Elevated Tumor Necrosis Factor-α and Interleukin-6 in Myocardium and Serum of Malfunctioning Donor Hearts

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Background—Myocardial dysfunction is a common and important problem in donor hearts. The mechanisms responsible remain unclear. We have studied the cytokines tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) in the myocardium and serum from donors with myocardial dysfunction (unused donors) and compared them with donors with good ventricular function (used donors) and patients with advanced heart failure (HF).

Methods and Results—Clinical details and ventricular function were assessed in 46 donors (31 used, 15 unused). Real-time reverse transcription–polymerase chain reaction, Western blotting, and immunocytochemistry were performed on myocardium and immunoassays on serum. TNF-α mRNA was 1.6-fold higher in unused than in used donors (P<0.005) and 1.74-fold higher than in 36 patients with HF. IL-6 mRNA was 2.4-fold higher in unused than in used donors (P<0.0001) and 4.67-fold higher than in HF (P<0.0001). Western blotting showed higher TNF-α in unused (218.3±6.4, n=4 versus 187.3±5.4, n=3 OD units) than used donors (P<0.05). TNF-α expression was localized to cardiac myocytes. Serum TNF-α was higher in unused (8.72±1.3 pg/mL, n=13) than in used (6.12±0.8 pg/mL, n=25, P<0.05) donors and HF (4.0±0.4 pg/mL, n=17, P<0.005). Serum TNF-α receptors did not differ between unused (4.3±0.8 and 8.6±1.6 ng/mL, n=10) and used (3.5±0.4 and 6.5±1.1 ng/mL, n=24) donors. There was a trend for higher serum IL-6 in unused (16.5±2.9 pg/mL, n=9) compared with used (13.9±1.6 pg/mL, n=26, P=NS) donors.

Conclusions—This study documented an increase in the expression of TNF-α and IL-6 in the myocardium of all donor hearts that was more marked in the dysfunctional (unused) donor hearts. This was accompanied by similar changes in the serum. This might have important therapeutic implications. (Circulation. 2000;102[suppl III]:III-352-III-358.)

Key Words: transplantation ■ myocardium ■ molecular biology

Myocardial dysfunction in the donor heart is a common problem and is so severe as to preclude its use for transplantation in 20% of cases. Brain death has been shown to result in various degrees of myocardial dysfunction,1 but the mechanisms remain poorly understood. Trabeculae and isolated myocytes from dysfunctional donors demonstrate a slowed relaxation and a poor force-frequency response in vitro comparable to that of hearts from patients with advanced heart failure.2

Patients with chronic heart failure due to a variety of causes have been found to have elevated expression of proinflammatory cytokines including tumor necrosis factor (TNF)-α both in the serum and myocardium.3–7 TNF-α expression has been demonstrated5–7 in the ventricles of patients with dilated cardiomyopathy, where it was seen in cardiac myocytes, endothelial cells, and in the vascular smooth muscle cells of intramyocardial blood vessels. Exogenous and endogenous TNF-α have been shown to produce myocardial depression and hemodynamic effects both in in vitro and in vivo models.4,5,8,9 Interleukin 6 (IL-6) is also elevated in patients with heart failure,3,5,10,11 and raised levels correlate with decreasing functional class, low ejection fraction, high right atrial pressures, and poor prognosis.5,10

The possible role of cytokines in producing donor dysfunction in humans has not been explored. In this study, we compare TNF-α and IL-6 expression in the myocardium together with circulating levels of TNF-α and its receptors, TNFR1 and TNFR2, and IL-6 in donors exhibiting myocardial dysfunction to donor hearts with good ventricular function used for transplantation. In an attempt to put these findings into perspective, we have also included myocardium from patients with advanced heart failure and serum from normal control subjects.

