Quantitative Changes In T-Cell Populations After Left Ventricular Assist Device Implantation
Relationship to T-Cell Apoptosis and Soluble CD95

Hendrik-Jan Ankersmit, MD; Niloo M. Edwards, MD; Michael Schuster, BSc; Ranjit John, MD; Alfred Kocher, MD; Eric A. Rose, MD; Mehmet Oz, MD; Silviu Itescu, MD

Background—Left ventricular assist devices (LVADs) are currently being evaluated as permanent therapy for end-stage heart failure. Because life-threatening infections limit successful long-term device implantation, we investigated the relationship between quantitative T-cell defects in LVAD recipients and CD95-mediated T-cell apoptosis.

Methods and Results—Immunological studies were performed in NYHA class IV patients awaiting cardiac transplantation who received either a TCI Heartmate left ventricular assist device (LVAD) or medical management. Fluorochrome-labeled Mabs were used in T-cell phenotypic analyses. T-cell apoptosis was measured by annexin V binding of T cells cultured in medium for 24 hours. Circulating serum levels of soluble CD95 were measured by ELISA. LVAD recipients had a relative lymphopenia and reduction in CD4 T-cell levels compared with NYHA class IV heart failure controls. These observations were confirmed in a longitudinal study in LVAD recipients, which showed that device implantation was accompanied by progressive and sustained reductions in circulating CD4 T-cell levels. These abnormalities in LVAD recipients were accompanied by increased levels of circulating soluble CD95 and by excessive CD4 and CD8 T-cell apoptosis. Susceptibility to induction of apoptosis was 2-fold greater for CD4 T cells than for CD8 T cells.

Conclusions—These results suggest that the reduction in CD4 T-cell levels accompanying LVAD implantation is a consequence of an augmented pathway of CD95-mediated apoptosis. The clinical consequences of these abnormalities may include increased prevalence of systemic infections. (Circulation. 1999;100[suppl II]:II-211–II-215.)

Key Words: assist devices, left ventricular n heart failure n transplantation n apoptosis

Congestive heart failure remains a major public health problem.1 As a result of the encouraging medium-term results with implantation of left ventricular assist devices (LVADs),2,3 such devices are being evaluated as a permanent therapeutic modality for patients with end-stage heart failure. However, LVAD implantation has been complicated by a high incidence of systemic infections, irrespective of the type of device used.4–9 In fact, prophylactic use of antifungal therapy has been advocated in selected LVAD recipients to reduce the risk of fungal infection.4 The high prevalence of fungal infection in LVAD recipients raises the possibility that LVAD implantation may be associated with the development of defects in host immunity.

In addition to possible defects in T-cell immunity, LVAD recipients develop prominent B-cell activation, as evidenced by heightened production of anti-HLA and antiphospholipid antibodies.10–12 A similar discordance between defects in T-cell immunity and B-cell hyperreactivity characterizes 2 other immunological disorders, systemic lupus erythematosus13–15 and infection with the human immunodeficiency virus type-1 (HIV-1).16,17 A proposed mechanism to account for the coexistence of T-cell defects and autoimmunity in these disorders is inappropriate induction of apoptotic T-cell death18–22 due to heightened interactions between CD95 (Fas) and CD95L (FasL).23–26

In this study, we initially performed cross-sectional and longitudinal quantitative analyses of T-cell populations in LVAD recipients. We then investigated whether quantitative T-cell defects were related to induction of T-cell apoptosis in LVAD recipients and whether this involved a CD95-dependent activation pathway. Our studies demonstrate heightened susceptibility of CD4 T cells from LVAD recipients to apoptosis and suggest that these abnormalities may be related to the high prevalence of infectious complications that accompany LVAD implantation.

Methods

Study Patients
40 NYHA class IV patients awaiting cardiac transplantation who received either a TCI Heartmate LVAD (n=20) or medical management (n=20) were available for immunological evaluation. No patients had any infection at the time of study, and none received immunosuppression therapy. All immunological studies were performed at least 1 month after implantation to limit the confounding effects of surgery.

From the College of Physicians and Surgeons of Columbia University, New York, NY.
Correspondence to Silviu Itescu, MD, Transplantation Immunology Department of Surgery, College of Physicians & Surgeons of Columbia University, 622 W 168th St, PH 14 W, Room 1485, New York, NY 10032.
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Immunophenotypic Analysis of Circulating T Cells

Cross-sectional immunophenotypic analyses were performed in 12 LVAD recipients and 20 controls, and serial studies were performed in 6 LVAD recipients. Cell counts from LVAD recipients and controls were acquired by Coulter counter analysis. Fluorochrome-labeled monoclonal antibodies (Mabs) against CD4 and CD8 (Becton Dickinson Systems) were used in 2-color immunofluorescence analyses. Log fluorescence was measured using a FACScan 500 flow cytometer (Becton Dickinson).

