, ischemia, and pressure overload. Details are given in the Data Supplement.

**CXCL16 Production in Isolated Cells After MI**

In a separate set of experiments, we investigated expression of CXCL16 in cardiomyocytes and noncardiomyocytes isolated from rat hearts 7 days after MI. Details are given in the Data Supplement.

**Isolation and Stimulation of Neonatal Cardiomyocytes and Myocardial Fibroblasts**

Primary neonatal cardiomyocytes and fibroblasts were isolated from 1- to 3-day-old Wistar rats (Taconic, Skensved, Denmark). Details are given in the Data Supplement.

**Protein Synthesis Assessed by [3H]Leucine Incorporation**

Cardiomyocytes and myocardial fibroblasts were plated on 24-well culture dishes (Corning Int.), and after 24 hours with or without recombinant CXCL16 treatment (200 ng/mL, chemokine domain of mouse CXCL16; R&D Systems), the culture medium was supplemented with 5 µCi/mL [3H]leucine (American Radiolabel Chemicals, St. Louis, Mo) and further incubated for 24 hours. Cardiomyocytes were fixed and washed in chilled 95% ethanol and solubilized in 0.2 mol/L NaOH, as previously described. The total protein-associated radioactivity was measured in a TRI-CARB 2000 TR Scintillation Counter (Packard, Meriden, Conn).

**Cardiomyocyte Measurements**

Cardiomyocyte size was measured as previously described and analyzed with ImageJ downloaded at http://rsb.info.nih.gov/ij/.

**Zymography**

Gelatinolytic activity was detected in conditioned media from fibroblasts and cardiomyocytes after incubation for 24 and 48 hours, respectively. Details are given in the Data Supplement.

**Real-Time Quantitative RT-PCR**

Total RNA was extracted by use of acid-phenol extraction in the presence of chaotropic salts (TRizol; Invitrogen, San Diego, Calif) and subsequent isopropyl alcohol–ethanol precipitation. All total RNA samples were subjected to DNase I treatment (RQI DNase; Promega, Madison, WI) and stored in RNA storage solution (Ambion, Austin, Tex) at −80°C. Sequence-specific PCR primers for CXCL16, matrix metalloproteinase (MMP)-2, MMP-9, α-skeletal actin, atrial natriuretic peptide, and brain natriuretic peptide were designed with Primer Express software version 1.5 (Applied Biosystems). Gene expression of the housekeeping proteins, β-actin, atrial natriuretic peptide, and brain natriuretic peptide were measured with ABI Prism 7500 (Applied Biosystems). Gene expression of the housekeeping proteins, β-actin, atrial natriuretic peptide, and brain natriuretic peptide were measured with ABI Prism 7500 (Applied Biosystems).

**Table. Clinical and Hemodynamic Characteristics of the Population With HF**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
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<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>56±13</td>
</tr>
<tr>
<td><strong>Gender, male/female</strong></td>
<td>148/40</td>
</tr>
<tr>
<td><strong>Etiology (CAD/DCM/others)</strong></td>
<td>43/54/3</td>
</tr>
<tr>
<td><strong>NYHA class (II/III/IV)</strong></td>
<td>27/43/30</td>
</tr>
<tr>
<td><strong>History, %</strong></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14</td>
</tr>
<tr>
<td>Hypertension</td>
<td>16</td>
</tr>
<tr>
<td>Previous myocardal infarction</td>
<td>36</td>
</tr>
<tr>
<td><strong>Biochemical values</strong></td>
<td></td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>101±46</td>
</tr>
<tr>
<td>Nt-proBNP, pmol/L</td>
<td>437±547</td>
</tr>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
</tr>
<tr>
<td>LVEF, %</td>
<td>30±12</td>
</tr>
<tr>
<td>PCW, mm Hg</td>
<td>18±8</td>
</tr>
<tr>
<td>Cardiac index, L/min/m²</td>
<td>2.1±0.6</td>
</tr>
<tr>
<td><strong>Medication, %</strong></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>66</td>
</tr>
<tr>
<td>ARB</td>
<td>20</td>
</tr>
<tr>
<td>β-blocker</td>
<td>77</td>
</tr>
<tr>
<td>Diuretics</td>
<td>68</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>41</td>
</tr>
<tr>
<td>Statins</td>
<td>27</td>
</tr>
<tr>
<td>Warfarin</td>
<td>45</td>
</tr>
</tbody>
</table>