Methods

Donors
The protocol for this study was approved by the local ethics committee. Donor hearts were assessed by transesophageal echocardiography before retrieval. The transgastric short-axis area was used to assess fractional shortening and hence ejection fraction (EF). Donors with an EF <30% coupled with 2 of the following poor hemodynamic parameters were judged unsuitable for transplantation:
Donor Details

<table>
<thead>
<tr>
<th>Used Donors (n=31)</th>
<th>Unused Donors (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>32.9±2.8</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>17:14</td>
</tr>
<tr>
<td>Mean No. of isotope per patient (range)</td>
<td>0.92±0.1 (0–2)</td>
</tr>
<tr>
<td>Central venous pressure (range), * mm Hg</td>
<td>6.9±0.7 (2–11)</td>
</tr>
<tr>
<td>Mean ventilation period, d</td>
<td>2.32±0.6</td>
</tr>
<tr>
<td>Smokers, yes:no</td>
<td>10:21</td>
</tr>
<tr>
<td>Evidence of infection, n</td>
<td>10/31 (32.3%)</td>
</tr>
<tr>
<td>Cardiac arrest, n</td>
<td>2/31 (6.5%)</td>
</tr>
</tbody>
</table>

*Significant difference between the two groups, P<0.05.

(unused donors): mean blood pressure <50 mm Hg, mean left atrial pressure >14, inotropic support (norepinephrine and/or epinephrine) >0.4 μg · kg⁻¹ · min⁻¹ necessary to maintain systolic blood pressure >90 mm Hg. This group was compared with a group of donors with good ventricular function used for transplantation. Donor clinical details, cause of death, period of ventilation, and any evidence of systemic infection were recorded. Infection in the donor was defined as a raised white cell count combined with either pyrexia or evidence of consolidation on chest radiography.

Unused Donor Hearts

Donor hearts unused for transplantation because of poor myocardial function (n=15) were retrieved normally after cold crystalloid cardioplegia with 20 mL/kg St Thomas cardioplegic solution. Left ventricular samples were taken from the apical area and transported to the laboratory in cardioplegia, where they were stored at −80°C. The mean time of transport to the laboratory was 137.9±12.6 minutes. Causes of death were subarachnoid hemorrhage (6), road traffic accident (4), intracranial bleed (2), epileptic seizure (1), meningitis (1), and astrocytoma (1). Clinical details are shown in the Table.

Used Donor Hearts

Ventricular endomyocardial biopsies were obtained immediately before implantation from 31 donors used for transplantation during the same period, frozen in liquid nitrogen, and stored at −80°C. The donor heart was preserved by infusion of St Thomas’ solution at 4°C. Mean ischemia time was 163±10.2 minutes. Causes of death were subarachnoid hemorrhage (13), road traffic accident (8), intracranial bleed (4), asthma (1), astrocytoma (1), and carbon monoxide poisoning (1), and 3 were domino hearts from patients undergoing heart-lung transplantation for cystic fibrosis. All had normal ECGs. Clinical details are shown in the Table. Hearts were reassessed by transthoracic echocardiography 1 week after transplantation, and all had good ventricular function (mean EF 72.8±1.4%).

Heart Failure

Left ventricular samples were obtained from 36 patients with advanced heart failure undergoing heart transplantation. Thirty were men and 6 were women. Mean age was 46±3.4 years. Diagnosis was dilated cardiomyopathy (18), ischemic heart disease (15), postpartum cardiomyopathy (2), and myocarditis (1). New York Heart Association class was III in 28 and IV in 8. Mean fractional shortening was 13.0±1.2%.

Serum

Blood was taken from 26 of the used and 13 of the unused donors immediately before retrieval. Blood was also taken from 17 of the advanced heart failure patients before transplantation and from 8 normal control subjects (5 men and 3 women, 44.2±3.2 years of age). Blood was spun within 4 hours of collection at 2500 rpm for 10 minutes, and the serum supernatant stored at −40°C for further analysis.

**Methods**

Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction

TNF-α and IL-6 mRNA were detected by polymerase chain reaction (PCR) amplification and quantified by 5’ nuclease assay with fluorescent-labeled TaqMan probes analyzed with the use of real-time quantitative PCR as follows.

Total RNA was extracted with the Qiagen RNeasy minicolumn procedure and eluted in diethylpyrocarbomate-treated dH₂O, following the manufacturer’s instructions. RNA quality and quantity was assessed by EthBr-agarose gel electrophoresis and by relative absorbance at 260 nm versus 280 nm. cDNA was synthesized from 150 ng of total RNA in a volume of 10 μL with the PE Biosystems reverse transcriptase kit (Cat No. N8080234) with random hexamer primers. Reactions were diluted to 100 μL with sterile dH₂O and stored at −20°C.