Assay for Quantification of T-cell Apoptosis

Peripheral blood mononuclear cells from LVAD recipients or heart failure controls were isolated from heparinized whole blood by Ficoll reagent, cultured in RPMI medium for 24 hours, and analyzed for apoptosis with a flow cytometric apoptosis detection kit (Becton Dickinson Systems). Briefly, after 24 hours of culture, $3 \times 10^6$ peripheral blood mononuclear cells were stained with fluorochrome-conjugated anti-CD3, anti-CD4, and anti-CD8 and then costained with 10 $\mu$L of FITC-conjugated annexin V (R&D systems) to detect phosphatidylserine expression on cells during early apoptotic phases.22,23 The samples were then analyzed by FACStar 500.

 Assay for Quantification of Soluble Circulating CD95 Levels

Serum samples were obtained from 20 LVAD recipients (at a uniform time point of 1 month after LVAD implantation), 20 NYHA class IV controls, and 13 normal individuals. Circulating serum levels of soluble CD95 were measured in a commercial ELISA using polyclonal antibodies against human CD95 (Cytoscreen, BioSource International, Inc). The sensitivity of the ELISA is 20 pg/mL. The amount of protein in each serum sample was calculated according to a standard curve of optical density values constructed for known levels of soluble CD95 protein.

Results

LVAD Recipients Have Relative Lymphopenia and Reduction in CD4 T-Cell Levels Compared With Heart Failure Controls

As shown in the Table, in a cross-sectional analysis of circulating leukocytes, LVAD recipients had reduced proportions of lymphocytes compared with NYHA class IV controls awaiting cardiac transplantation (mean lymphocyte proportion 10$\pm$2% versus 17$\pm$2%, $P<0.05$). This relative lymphopenia in LVAD recipients was accompanied by significant reductions in both the mean CD4/CD8 T-cell ratios (2.0$\pm$0.3 versus 4.1$\pm$0.4, $P<0.001$) and in the mean number of circulating CD4 T cells (374$\pm$6 versus 624$\pm$59 per cubic millimeter, $P<0.01$). In contrast, mean CD8 T-cell counts were not different between the 2 groups (230$\pm$1 versus 194$\pm$32 per cubic millimeter, $P=\text{NS}$).

LVAD Recipients Develop Progressive Reduction in CD4 T-Cell Levels After Device Implantation

To investigate these observations sequentially, in 6 LVAD recipients, serial measurements of T-cell subsets were performed at 1 and 2 months after implantation and compared with preimplantation levels. As shown in Figure 1, the mean CD4/CD8 T-cell ratio was reduced from 4$\pm$0.5 at baseline preimplantation to 1.2$\pm$0.2 by 2 months after implantation. As shown in Figure 2, this was a result of a progressive reduction in mean percentage of CD4 T-cell levels, from 55$\pm$3% at baseline preimplantation to 42$\pm$2% by 2 months after implantation, a mean reduction of 24%. In contrast, mean percentage of CD8 T-cell levels were not reduced during this period.

CD4 T Cells in LVAD Recipients Have Heightened Susceptibility to Apoptosis

We next investigated whether this progressive reduction in circulating CD4 T cells in LVAD recipients could be explained on the basis of differential susceptibility to induction of apoptosis. T-cell apoptosis after 24 hours of culture was measured in NYHA class IV controls and LVAD recipients by surface expression of phosphatidylserine, which is translocated from the internal leaflet to the external leaflet of the plasma membrane after initiation of a death-inducing activation process.27,28 As shown in Figure 3, CD4 T cells from heart failure controls demonstrated a relative resistance to apoptosis after 24 hours of culture versus CD8 T cells. Surface expression of phosphatidylserine, as defined by annexin V binding, was 26$\pm$3% for CD8 T cells, compared with only 10$\pm$2% for CD4 T cells ($P<0.01$). In contrast, both CD8 and CD4 T cells from LVAD recipients expressed high levels of phosphatidylserine after 24 hours of culture (71$\pm$1% and 67$\pm$2%, respectively). Figure 4 shows the relative increase in apoptosis of CD8 and CD4 T cells between LVAD recipients and heart failure controls. Whereas apoptosis of CD8 T cells increased by a mean of 2.7-fold in LVAD recipients, apoptosis of CD4 T cells increased by a mean of 6.9-fold ($P<0.05$). These results indicate that LVAD recipients have greater susceptibility to apoptosis of CD4 T cells than CD8 T cells.

| Selective Reduction in CD4 T Cells in LVAD Recipients |
|----------------|----------------|
|                | NYHA Class IV Controls (n=20) | LVAD Recipients (n=12) | $P$ |
| Lymphocytes, % | 17$\pm$2 | 9.55$\pm$2.4 | 0.043 |
| CD4/CD8 ratio  | 4.1$\pm$0.4 | 2$\pm$0.3 | 0.0008 |
| CD4 levels, n/mm$^3$ | 624$\pm$59 | 374$\pm$6 | 0.009 |
| CD8 levels, n/mm$^3$ | 194$\pm$32 | 230$\pm$93 | 0.26 |

