The Interleukin-6/Interleukin-6-Receptor System Is Activated in Donor Hearts

Gabriele Plenz, PhD, MSC;² Heike Eschert, PhD, MSC;² Michael Erren, MD;§
Thomas Wichter, MD,§ Michael Böhm, MD,∥ Markus Flesch, MD,¶ Hans H. Scheld, MD,*
Mario C. Deng, MD, FACC, FESC*#

Muenster and Homburg/Saar, Germany; Houston, Texas; and New York, New York

OBJECTIVES
To assess the potential of the donor heart to respond to interleukin-6 (IL6), the present study investigated the expression of IL6 receptor components in the myocardium of donor hearts before transplantation.

BACKGROUND
Donor heart dysfunction early after transplantation has been associated with the cytokine storm after donor brain death. Proinflammatory cytokines are thought to play a central role in this process. Interleukin-6 (IL6) is of specific interest because it has been associated with cardiac allograft dysfunction and is related to an impaired prognosis. Its action requires expression of the specific IL6 receptor (IL6R), and the common signal transducer of the IL6 family glycoprotein 130 (gp130) in the donor heart.

METHODS
The activation of IL6, IL6R and gp130 messenger ribonucleic acid (mRNA) and protein was studied via reverse transcription-polymerase chain reaction (RT-PCR) and immunohistology in donor hearts (n = 6) and compared with patients undergoing evaluation of ventricular arrhythmias (control, n = 9) or with advanced heart failure (n = 20).

RESULTS
Messenger RNA of IL6, IL6R and gp130 was strongly expressed in all chambers of donor hearts, whereas right ventricles of control patients did not show any expression (donor vs. control: p < 0.005). Right ventricles of failing hearts showed IL6, IL6R and gp130 mRNA levels comparable with those found in donor hearts. Immunohistochemistry paralleled the RT-PCR data on the protein level. While IL6 was mainly expressed by myocytes, both receptor components were preferentially found mainly on interstitial cells.

CONCLUSIONS
The expression of the IL6 receptor components in the donor heart before transplantation establishes the condition sine qua non for the response of the donor heart to circulating IL6. This mechanism may explain the close association of elevated IL6 serum levels to acute cardiac allograft dysfunction in the early perioperative period. (J Am Coll Cardiol 2002;39:1508–12 © 2002 by the American College of Cardiology Foundation

Primary cardiac allograft failure, that is, myocardial failure unexplained by hyperacute rejection, acute rejection or surgical problems, accounts for roughly 25% of early deaths after cardiac transplantation (1). One potential mechanism to explain nonspecific graft failure is donor heart dysfunction after the neurohormonal and cytokine storm associated with brain death. Expression of proinflammatory cytokines in the hearts of brain-dead rats has been demonstrated (2,3).

Cardiac dysfunction is mediated by several proinflammatory cytokines. Besides interleukin-1 (4–7) and tumor necrosis factor (8–11), interleukin-6 (IL6) has been extensively investigated. Interleukin-6 levels are elevated in the circulation of patients with asymptomatic left ventricular dysfunction and symptomatic congestive heart failure (12–14). In patients with advanced heart failure undergoing transplantation, IL6 serum levels are correlated with a reduction in myocardial contractility (15). Interleukin-6 and its specific receptor (IL6R) are upregulated in the failing myocardium, and both are modulated in the process of cardiac remodeling after left ventricular assist device implantation (16–18).

After transplantation, IL6 is of specific interest because it has been associated with cardiac allograft dysfunction early after cardiac transplantation in the absence of cellular rejection and related to an impaired prognosis (15,19). Interleukin-6 serum levels are increased not only in the recipient of the graft but also in the brain-dead donor (20,21). Furthermore, IL6 messenger ribonucleic acid (mRNA) is expressed in donor hearts before alloantigen contact (21). Substitution of hormones antagonistically influencing IL6 effects including corticosteroids (22), triiodothyronine (23) and arginine vasopressin (24), has been shown to be of benefit in the maintenance of donor heart function after brain death before explantation. Taken together, these data indicate that IL6 may impact negatively on donor heart function.

Interleukin-6 is a member of a structurally related cytokine family—including IL6, interleukin-11, cardiotrophin-1, oncostatin-M, leukemia-inhibitory factor and ciliary neurotro-
phic factor—with overlapping biologic effects (25). The redundancy and functional pleiotropy of the IL6 cytokine family in a wide variety of tissues and cells can be explained on the molecular level by the use of shared receptor subunits (26). Interleukin-6 signals through a receptor complex that is composed of the IL6R and the common signal transducer glycoprotein 130 (gp130).