Primers and TaqMan probe for human IL-6 were designed with the use of the Primer Express Software (PE Biosystems) from published mRNA sequence (EMBL/GenBank accession No. M54894) with flanking primers located on separate exons giving an amplicon size of 96 bp and with the TaqMan probe straddling the exon-exon junction: Forward primer (Tm=58°C) 5’-TGCACAAAAATTCGGTACATCT-3’, reverse primer (Tm=60°C) 5’-AGTGCCCTTTGCTGTTTTAC-3’, TaqMan probe (Tm=68°C) 5’-TACCTCCTGTAATCCTCCTTTCTCAGGGCTG-3’. Internal control 18S rRNA primers and TaqMan probe were provided as a preoptimized kit (Cat No. 4310893E).

**Western Blot Analysis**

Total protein extracts were prepared by homogenizing myocardial biopsies in lysis buffer (1% SDS, 1 mmol/L phenethylsulfonyl fluoride, 10 μg · mL⁻¹ aprotinin, and 10 μg · mL⁻¹ leupeptin). Protein (40 μg per sample) was mixed with sample buffer and loaded onto a 12.5% SDS-polyacrylamide gel. After electrophoresis, proteins were transferred onto Hybond-C super nitrocellulose membranes. Membranes were immersed in PBS-Tween 20 and 5% milk protein overnight at 4°C to block nonspecific binding. An affinity-purified TNF-α polyclonal antibody (Santa-Cruz Biotechnology), diluted 1:500 (vol/vol) in PBS-T/5% milk protein, was added to the membranes for 60 minutes. After washing, membranes were incubated with a horseradish peroxidase–conjugated rabbit anti-goat secondary antibody (DAKO) diluted 1:1000 (vol/vol) in PBS-T/35% hydrogen peroxide. Membranes were washed and membranes were developed with 4-chloro-1-napthol (100 mg/mL in 0.05% substrate). Membranes were washed again, dried, and exposed overnight to X-ray film. Membranes were then scanned into a Macintosh using a Sony video camera and a Macintosh laser scanner. Membranes were stripped of antibody with a 0.1% Tween-20 wash. Membranes were reprobed for IL-6 using the same protocol.
milk protein for 1 hour. The immunoreactive bands were visualized with the use of Amersham enhanced chemiluminescence reagents and were scanned with Image Analysis 1000 software (Alpha Innotech).

**Immunocytochemistry**

Immunocytochemistry was performed on formalin-fixed paraffin-embedded sections to localize the cell type producing TNF-α. The avidin-biotin-peroxidase complex method was used. Endogenous peroxidase was blocked with a solution of 0.03% (vol/vol) hydrogen peroxide in methanol for 20 minutes. After incubation with normal goat serum (1:30, 30 minutes), sections were incubated overnight at 4°C with primary rabbit antibodies to TNF-α (Antigenex America Inc) diluted 1:100. Immunoreaction sites were visualized with the use of the appropriate biotinylated secondary antibodies and the avidin-biotin-peroxidase complex procedure (Vector Labs). Peroxidase activity was revealed with a solution of diaminobenzidine as chromogen with 0.2% (vol/vol) hydrogen peroxide in PBS to produce a brown reaction product and sections counterstained with Harris’ hematoxylin. Controls consisted of replacement of primary antibodies with nonimmune rabbit serum.

**Serum Immunoassay**

Measurements of TNF-α, its receptors TNF R1 and TNF R2, and IL-6 were performed with commercially available immunoassay kits (Quantikine HS, R & D Systems).

**Statistical Analysis**

Variables are expressed as mean±SEM. Significance was assessed on grouped data with either the Student’s t test or the nonparametric Mann-Whitney U test. Fisher’s exact test was used to compare some of the clinical variables. A value of P<0.05 was considered significant.

**Results**

**Myocardium**

**TNF-α mRNA**

TNF-α mRNA was measured in myocardial samples relative to 18S ribosomal RNA (Figure 1). TNF-α mRNA expression in the 15 unused donors was 1.6-fold higher than in the 31 donors used for transplantation (P<0.005, Figure 2A). TNF-α mRNA in the 15 unused donors was also 1.74-fold higher than in the 36 patients with advanced heart failure (P<0.001, Figure 2A). TNF-α mRNA levels in the used donor group were similar to levels in advanced heart failure (1.09-fold, P=NS).

TNF mRNA levels were not influenced by donor sex, cause of death, central venous pressure, the presence of infection in the donor, or ischemia time. In addition TNF,
mRNA levels were not influenced by the number of inotropes used (1, 2, or 3) or the use of norepinephrine.