Data are presented as the mean±SD or number or percentage of subjects. PCW indicates pulmonary capillary wedge pressure; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.
gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization (Applied Biosystems).

Immunohistochemistry

Immunohistochemical analysis was performed on left ventricular (LV) myocardial tissue from human cardiac explants (n=110052) and nonfailing donor hearts (n=110052) as previously described.17 Details are given in the Data Supplement.

Western Blotting

Western blotting was performed as described previously18 with minor modifications. Details are given in the Data Supplement.

A description of tissue sampling from human myocardium, multiplex suspension array, fibroblast proliferation, [3H]proline incorporation, total MMP activity, enzyme immunoassay, ethics, and statistical analysis is given in the Data Supplement.

Results

Plasma Levels of CXCL16 in Human HF

To examine whether CXCL16 is upregulated in human HF, we first measured the plasma levels of CXCL16 in patients with HF (n=188) and in sex- and age-matched healthy individuals (n=20). As shown in Figure 1A, the patients with the most severe HF (ie, NYHA class III/IV) had significantly increased CXCL16 levels compared with healthy controls and patients in NYHA class II, with no difference between NYHA class III and IV (data not shown). Moreover, in the patient group as a whole, we found that CXCL16 levels were negatively correlated with LV ejection fraction (r=−0.30, P<0.02) and positively correlated with plasma levels of N-terminal pro–brain natriuretic peptide (r=0.30, P<0.02). Within NYHA class II, we found significantly increased CXCL16 levels in those with ischemic compared with those with nonischemic cardiomyopathy (Figure 1B). In contrast, no difference in CXCL16 levels was found when comparing these 2 etiologic subgroups in NYHA class III and IV (Figure 1B).

The Expression of CXCL16 in Failing Human Myocardium

We then examined whether the human myocardium itself could produce CXCL16 during HF. By using immunohistochemistry, we found CXCL16 immunoreactivity in cardiomyocytes, vascular SMCs, and endothelial cells (Figure 2). Immunostaining of its corresponding receptor CXCR6 was observed in the same types of cells, and in addition, fairly strong CXCR6 immunostaining was seen in vimentin-positive fibroblasts (Figure 2). To obtain a more quantitative assessment of the production of CXCL16 and CXCR6 in the failing human myocardium, we analyzed the protein levels of these components in LV myocardial tissue by Western blotting. As depicted in Figure 3, protein expression of CXCL16 and CXCR6 were upregulated in failing (n=8) compared with nonfailing (n=6) myocardium, although the increase in CXCR6 did not reach statistical significance. The same pattern was seen in ischemic (n=5) and nonischemic (n=3) cardiomyopathy (data not shown).