Although several authors hypothesize an impact of elevated circulating IL6 levels on donor heart dysfunction as mentioned in the preceding text, little is known about the expression of these components in the donor heart before explantation are not available. Therefore, the present study evaluates the expression of IL6R and gp130 together with IL6 in the donor heart.

METHODS

Human myocardial tissue. Nonfailing human myocardium (normal ejection fraction by echocardiographic examination; absence of cardiovascular medication in patient history; inotropic support only with dopamine, but not high-dose isoprenaline or noradrenaline) was obtained from six donors who suffered from brain death as a result of traumatic injury but could not be transplanted for nonmedical reasons. Before acceptance and explantation of the donor heart, functional data demonstrating adequate quality were given to the attending cardiologist and the cardiac surgeon in the transplant center. Subsequent to explantation, these nonfailing hearts could not be used for transplantation because of technical or logistic reasons (27). Nine heart specimens of patients with normal ventricular function and absence of cardiovascular medication in patient history; inotropic support only with dopamine, but not high-dose isoprenaline or noradrenaline) served as controls. In addition, RV myocardium of failing hearts (patients suffering from advanced heart failure) or in the biopsy suite immediately after removal, specimens representing the left and right ventricle were snap frozen in liquid nitrogen. Care was taken in ischemic cardiomyopathy patients to avoid sampling of scarred areas. Until use, the tissue was stored at −80°C.

Cytokine detection. Interleukin-6, IL6R, gp130 and glyceraldehyde-3-phosphate-dehydrogenase (G3PDH) mRNA in heart specimens were assessed by reverse transcription-polymerase chain reaction (RT-PCR). Total ribonucleic acid of myocardial specimens (donor and failing hearts) was isolated, as previously described (28). To isolate mRNA from biopsies, the MACS system (Miltenyi Biotec, Nordrhein-Westfalen, Bergisch Gladbach, Germany) was used. Each ribonucleic acid sample was reverse transcribed using Superscript II according to the manufacturer’s instructions (Life Technologies, Eggenstein, Germany). The polymerase chain reaction (PCR) was done in a total volume of 100 μl using 1 × buffer (20 mM Tris-HCl [pH 8.4], 50 mM KCl), 1.5 mM MgCl2, 0.1 mM of each dNTP, 2.5 U Taq Polymerase (Life Technologies) and 0.5 μM of each primer (IL6: left primer: CACAGACAGC-CACCTCACCTCCTC; right primer: CTGGCCGAATGAGTGTGTTC; IL6R: hINF3G-IL6R, MB, St. Leon-Rot, Germany; gp130: left primer: CCACCCGATCT-TCATTCATC; right primer: TAGTTCAATTCCAGCCA-AAGC; G3PDH: left primer: GTGTCGGGTGACCT-GATCT; right primer: TGGGATTGACAAAGTGGTC). Product sizes were 464 base pair (bp) (IL6), 300 bp (IL6R), 498 bp (gp130) and 212 bp (G3PDH). Amplification was carried out in an UNO thermocycler (Biotop, Göttingen, Germany) after an initial denaturation at 95°C for 5 min. The following cycle profile was used: denaturation at 95°C for 60 s, primer annealing at 55°C for 10 min, primer extension at 72°C for 60 s and a final extension of 72°C for 10 min. Aliquots of the PCR products were analyzed using standard agarose gel electrophoresis, a video documentation system and ImageQuant software (Molecular Dynamics, Krefeld, Germany).

Immunohistochemistry. The IL6 system was detected as follows: 1) for IL6, rabbit anti-human IL6 (H-183, sc-7920; Santa Cruz, Heidelberg, Germany); 2) for IL6R, rabbit anti-human IL6R (C-20, sc-661; Santa Cruz); and 3) for gp130, rabbit anti-human gp130 (C-20, sc-655, Santa Cruz) were used. The secondary antibody/detection system was donkey anti-rabbit immunoglobulin conjugated to Texas Red (Chemicon International, Hofheim, Germany). The immunohistochemical staining procedure was performed with modifications, as described (29). In brief, cryosections were incubated with the respective antibody (1:200) at room temperature for 1 h. After washing thoroughly in PBS, sections were treated with the secondary antibody (1:500) for 1 h at room temperature. After
Myocardial mRNA expression of IL6, IL6R and gp130.

RESULTS

washing again, the slides were mounted with fluoromount mounting medium (BDH, Poole, United Kingdom). Negative controls included substitution of the primary antibody by rabbit immunoglobulins or omission of the primary antibody. Results were documented using a fluorescence microscope (AXIOPHOT2, Zeiss, Jena, Germany) fitted with the appropriate filter blocks for detection of Texas Red fluorescence.