**IL-6 mRNA**

IL-6 mRNA expression was 2.4-fold higher in the unused donors than in donors used for transplantation \((P<0.0001, \text{ Figure 1B})\). IL-6 mRNA in the unused donors was 4.67-fold higher than in the 36 patients with advanced heart failure \((P<0.0001, \text{ Figure 1})\). IL-6 mRNA levels in the used donor group were 1.96-fold higher than in advanced heart failure \((P<0.0001)\).

IL-6 mRNA levels were not influenced by donor sex, the presence of infection in the donor, or ischemic time but correlated with the quantity of inotropes used (levels being 3.18-fold in donors with 1 inotrope compared with those with none, \(P<0.0001\), and 1.58-fold with 2 inotropes compared with 1, \(P<0.0001\)). IL-6 levels were 1.5-fold higher in those receiving norepinephrine compared with those not receiving \((P<0.005)\).

**TNF-α Protein Expression by Western Blotting**

TNF-α expression was significantly higher in specimens examined from unused donor hearts (mean 218.3±6.4 OD units, \(n=3\)) compared with donor hearts used for transplantation (187.3±5.4 OD units, \(n=4, P<0.05\)).

**TNF-α Immunocytochemistry**

TNF-α was strongly expressed in unused donor hearts (Figure 3, top) compared with used donor hearts (Figure 3, bottom). TNF-α was immunolocalized to cardiac myocytes and was not expressed in endothelial cells and only in occasional vascular smooth muscle cells.

**Serum**

**Serum TNF-α**

Serum TNF-α was significantly higher in 13 unused donors \((8.72±1.3 \text{ pg/mL}, n=3)\) compared with 25 used donors \((6.12±0.8 \text{ pg/mL}, P<0.05, \text{ Figure 4})\). Serum TNF-α in both unused and used donors was significantly higher than in 8 normal subjects \((2.58±0.45 \text{ pg/mL}, P<0.0001 \text{ for unused and } P<0.001 \text{ for used donors})\). Serum TNF-α in both donor groups was also significantly higher than in advanced heart failure \((4.0±0.4 \text{ pg/mL}, n=17, P<0.005 \text{ for unused and } P<0.05 \text{ for used donors})\). As expected, serum TNF-α in patients with advanced heart failure was higher than in normal subjects \((P<0.05)\).

**Serum TNFR1**

There was no significant difference between serum TNFR1 levels in unused \((4.3±0.8 \text{ ng/mL}, n=10)\) and used donors \((3.5±0.4 \text{ ng/mL}, n=24, \text{ Figure 5A})\). There was also no significant difference between serum TNFR1 levels in unused \((3.5±0.4 \text{ ng/mL}, n=24)\) and unused \((4.3±0.8 \text{ ng/mL}, n=10)\) donors than in heart failure \((2.8±0.3 \text{ ng/mL}, n=17, P=\text{NS for both})\). TNFR1 levels were higher in both unused and used donors and in patients with heart failure than in normal subjects \((1.1±0.4 \text{ ng/mL}, n=8; P<0.0001, P<0.0005, \text{ and } P=0.0001, \text{ respectively})\).

**Serum TNFR2**

Serum TNFR2 levels were significantly higher in patients with heart failure \((9.0±0.8 \text{ ng/mL}, n=17)\) than in normal subjects \((4.7±0.5 \text{ ng/mL}, n=8, P<0.005)\) but were not significantly higher in either used \((6.5±1.1 \text{ ng/mL}, n=24)\) or unused \((8.6±1.6 \text{ ng/mL}, n=10)\) donors than in normal...
subjects. There was no difference between TNFR2 in unused (8.6 ± 1.1 ng/mL, n = 10) and used (6.5 ± 1.1 ng/mL, n = 24) donors (Figure 5B). TNFR2 levels were lower in used donors than in patients with heart failure ($P < 0.01$). TNFR2 levels were nonsignificantly lower in unused donors than in patients with heart failure.

**Serum IL-6**

There was a trend for higher serum IL-6 in unused (16.5 ± 2.9 pg/mL, n = 9) compared with used (13.9 ± 1.6 pg/mL, n = 26) donors (Figure 6) and in both unused and used donors compared with patients with heart failure (12.7 ± 2.6 pg/mL, n = 17). Serum IL-6 levels were significantly higher in unused (16.5 ± 2.9 pg/mL, n = 9) and used (13.9 ± 1.6 pg/mL, n = 26) donors compared with normal subjects (1.55 ± 0.6 pg/mL, n = 7; $P < 0.0005$ and $P < 0.0001$, respectively) and in heart failure compared with normal subjects ($P < 0.001$).