Values are mean$\pm$SEM.
High Circulating Levels of Soluble CD95 in the Serum of LVAD Recipients Compared With Heart Failure Controls

Translocation of phosphatidylserine as an early component of T-cell apoptosis occurs after ligation of CD95 (Fas). To investigate whether the heightened susceptibility of T cells from LVAD recipients to apoptosis was a consequence of CD95-mediated T-cell activation, we measured circulating levels of soluble CD95 in sera of LVAD recipients and NYHA class IV heart failure controls. As shown in Figure 5, mean serum levels of soluble CD95 were significantly higher in LVAD recipients than in heart failure controls (14 ± 2 versus 7 ± 1 ng/mL; P < 0.01). These results are consistent with a heightened state of T-cell activation via a CD95-dependent pathway accompanying LVAD implantation relative to heart failure.

Discussion

In this study, we have shown in a cross-sectional analysis that LVAD recipients demonstrate a relative lymphopenia and reduction in CD4 T-cell levels in comparison to NYHA class IV heart failure controls who were treated medically. These observations were confirmed in a longitudinal study in LVAD recipients, which showed that device implantation was accompanied by progressive and sustained reductions in circulating CD4 T-cell levels. These abnormalities were accompanied by excessive T-cell apoptosis and increased levels of circulating soluble CD95. Because high circulating serum levels of soluble CD95 are detected in other diseases associated with increased T-cell apoptosis, such as systemic lupus erythematosus,29–31 these results suggest that reduction in CD4 T-cell levels accompanying LVAD implantation may be a consequence of an augmented pathway of CD95-mediated apoptosis. The clinical consequences of these abnormalities appear to reflect a state of reduced immune competency, with increased prevalence of Candida and other systemic infections.4–9

Progressive CD4 T-cell depletion and increased prevalence of infections associated with defects in cell-mediated immu-
nity are features that are common to HIV-infected individuals. One proposed mechanism to account for these abnormalities in HIV-1 infection is inappropriate induction of apoptotic T-cell death resulting from HIV-mediated interactions between CD95 (Fas) and CD95L (FasL).21,22,32,33 HIV-infected dendritic cells appear to be particularly effective at inducing T-cell expression of CD95 and delivering apoptosis-inducing signals to uninfected T cells.25,26 In this regard, cells of monocyte or dendritic lineage are present on the LVAD surface at the time of explantation34 and are functionally activated as defined by nuclear factor-xB expression35 and augmented production of cytokines and coagulation factors.27 These results suggest that antigen-presenting cells, which are aberrantly activated by the implanted LVAD, deliver excessive costimulatory signals to T cells, inducing T-cell apoptosis by a way of a CD95-dependent pathway.

Although both CD4 and CD8 T cells from LVAD recipients demonstrated greater levels of apoptosis than T cells from heart failure controls, susceptibility to induction of apoptosis was >2-fold greater for CD4 T cells from LVAD recipients than for CD8 T cells. The explanation for this may lie in the selective susceptibility of CD4 T cells to CD95-mediated apoptosis. After cross-linkage of CD95, the cytoplasmic domain of this receptor binds the adaptor molecule FADD,28,36 enabling interactions with another protein, called FLICE (caspase-8). Activation of FLICE leads to catalytic activation of a cascade of caspases, with the ultimate result of cellular apoptosis.37,38 The binding of FLICE to FADD can be competitively inhibited by a negative regulator of apoptosis, FLIP.39–40 Because IL-2 inhibits transcription of FLIP and enhances transcription of CD95 ligand (FasL),41 IL-2–producing Th1 CD4 cells are selectively susceptible to apoptosis after CD95 engagement.42–45 This provides a mechanism to account for the progressive reduction in CD4 T-cell levels in LVAD recipients and for the high prevalence of infections associated with defects in cellular immunity. Due to the cross-sectional nature of this study and the relatively small number of patients studied, we were not able to make specific correlations among degree of CD4 T-cell apoptosis, soluble CD95 levels, and infectious episodes. A separate longitudinal study is required to address the relationship between CD95-mediated T-cell apoptosis, reduction in CD4 T-cell numbers, and infectious complications in LVAD recipients. Because CD95-mediated apoptosis is an IL-2–dependent process, one potential approach to prevent T-cell depletion and defects in cellular immunity in LVAD recipients is the use of cyclosporine A and FK506, 2 drugs that inhibit mRNA transcription of IL-2 and consequently of CD95 ligand.46 We are currently evaluating the use of these and other agents in studies aimed at reducing aberrant immune activation in LVAD recipients.

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