Gene Expression of CXCL16 in Experimental HF

Mouse models of experimental HF allow us to study the myocardial expression of CXCL16 at an early stage in the development of HF. We, therefore, next examined the expression of CXCL16 by means of real-time RT-PCR in 2 experimental mouse models of HF, ie, post-MI HF and HF secondary to pressure overload (aortic banding), representing 2 important etiologic subgroups of HF. As depicted in Figure 4A, we found that mice with post-MI HF had markedly increased...
levels of CXCL16 mRNA in both the ischemic and the nonischemic region of the LV already 3 days after MI compared with the LV in the sham-operated group. Increased expression was also found at 7 and 21 days after MI, with particularly high expression in the infarcted area (7 days, Figure 4A). Furthermore, in mice subjected to LV pressure overload, the level of CXCL16 mRNA was increased, indicating that enhanced expression of CXCL16 in the failing myocardium is not related to ischemia alone (Figure 4B). Although both mice with decompensated and mice with compensated hypertrophy had significantly enhanced expression of CXCL16 compared with the sham group, particularly high levels of CXCL16 were found in those with overt HF (ie, decompensated myocardial hypertrophy) (Figure 4B). Although mRNA levels of CXCL16 in the nonischemic part of LV (post-MI HF) were quite similar to the myocardial expression of CXCL16 in mice with HF secondary to aortic banding, the myocardial CXCL16 response seems to be markedly increased within the infarcted area in rats with post-MI HF, potentially reflecting the involvement of CXCL16 in repair processes (Figure 4A and B).

Gene Expression of CXCL16 in Cardiomyocytes and Noncardiomyocytes During Experimental Post-MI HF
To examine whether the increased expression of CXCL16 during experimental HF was localized to cardiomyocytes or other myocardial cells, we examined the mRNA level of CXCL16 in a rat model of post-MI HF, allowing us to separate the nonischemic LV from rats with HF (n=7) and sham rats (n=7) into cardiomyocytes and noncardiomyocytes (primarily consisting of fibroblasts) 7 days after the induction of MI or sham operation. As shown in Figure 5, both cardiomyocytes and noncardiomyocytes showed in-
creased expression of CXCL16 in nonischemic LV in post-MI rats compared with sham rats.

**Regulation of CXCL16 in Neonatal Rat Cardiomyocytes**

Given the increased expression of CXCL16 in cardiomyocytes during post-MI HF, we next examined factors that might be responsible for varying regulation of CXCL16 in these cells using a well-established neonatal rat cell system. The expression of CXCL16 was measured by means of real-time quantitative RT-PCR after stimulating cardiomyocytes for 24 hours with various stimuli with relevance to HF, such as the inflammatory cytokines tumor necrosis factor-α, interleukin (IL)-1β, and interferon-γ; mediators with potential hypertrophic effects such as leukemia inhibitory factor and endothelin-1; neurohormones such as noradrenaline; and toll-like receptor (TLR)2 and TLR4 agonists. Endothelin-1, noradrenaline, and the hypertrophic cytokine leukemia inhibitory factor did not regulate CXCL16 expression. In contrast, tumor necrosis factor α, IL-1 β, and interferon-γ as well as TLR2 and TLR4 activation induced increased CXCL16 mRNA levels, with a particular enhancing effect of IL-1 β (supplemental Figure I).

**Effects of CXCL16 on Neonatal Rat Cardiomyocytes and Myocardial Fibroblasts**

To study the potential pathogenic consequences of the enhanced CXCL16 expression during development of HF, we examined effects of CXCL16 on neonatal rat cardiomyocytes and myocardial fibroblasts, both shown to express significant amounts of CXCR6 as assessed by Western blotting (Figure 6A). In cardiomyocytes, CXCL16 promoted an increase in protein synthesis as assessed by a modest, but significant, increase in [3H]leucine incorporation (Figure 6B). However, CXCL16 had no effect on size (area; 1300 ± 1475 μm² versus 1475 ± 307 μm²) or expression of hypertrophy markers (brain natriuretic peptide [1.05 ± 0.05 versus 0.92 ± 0.05], atrial natriuretic peptide [1.02 ± 0.05 versus 0.98 ± 0.04], α-skeletal actin [1.08 ± 0.06 versus 1.13 ± 0.02] in cardiomyocytes (data are given as CXCL16 stimulated and unstimulated, respectively). In fibroblasts, CXCL16 markedly increased cell proliferation as assessed by [3H]thymidine incorporation (Figure 6C). To examine the signaling pathways involved in the effects of CXCL16 on myocardial fibroblasts, showing the most prominent cellular response to CXCL16, we examined the effect of CXCL16 on fibroblasts by multiplex suspension array technology that detects phosphorylation of intracellular signaling molecules. CXCL16 induced enhanced phosphorylation of JNK and p38 mitogen-activated protein kinase (MAPK) and, to some degree, of extracellular signal regulated kinase (ERK) 1/2 in fibroblasts after 10 minutes of stimulation (Figure 7A and C). Moreover, blocking of JNK, but not of MAPK/ERK (data not shown), completely abolished the enhancing effect of CXCL16 on fibroblast proliferation (Figure 7D). There were no differences in cell toxicity between cell cultures with and without the JNK and MAPK/ERK blockers (LDH leakage assay, data not shown).