**Discussion**

This study has shown an elevation of the cytokines TNF-α and IL-6 in both myocardium and serum of donor hearts with poor myocardial function compared with donor hearts with good ventricular function. Elevated TNF-α and IL-6 levels were observed in all donor hearts, but the elevation was more marked in those with poor ventricular function.

Myocardial dysfunction has previously been shown in donor hearts and may have a variety of causes that include the effects of brain death, donor management, and myocardial preservation. Studies after brain death in experimental animals have shown decreased biventricular systolic function and contractility. Although several humoral and neurogenic factors have been implicated, the mechanisms underlying this remain unclear.

Our study suggests that the cytokines TNF-α and IL-6 might play a major role. This is supported by the fact that TNF-α was produced in the dysfunctional donor hearts in our study at levels higher than that in advanced heart failure. Levels of TNF-α were higher in both the myocardium and serum in unused compared with used donors and compared with advanced heart failure. Infusion of TNF-α into rats at similar levels to those found in patients with end-stage heart failure results in depression of left ventricular function, cardiac myocyte shortening, and left ventricular dilation.9 In our study, TNF-α expression was demonstrated in cardiac myocytes in the dysfunctional donor hearts. Cardiac myocytes have previously been shown to produce substantial amounts of TNF-α.12 It is therefore likely that the TNF-α produced by cardiac myocytes plays an important role in causing donor heart dysfunction.

To the best of our knowledge, no other studies in humans have examined the relation between cytokine expression and donor heart malfunction. However, Takada and colleagues have shown elevated TNF-α and IL-6 mRNA in the myocardium and serum of rats after explosive brain death along with other cytokines produced by macrophages. They have also found cytokine elevation in the kidney, brain, liver, and spleen.

The factors causing cytokine induction in donor hearts are unclear. There was no relation between cytokine expression and the cause of brain death in our study, with all types of brain injury causing elevation of these cytokines. Brain death is known to be associated with marked hemodynamic changes. We did not monitor hemodynamic changes during the process of brain death, but after brain death we observed no correlation between TNF-α and the donor central venous
pressure, even though TNF-\(\alpha\) production can be stimulated by pressure overload. Left ventricular samples from unused donors and used donor hearts were both transported in cardioplegia, but only the used donors were transported as the whole organ, and the samples from patients with end-stage heart failure were not transported at all. However, this is unlikely to have affected cytokine measurement, particularly because both TNF and IL-6 mRNA expression were independent of ischemia time. Other potential causes of TNF-\(\alpha\) elevation in the donor heart include drug therapy, neurohumoral factors, and infection, although TNF expression was not related to infection in our study.

TNF-\(\alpha\) acts on 2 cell surface receptors, TNFR1 (55 kDa) and TNFR2 (75 kDa), which are thought to mediate and regulate most of the effects of TNF-\(\alpha\), and both have been immunolocalized to cardiac myocytes.\(^1\) TNF-\(\alpha\) produced in these donor hearts may therefore act locally in either an autocrine or paracrine fashion by binding to receptors situated on surrounding myocytes. The extracellular domain fragments of both receptors are shed from cell surfaces and can be detected as soluble forms (sTNFR1 and sTNFR2) in blood and urine. Both soluble receptors have been shown to be elevated in heart failure,\(^1\) which we also found in our study. Although the exact biological role for these soluble TNF-binding proteins is not known, it has been suggested that they may serve as biological buffers that neutralize the highly cytotoxic activity of TNF-\(\alpha\), and experimentally it has been shown that soluble TNF receptors are sufficient to both block and reverse the negative inotropic effects of TNF-\(\alpha\).\(^16\) It has also been postulated that in the long term, soluble TNF receptors may stabilize TNF-\(\alpha\) as a homotrimer and hence increase TNF bioactivity relative to unstabilized TNF-\(\alpha\), which dissociates into inactive monomers. Interestingly, although raised levels of TNF-\(\alpha\) in the unused relative to the used donors were detected in our study, the levels of the receptors were no higher in the unused than the used donors, suggesting that the rise in TNF-\(\alpha\) in the unused donors is not neutralized by a similar rise in the TNF receptors.

