Alterations in Janus Kinase (JAK)-Signal Transducers and Activators of Transcription (STAT) Signaling in Patients With End-Stage Dilated Cardiomyopathy

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Background—Experimental studies indicate that interleukin-6 (IL-6)–related cytokines, signaling via the shared receptor gp130, Janus kinases (JAKs), and signal transducers and activators of transcription (STATs), provide a critical cardiomyocyte survival pathway in vivo. Little is known about the activation of this signaling pathway in the myocardia of patients with end-stage dilated cardiomyopathy (DCM).

Methods and Results—We performed a comprehensive expression and activation analysis of IL-6–related cytokines, receptors, signal transducers, and signal transduction inhibitors in left ventricular (LV) myocardia from patients with DCM (n = 10) and non-failing (NF) donor hearts (n = 5). Differential expression (DCM versus NF) was observed by immunoblotting at each level of the signaling cascade, including receptor ligands (IL-6: −59%, P < 0.01; leukemia inhibitory factor [LIF]: +54%, P < 0.05), receptor subunits (LIF receptor: −16%, P < 0.05), signaling molecules (the Janus kinase TYK2: −48%, P < 0.01; STAT3: −47%, P < 0.01), and suppressors of cytokine signaling (SOCS1: +97%, P < 0.05; SOCS3: −49%, P < 0.01). Tyrosine-phosphorylation status of gp130 was increased (+60%, P < 0.05), whereas tyrosine-phosphorylation status of Jak2 was reduced in DCM (−72%, P < 0.01). Moreover, as shown by immunohistochemistry, the number of STAT3-positive cardiomyocytes was reduced in DCM (−42%, P < 0.01).

Conclusion—Signaling via gp130 and JAK-STAT is profoundly altered in DCM. Importantly, tyrosine-phosphorylation of JAK2 is reduced in the face of increased gp130 phosphorylation, indicating impaired downstream activation of this critical pathway in DCM. *(Circulation. 2003;107:798-802.)*

Key Words: cardiomyopathy • interleukins • signal transduction

Dilated cardiomyopathy (DCM) represents a common end-stage disease state of the myocardium in response to different environmental and genetic factors, a fact that has led to the proposition of shared signaling pathways for cardiac dilation and failure.1 In this regard, a growing body of evidence indicates that interleukin-6 (IL-6)–related cytokines signaling via the shared receptor gp130 provide a critical myocyte survival pathway in vivo. Most notably, gene-targeted mice with a cardiomyocyte-restricted deletion of gp130 develop massive cardiomyocyte apoptosis and dilated cardiomyopathy when subjected to biomechanical stress.2

A prevailing concept predicts that an intricate balance between cardiomyocyte hypertrophy and apoptosis determines heart failure progression.1 In this regard, the Janus kinases–signal transducers and activators of transcription (JAK-STAT) signaling pathway has been shown to mediate hypertrophic and cytoprotective effects of gp130 activation in cardiomyocytes.2–8 IL-6–related cytokines potently activate gp130, which in turn promotes tyrosine-phosphorylation (ie, activation) of JAKs and cytoplasmic latent transcription factors of the STAT family.9 Signaling via gp130 and JAK-STAT is controlled in a negative-feedback fashion by a family of proteins referred to as suppressors of cytokine signaling, including SOCS1 and SOCS3.10,11

Despite increasing evidence implicating IL-6–related cytokines, gp130, and JAK-STAT as a critical myocyte survival pathway, little is known regarding expression and activation of this pathway in patients with DCM. In the present study, we have conducted a comprehensive expression and activation analysis of the gp130-JAK-STAT signaling cascade in left ventricular (LV) myocardia from patients with DCM.

Methods

**Patient Population**

LV myocardium was obtained from patients undergoing heart transplantation because of end-stage DCM (n = 10; mean age: 44 ± 13 years; New York Heart Association functional classes III and IV; LV ejection fraction: 16 ± 7%; LV end-diastolic diameter: 65 ± 14 mm).
Seven patients had been treated with angiotensin-converting enzyme (ACE) inhibitors, 6 patients with diuretics, 7 patients with digoxin, and 5 patients with β-blockers. For comparison, we studied LV tissue samples from non-failing (NF) donor hearts that could not be transplanted for technical reasons (n=5; mean age: 42±1 years). The non-failing state of these hearts was confirmed by low LV mRNA expression levels of B-type natriuretic peptide (BNP) as assessed by Northern-blot (0.07±0.02 densitometric units in NF versus 0.44±0.05 in DCM).