Statistics. For statistical analysis, the Statistical Package for the Social Sciences (SPSS 9.0, SPSS Inc., Chicago, Illinois) was used. Comparisons between optical density ratios of cytokine (receptor)/G3PDH mRNA of donor and control tissues were carried out by Mann-Whitney U test for independent samples. P values of <0.05 were considered significant.

RESULTS

mRNA expression of IL6, IL6R and gp130. Myocardial samples of all chambers of donor hearts, RV biopsy specimens of patients undergoing evaluation of ventricular arrhythmias (control) and the right ventricle of patients undergoing heart transplantation were analyzed for the expression of IL6, IL6R and gp130 mRNA by RT-PCR (Table 1). The RV control biopsies did not show detectable expression of IL6, IL6R and gp130 mRNA. In contrast with the controls, donor hearts and failing hearts strongly expressed the mRNAs of IL6, IL6R and gp130 (p < 0.005). In donor hearts, the components of the IL6 system were strongly expressed in all right and left ventricles and atria. Differences between the chambers were not observed. The right ventricles of patients suffering from advanced heart failure showed high IL6, IL6R and gp130 mRNA levels. In comparison with the expression levels found in failing hearts, the RV mRNA levels of IL6, IL6R and gp130 in donor hearts were not significantly lower.

Immunohistochemical demonstration of the IL6, IL6R and gp130 distribution patterns. In order to demonstrate that the elevated mRNA levels of IL6, IL6R and gp130 in donor hearts were translated into protein, sections from the right and left ventricular myocardium of donor hearts and the RV biopsies controls were immunostained with rabbit anti-IL6, IL6R and gp130 antibodies (Fig. 1). The myocardium of the control biopsies were devoid of immunolabeling for IL6, IL6R and gp130. In the ventricles of donor hearts, IL6 and both of its receptor components were found. The immunostaining of all components of the IL6 system was distributed in patchy fashion over the entire myocardium and bound to cellular structures. Interleukin-6 was mainly produced by a subpopulation of cardiomyocytes but was also strongly expressed by some interstitial cells, probably fibroblasts. In comparison, strong labeling for IL6R and gp130 was located mainly on interstitial cells; only few myocytes stained weakly. In failing hearts, the distribution patterns were similar to those found in donor hearts.

Table 1. Expression Levels of IL6, IL6R and gp130 in CTRL Biopsies, All Chambers of Donor Hearts and RV of FH

<table>
<thead>
<tr>
<th>mRNA Ratio</th>
<th>CTRL (n = 9)</th>
<th>FH (n = 20)</th>
<th>Donor Hearts (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RV</td>
<td></td>
<td>RV</td>
</tr>
<tr>
<td>IL6/G3PDH</td>
<td>0</td>
<td>2.2 ± 1.2†</td>
<td>1.3 ± 0.9†</td>
</tr>
<tr>
<td>IL6R/G3PDH</td>
<td>0</td>
<td>1.9 ± 1.0†</td>
<td>1.3 ± 0.5†</td>
</tr>
<tr>
<td>gp130/G3PDH</td>
<td>0</td>
<td>2.0 ± 1.2†</td>
<td>1.3 ± 0.4†</td>
</tr>
</tbody>
</table>

Versus control *p < 0.05; †p < 0.005.

CTRL = control hearts, myocardium of patients undergoing evaluation of ventricular arrhythmias; FH = failing hearts, myocardium of patients undergoing heart transplantation; G3PDH = glyceraldehyde-3-phosphate-dehydrogenase; gp130 = glycoprotein 130; IL6 = interleukin-6; IL6R = interleukin-6-receptor; LA = left atrium; LV = left ventricle; mRNA = messenger ribonucleic acid; RA = right atrium; RV = right ventricle.
However, the expression by interstitial cells was more pronounced for all components of the IL6 system (data not shown).

### DISCUSSION

Our data demonstrate for the first time that, concomitantly with IL6, both components of the IL6 receptor, that is, the specific subunit IL6R and the common signal transducer gp130: 1) are induced in all cardiac chambers of brain-dead donors before removal for transplantation; 2) are expressed at levels comparable to failing hearts in hearts of brain-dead donors; and 3) show similar distribution patterns on the cellular level in donor hearts and failing hearts.