**The Effect of CXCL16 on MMP Activity**

Matrix degradation plays an important role in myocardial remodeling, and we have previously shown enhanced expression of MMPs in CXCL16-stimulated vascular SMC. To elucidate whether similar effects potentially could be seen within the myocardium, we examined MMP activity in myocardial fibroblasts and neonatal rat cardiomyocytes that had been exposed to CXCL16 for 24 and 48 hours, respectively. CXCL16 induced a significant increase in total MMP activity in both fibroblasts and cardiomyocytes (Figure 8A and B). The CXCL16-induced increase in total MMP activity was accompanied by an increase in MMP-2, but not in MMP-9, levels as assessed by enzyme immunoassay (Figure 8C and D), and in fibroblast, also by an increase in gelatinolytic activity (Figure 8E), suggesting that the CXCL16-mediated increase in MMP activity, at least partly, reflects increased MMP-2 levels. In cardiomyocytes, low or undetectable gelatinolytic activity was seen in both unstimulated and CXCL16-stimulated cells. In contrast to the effect on protein levels, CXCL16 had no effect on mRNA levels of MMP-2 and MMP-9 in either fibroblasts or cardiomyocytes (data not shown), suggesting that the CXCL16-mediated induction of MMP activity, or at least MMP-2, is regulated at the post-transcriptional level.

**CXCL16 Downregulates Proline Incorporation in Myocardial Fibroblasts**

Our findings suggest that CXCL16 may influence myocardial extracellular matrix remodeling. To further elucidate these issues, we examined the ability of CXCL16 to modulate
proline incorporation in myocardial fibroblasts as a marker of collagen synthesis in these cells. Although CXCL16 had no significant effect on leucine incorporation, CXCL16 significantly downregulated proline incorporation, suggesting an attenuating effect of CXCL16 on collagen synthesis in myocardial fibroblasts (supplemental Figure II).

**Discussion**

In this study, we demonstrate enhanced myocardial expression of CXCL16 in both experimental and clinical HF. In patients with HF, this increase was accompanied by increased plasma levels of CXCL16, which significantly correlated with LV ejection fraction and N-terminal pro–brain natriuretic peptide levels, both well-known markers for disease severity and predictors of outcome in HF. In a mouse model of post-MI HF, we found significantly increased gene expression of CXCL16 in both the ischemic and the nonischemic part of the LV as early as 3 days after MI. In a mouse model of pressure overload without ischemia (aortic banding), we also found markedly enhanced expression of CXCL16 in both compensated and, particularly, in decompensated hypertrophy. In the failing human myocardium, the upregulation of CXCL16 was accompanied by significant immunostaining of its corresponding receptor CXCR6 in cardiomyocytes and in fibroblasts. Our in vitro experiments suggest that CXCL16 could induce enhanced protein synthesis and MMP activity in cardiomyocytes and promote a proliferating and MMP activating phenotype in myocardial fibroblasts, accompanied by inhibition of collagen synthesis. If similar CXCL16/CXCR6 interactions also are operating in vivo within the failing myocardium, it could contribute to the pathological myocardial remodeling characterizing the development of HF.