Moreover, our group has previously demonstrated increased activity of the inhibitory G protein, \(\text{G}_{\text{i}}\), in donor hearts with myocardial dysfunction.\(^2\) Treatment of rat cardiomyocytes with TNF-\(\alpha\) causes a concentration-dependent increase in \(\text{G}_{\text{i}}\),\(^17\) and this previously documented rise in \(\text{G}_{\text{i}}\) may be a consequence of TNF-\(\alpha\) expression, thus providing a pathway linking elevated TNF-\(\alpha\) to impaired myocardial function. TNF-\(\alpha\) may act through several different mechanisms. Binding of TNF-\(\alpha\) to TNFR1 results in the production of sphingosine, which in turn decreases calcium transients that may lead to dysfunctional excitation-contraction coupling and to systolic and/or diastolic dysfunction.\(^18\) TNF-\(\alpha\) can induce cardiac myocyte apoptosis through a sphingosine-dependent mechanism,\(^19\) and recent data from our group suggests that activation of the apoptotic pathway occurs in dysfunctional donor hearts.\(^20\) TNF-\(\alpha\) can also induce expression of inducible nitric oxide synthase, resulting in the production of nitric oxide, which itself can be negatively inotropic.\(^3\) Last, it has the potential to lead to heart failure through its effects on the matrix metalloproteinases.\(^21\)

IL-6 levels were higher in the myocardium of unused donors compared with both used donors and patients with advanced heart failure in our study. Elevated IL-6 has been demonstrated in the peripheral circulation in heart failure,\(^10\) and recently, one study showed IL-6 mRNA expression in the myocardium\(^11\) of patients with heart failure. Like TNF-\(\alpha\), IL-6 can exert a negative inotropic effect.\(^22\) Transgenic mice overexpressing the IL-6 gene and its receptor, gp130, develop hypertrophy of the ventricular myocardium and increase in heart size,\(^23\) which may predispose to heart failure. TNF-\(\alpha\) can induce IL-6 gene and protein expression in a variety of cell types, and the rise in IL-6 seen in our study might be secondary to or independent of TNF-\(\alpha\) release. Elevated IL-6 levels have been found previously to correlate with elevated TNF-\(\alpha\) levels in heart failure,\(^10\) and it may be that a “cytokine cascade” is initiated. IL-6 levels also correlated with the amount of donor inotropic support in our study. IL-6 levels have been shown to correlate with norepinephrine levels in heart failure in previous studies,\(^10\) and it is possible that the IL-6 elevation seen in the donor hearts may be contributed to by the endogenous norepinephrine known to be elevated after brain death or to exogenous norepinephrine administered to these donors. IL-6 can be produced from leukocytes, endothelial cells, and vascular smooth muscle cells in vitro and has recently been detected in cardiac myocytes.\(^24\)

Modulation of the expression of these cytokines might not only improve the functional results after transplantation but might potentially increase the use of organs from underutilized “marginal” donors and enlarge the donor pool.

Etanercept is a p75 TNF receptor fusion protein that binds to TNF-\(\alpha\), functionally inactivating it. When given to patients with NYHA class III heart failure in a randomized double-blind trial, it increased quality of life, 6-minute walk distance, and EF.\(^25\) Pentoxifylline is a xanthine derivative that suppresses or reduces the production of TNF-\(\alpha\). Administration of pentoxifylline to patients with idiopathic dilated cardiomyopathy\(^26\) resulted in improved functional class, increased EF, and decreased TNF-\(\alpha\) levels.

Limitations of the Study
Although our results show an elevation of these cytokines in dysfunctional donor hearts to levels sufficient to cause myocardial dysfunction in other studies, a causal relation has not been established. Further studies will be required in which these cytokines are blocked and the functional results ascertained. Our study was limited by the necessarily small amount of tissue available from the donor hearts used for transplantation, which was insufficient to allow protein studies for IL-6.

Conclusions
In conclusion, increased expression of the cytokines TNF-\(\alpha\) and IL-6 was detected in the myocardium, with similar changes in the serum of donor hearts with poor myocardial function when compared with donor hearts with good ventricular function. Pharmacological modulation of the expression of these cytokines in organ donors might not only improve the functional results after transplantation but might...
increase the use of organs from “marginal” donors and possibly organs previously considered untransplantable.

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References
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