**Immunoblotting**

Total protein extracts were subjected to 10% SDS-PAGE, transferred to PVDF-membranes, and incubated with primary antibodies according to standard immunoblotting procedures. Antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif) (IL-6, leukemia inhibitory factor [LIF], LIF receptor [LIFR], JAK2, TYK2, SOCS1, SOCS3), R&D Systems (Minneapolis, Minn, cardiophrin-1 [CT1], gp130, IL-6R), Affinity Bioreagents (Golden, Colo, JAK1, phospho-JAK1, phospho-JAK2), and Cell-Signaling Technology (Beverly, Mass, STAT1, STAT3, phospho-TYK2, phospho-STAT1, phospho-STAT3). Equal loading was confirmed by Ponceau-S staining, and protein levels were normalized to total actin content. Tyrosine-phosphorylation of gp130 and STAT3 was evaluated by immunoprecipitation using an anti-phosphotyrosine antibody (Cell Signaling Technology) and protein A-agarose (Roche, Basel, Switzerland), followed by anti-gp130 and anti-STAT3 immunoblotting. Specific bands were visualized by enhanced chemiluminescence (Bio-Rad, Hercules, Calif). Images were scanned using the GelDoc 2000 documentation system and quantified with Quantity One software (Bio-Rad).

**Immunohistochemistry**

Immunohistochemistry was performed on serial sections from NF and DCM hearts using antibodies from New England Biolabs (Beverly, Mass, JAK2), Cell Signaling Technology (STAT3), Biomedia (Foster City, Calif, skeletal muscle α-actin), and Alexis (San Diego, Calif, myosin) and the Vectastain ABC elite kit from Vector Laboratories (Burlingame, Calif).

**Semi-Quantitative Reverse Transcription Polymerase Chain Reaction**

IL-6, LIF, and CT-1 mRNA expression levels were determined by reverse transcription polymerase chain reaction under linear amplification conditions. All RNA samples were tested for equal G3PDH content. The following primer pairs were used: CCATCCAGTTGCCTCCTTG/AGTGCATCATCGTTGTTCATAC (IL-6), CTGTTGCTGTA (G3PDH).

**Results**

Plasma levels of IL-6 and CT-1 in DCM are increased in patients with heart failure; however, little is known regarding the cellular source(s) of these cytokines (reviewed in 12). IL-6 is elaborated, at least in part, in the peripheral vascular bed in heart failure patients.13 As demonstrated here, myocardial IL-6 expression decreases, whereas myocardial CT-1 expression is unchanged in DCM, suggesting that circulating and tissue levels of IL-6 and CT-1 are controlled by distinct mechanisms.

Regulatory circuits controlling phospho-JAK and phospho-STAT abundance are highly complex.14 Our data indicate that decreases in protein levels are primarily responsible for reduced phospho-TYK2 and phospho-STAT3 levels in the failing heart. By contrast, differences in phosphorylation status per se account for the decreased phospho-JAK2 and increased phospho-gp130 abundance observed in patients.
with DCM. Angiotensin II activates the JAK-STAT pathway in cardiomyocytes, raising the possibility that decreases in JAK-STAT phosphorylation may be due to ACE-inhibitor treatment of patients with DCM. However, JAK2, TYK2, and STAT3 phosphorylation levels were not different in patients treated with (n=7) or without (n=3) ACE-inhibitors, making such a possibility unlikely (not shown).

JAK2 and STAT3 were readily detectable by immunohistochemistry in cardiomyocytes from NF and DCM hearts. The expression pattern of JAK2 was similar in failing and

Figure 1. Expression levels of IL-6, LIF, and LIFR were measured by immunoblotting; representative blots and bar graphs are shown in A. Likewise, expression levels of gp130, JAKs, STATs, phospho-JAKs, and phospho-STATs were determined by immunoblotting, whereas phospho-gp130 levels were assessed by immunoprecipitation; representative blots are shown in B. In each case, the expression ratio of tyrosine-phosphorylated protein/total protein was calculated (bar graphs in B). Protein levels of SOCS1 and SOCS3 were determined by immunoblotting; representative blots and bar graphs are shown in C. *P<0.05, **P<0.01 versus NF (NF, n=5; DCM, n=10).
non-failing hearts, supporting our immunoblotting data, which demonstrated no differences in JAK2 protein expression levels. By contrast, STAT3 protein abundance was significantly reduced in cardiomyocytes from failing hearts. This finding would support the concept that the decrease in STAT3 levels in patients with DCM reflects downregulation, mainly in the cardiomyocyte compartment. Moderate staining was also present in non-myocytes, however, suggesting that part of the alterations in gp130-JAK-STAT signaling may reflect changes in the non-myocyte compartment.

Recently Zolk et al.\(^\text{16}\) reported increased CT-1 and diminished gp130 protein levels in the face of increased gp130 mRNA levels in a heterogeneous group of patients with ischemic and dilated cardiomyopathy. We focused on patients with DCM, and, importantly, excluded NF hearts with significant BNP mRNA levels.

Experimental studies have provided ample evidence that the JAK-STAT pathway protects cardiomyocytes from apoptosis,\(^\text{2-5}\) induces hypertrophy\(^\text{6-8}\) and promotes expression of cardioprotective genes, including superoxide dismutase and...
vascular endothelial growth factor. Therefore, reduced JAK-STAT activation may play an important pathophysiological role in patients with end-stage DCM and may represent a target for therapeutic intervention.

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