Previously, increased levels of soluble CXCL16 have been reported in various autoimmune disorders,19–21 and although some discrepancies exist,22 increased plasma levels have also been found in coronary artery disease (CAD).6,23 We present novel evidence that also patients with HF are characterized by significantly increased plasma levels of CXCL16, with increasing levels according to disease severity as assessed by clinical, hemodynamic, and neurohormonal parameters. Although patients with HF classified as NYHA II with CAD etiology had significantly increased CXCL16 levels, patients with dilated cardiomyopathy had plasma levels of CXCL16 similar to those of healthy controls. These results may support earlier findings of increased CXCL16 levels in CAD, but our study also indicates that the increased plasma levels of CXCL16 in HF do not merely reflect accompanying atherosclerosis. Hence, in more severe HF, CXCL16 levels were markedly increased in both ischemic and nonischemic cardiomyopathy, not only in plasma, but also within the failing myocardium. On the basis of the plasma data, showing no (dilated cardiomyopathy) or a modest (CAD) increase in NYHA class II, one could suggest that CXCL16 is not a pathogenic mediator at the early stage of HF. However, plasma levels may not necessarily reflect the myocardial expression of CXCL16, and our data from experimental HF may suggest an early upregulation of CXCL16 within the failing myocardium. Nonetheless, future studies should more precisely address the role of CXCL16 at the early stage of human HF.
To our knowledge, production of CXCL16 in myocardial tissue has not been demonstrated previously, but Yamauchi et al. showed CXCL16 expression in endothelial cells of the cardiac valves during murine development. On the other hand, CXCL16 expression in human cardiac valves was undetectable in the absence of valvular inflammation. Herein, we show enhanced expression of CXCL16 accompanied by significant immunostaining of its corresponding receptor within the failing human myocardium. We further demonstrate markedly increased levels of CXCL16 mRNA in 2 different experimental models of HF. Mice with post-MI HF showed a significant and early increase in gene expression of CXCL16 in both the ischemic and the nonischemic region of the LV. Moreover, the increase in myocardial CXCL16 expression in mice subjected to pressure overload shows that atherosclerosis or ischemia is not necessary for the myocardial induction of CXCL16. Our findings indicate that the failing myocardium itself also contributes to the increased systemic CXCL16 levels in HF, in particular in decompensated myocardial failure. However, although these findings clearly show that the failing myocardium produces and expresses CXCL16, the increased plasma levels of CXCL16 in chronic HF could also, at least partly, reflect enhanced release secondary to systemic inflammation and endothelial cell activation.

Inflammatory cytokines such as tumor necrosis factor-α and interferon-γ have previously been found to enhance CXCL16 expression in endothelial cells, SMC, and macrophages, and we have recently shown that IL-1β has similar properties in endothelial cells and peripheral blood mononuclear cells. In this study, we show that these inflammatory cytokines as well as TLR2 and TLR4 agonists induce the expression of CXCL16 in neonatal rat cardiomyocytes, with particularly enhancing effects of IL-1β. There are several reports of increased levels of these inflammatory cytokines, including IL-1β, during HF. TLR2 and TLR4 activation has also been linked to the development of myocardial failure. It is therefore conceivable that these inflammatory mediators could be operating within the failing myocardium, contributing to the enhanced myocardial expression of CXCL16 in overt HF, being part of the inflammatory network that could contribute to the progression of myocardial failure.