**Brain death activates the cardiac IL6 system.** The lack of expression of IL6 receptor components in control biopsies obtained from arrhythmia patients with hemodynamic and echocardiographic evidence of normal heart function suggests that the expression of the IL6 receptor components as well as IL6 in donor hearts may be induced by the sequelae of brain death. Brain death itself causes a massive disruption in the hemodynamic, hormonal and cytokine homeostasis. In the rat model, donor brain death stimulates expression of inflammatory cytokines, including IL6 in the heart and other peripheral organs. Furthermore, a differential expression has been suggested between organs from living donors compared with organs from cadaveric donors regarding the cytokines tumor necrosis factor-alpha, IL6 and interleukin-8. In the human system, analogies can be drawn from the field of renal transplantation. Renal allografts obtained from cadaveric donors show elevated cytokine levels compared with living donations. This is reflected in our study by the massive activation of IL6 and its receptor components in donor hearts compared with endomyocardial biopsies obtained from control patients. With this demonstration of an upregulation of the IL6 receptor system in the donor heart after brain death, it is likely that circulating IL6, either generated by the donor heart itself as shown here or by the recipient due to advanced heart failure or after cardiac surgery, exerts its negative inotropic effects on the donor myocardium, thereby producing the clinical syndrome of early perioperative donor heart dysfunction. Thus, the responsiveness of the donor heart to circulating IL6 in the immediate perioperative period based on the pre-existent expression of IL6 receptor components may explain the phenomenon of early “unexplained” graft failure.

**Activation of donor hearts is comparable to failing hearts.** Our study extends recent data demonstrating similar left ventricular IL6 mRNA expression levels in donor and failing hearts. While our data show comparable levels of gp130 in all chambers of donor hearts, the study of Eicken et al. revealed a stronger expression of gp130 in left atria than in left ventricles. This discrepancy may be due to differences in the recruitment of the samples. Investigating donor heart malfunction, Birks et al. demonstrated that transplanted donor hearts—comparable to our donor cohort—and failing hearts expressed similar levels of tumor necrosis factor-alpha and IL6 mRNA. In their study, circulating levels of IL6 were increased eight- to ninefold in comparison with normal subjects. Taken together, these data indicate that the donor heart is able to respond to circulating IL6 in the immediate perioperative period and, therefore, support a role of the IL6 system in the syndrome of early unexplained allograft failure.

**The IL6 system is activated in cardiomyocytes and fibroblasts.** In our study, upregulation of mRNA was paralleled by corresponding protein distribution patterns of all components of the IL6 system. Immunohistochemistry revealed that the patterns are comparable in donor hearts and failing hearts, while controls did not show immunolabeling. The predominant expressing cell population was located in the interstitium, preferentially fibroblasts. It has been shown previously in cell culture studies that IL6 and other members of this cytokine family are expressed by interstitial fibroblasts. However, no data are available on the expression of the IL6 receptor components in cardiac interstitial fibroblasts. As additional expressing cell types, myocytes and microvascular endothelial cells were identified. Expression of IL6 in the vascular tissue has been reported before. Studies on the role of interleukin-1-beta—an another proinflammatory cytokine—in congestive heart failure showed similar cellular distribution patterns as those described in the present study for IL6, that is, expression by cardiac monocytes and fibroblast, vascular cell types and infiltrating cells. On the other hand, Eicken et al. demonstrated only cardiomyocytes as major producers of IL6 and gp130 in failing hearts and suggest a similar expression pattern in donor hearts. This discrepancy in the cell populations producing IL6 and gp130 may be due to the different methodologic approaches used, that is, differences in fixation or in the embedding procedure. Nevertheless, our data demonstrate that both IL6 and its receptor components are induced not only in myocytes but also in fibroblasts. Therefore, we postulate that in donor hearts as well as failing hearts both cell types harbor the potential to respond to circulating and locally produced IL6 and, through these complementary pathways, may mediate not only acute cardiac allograft dysfunction but also set the stage for chronic transplant vasculopathy.

**Conclusions.** Expression of IL6 and its receptor components is intensely stimulated in the myocardium of brain dead donors comparable to patients with advanced heart failure in comparison with a control group not compromised by the sequelae of brain death. The induction of the IL6 receptor system in the donor heart before transplantation establishes the condition sine qua non for the response of these hearts to circulating and locally produced IL6 in the immediate perioperative period, which may explain the close association of IL6 serum levels to acute cardiac allograft dysfunction. This might have important therapeutic implications.
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Reprint requests and correspondence: Dr. Gabriele Plenz, Department of Cardiothoracic Surgery, Muenster University, Albert-Schweitzer-Str. 33, D-48149 Muenster, Germany. E-mail: plenz@uni-muenster.de.

REFERENCES