Cardiomyocyte hypertrophy and an imbalanced regulation of the extracellular matrix are important features of myocardial remodeling during development of HF. In this study, we show that CXCL16 increases protein synthesis in neonatal rat cardiomyocytes, but no increase was found in the expression of hypertrophic markers or cardiomyocyte size. On the other hand, CXCL16 markedly increased MMP activity in both cardiomyocytes and fibroblasts. MMPs are a family of proteolytic enzymes that are regulated by inflammatory signals to mediate changes in extracellular matrix. Enhanced MMP activity, and in particular increased MMP-2 and
MMP-9 activity, has been recognized to play an important role in myocardial remodeling contributing to the development of myocardial failure.30,31 CXCL16 has previously been shown to promote MMP activity in vascular SMC and prostate cancer cell lines.5,32 In this study, we show that CXCL16 is an inducer of MMP activity, at least partly reflecting increased MMP-2 levels, in both myocardial fibroblasts and cardiomyocytes, potentially promoting extracellular matrix remodeling. The ability of CXCL16 to attenuate proline incorporation in myocardial fibroblasts, potentially reflecting impaired collagen synthesis, may further support such a notion. Because we found fairly strong CXCR6 immunoreactivity in these cell types within the failing human myocardium, it is tempting to hypothesize that CXCL16/CXCR6 interaction could also be operating in clinical HF to alter the extracellular matrix through enhancement of MMP activity and downregulation of collagen synthesis.

CXCL16 also induced proliferation of myocardial fibroblasts, which could be blocked by inhibition of JNK, MAPK and ERK pathways have been implicated in the CXCL16-mediated cell recruitment to rheumatoid arthritis synovial tissue and the CXCL16-mediated differentiation of glial precursor cells.33 In our study, we confirmed that CXCL16 stimulation induces phosphorylation of p38 MAPK and ERK 1/2, but importantly, in neonatal myocardial fibroblasts, CXCL16 also induced JNK activation, and only JNK inhibition abolished the CXCL16-induced proliferation of these cells. JNK activation seems to play an important role in myocardial remodeling,34,35 and our data suggest that CXCL16 acts, at least partly, through this signaling pathway to alter fibroblast function and thus potentially also extracellular matrix.

In this study, we show increased expression of CXCL16 in both clinical and experimental HF, and in patients with overt HF, LV myocardial tissue showed enhanced protein levels of CXCL16 and significant immunoreactivity for its corresponding receptor CXCR6 in cardiomyocytes and fibroblasts. Our in vitro experiments demonstrated that CXCL16 stimulated MMP activity in cardiomyocytes and fibroblasts and attenuated proline incorporation as a marker of collagen synthesis in fibroblasts. Although future studies, including the use of a rodent transgenic/null model, are needed to further establish a role for CXCL16 in the development of HF, our findings showing a combined effect on cardiomyocytes and myocardial fibroblasts may suggest a role for CXCL16 in extracellular matrix remodeling and ultimately in the development of HF as well.

**Acknowledgments**

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**Disclosures**

None.

**References**


**CLINICAL PERSPECTIVE**

Chronic heart failure (HF) is an important and increasing cause of cardiovascular morbidity and mortality. Despite the introduction of new therapeutic options, the 5-year mortality is above 50%, suggesting that important pathogenic mechanisms remain unchallenged by current treatment modalities. Regardless of optimal treatment, patients with HF are characterized by persistent inflammation, both systemically and within the failing myocardium, potentially contributing to myocardial remodeling. In this study, we identify the chemokine CXCL16 as an important mediator in these processes. We show that patients with chronic HF have increased plasma levels of CXCL16, significantly correlated with disease severity. In addition, left ventricular tissue from patients with end-stage HF shows enhanced CXCL16 levels compared with nonfailing left ventricular. Enhanced left ventricular expression of CXCL16 was also seen in experimental HF, as shown in HF secondary to myocardial infarction as well as in HF secondary to pressure overload. Within cardiomyocytes, several inflammatory mediators, and particularly interleukin-1, markedly enhanced the expression of CXCL16, linking this chemokine to the myocardial cytokine network. Our in vitro experiments show that CXCL16 induces matrix metalloproteinase activity in myocardial fibroblasts and cardiomyocytes and attenuates collagen synthesis in fibroblasts. Our findings imply a role for CXCL16 in myocardial remodeling, and the combined effect on cardiomyocytes and myocardial fibroblasts may suggest a role for CXCL16 in myocardial remodeling and ultimately in the development of HF.
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