Transitory immunologic response after implantation of the DeBakey VAD continuous-axial-flow pump

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Background: The development of local and systemic infection is a significant risk factor associated with implantation of a ventricular assist device. The immunologic consequence of continuous-flow rotary blood pumps is not known.

Methods: Six male adult patients (mean age 47 ± 10.3) with end-stage left heart failure received a DeBakey VAD axial-flow pump for use as a bridge to transplantation. (Four patients underwent transplantation after a mean 115 ± 14 days; 2 patients are still waiting for the allograft.)

Results: We prospectively monitored T-cell populations and apoptosis-specific aberrant T-cell activation via CD95 triggering and annexin V binding to lymphocytes, identifying T cells undergoing early phases of apoptosis, within the first 10 weeks. Moreover, soluble death-inducing receptors soluble CD95 and soluble tumor necrosis factor-R1 were evaluated by enzyme-linked immunosorbent assay.

Conclusion: Patients bridged to transplantation by a nonpulsatile ventricular assist device demonstrated an initial pronounced apoptosis-specific immune alteration by increased annexin V binding to CD3 T cells and death-inducing receptors soluble CD95/tumor necrosis factor-R1 (all $P < .001$). All parameters normalized after 7 weeks to baseline. No blood-borne sepsis was detected, as defined by blood culture, within the first 10 weeks of the cohort study. These results indicate a biphasic immunologic response in patients with end-stage heart failure treated with nonpulsatile ventricular assist devices.

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Congestive heart failure is a major public health issue, and the pulsatile ventricular assist device (VAD) has been proven to be beneficial in bridging patients to allograft transplantation with encouraging medium-term results.^{1,2} A prospective, randomized, multicenter trial of the permanent implantation of a left ventricular assist device (LVAD) for patients with end-stage heart failure is under way.³ The clinical success of LVAD implantation has been accompanied by complications such as thromboembolic events ranging from 5% to 30% of cases⁴⁻⁶ and an increased rate of systemic infections, which has been reported in 25% to 40% of VAD recipients irrespective of device type used.^{7,8} Although some of these infections were attributed to drive line, pocket, and perioperative complications, the frequent identification of fungal and bacterial infections has led to investigations in the genesis of immune defect caused by VAD-immune system interaction. These observations have led to the conclusion that LVAD implantation is accompanied by progressive defects in cellular immunity and was associated with an induction of an aberrant T-cell activation involving the CD95 (Fas) pathway and activation-induced cell death of CD4 T cells.⁹

TABLE 1. Demographic and clinical features of patients

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Sex	Male	Male	Male	Male	Male	Male
Age	65	46	45	37	38	52
Diagnosis	Dilatative CMP, s/p MI	Ischemic CMP	Ischemic CMP	s/p myocarditis	Idiopathic CMP	Ischemic CMP
Indication	Terminal cardiac insuf.	s/p MI; terminal cardiac insuf.	Terminal cardiac insuf.	DCM dec.	DCM dec.	Terminal cardiac insuf.
Duration (d)	120	96	Ongoing	Ongoing	116	130
Weaning HTX	Yes	Yes	No	No	Yes	Yes
Survival	Yes	Yes	Yes	Yes	Yes	Yes
Blood-borne infection	No	No	No	No	No	No

CMP, Cardiomyopathy; s/p, status post; MI, myocardial infarction; insuf., insufficiency; dec., ***; HTX, heart transplantation.

Axial-flow impeller pumps, with their potential for small size, low noise, and absence of a compliance chamber, have been developed for clinical use during the past 10 years. Of 4 potential candidates (Jarvik 2000 [CardioWest Technologies, Inc, Tucson, Ariz], DeBakey VAD [MicroMed Technology, Inc, Houston, Tex], Nimbus/Pittsburgh axial-flow blood pump [Nimbus, Inc, Rancho Cordova, Calif], and Sun Medical/HIJ, Waseda/Pittsburgh intraventricular axial-flow blood pump),¹⁰⁻¹³ the DeBakey VAD was the first clinically used with successful consecutive transplantation.¹⁴ To assess the long-term rather than perioperative immunologic impact, we aimed to analyze the recipient's immune status, presence of death-inducing receptors, and apoptosis of the recipient's T cells before as well as during the first 10 weeks in a sequential manner. Blood-borne sepsis was defined by positive blood culture.

Materials and Methods

DeBakey VAD

The electromagnetically actuated DeBakey VAD is a miniaturized pro-peritoneal implantable titanium axial-flow blood pump. A titanium inflow cannula connects the pump to the apex of the left ventricle, and a Vascutek Gelweave vascular graft (Vascutek, Renfrewshire, Scotland) as an outflow conduit connects the pump to the ascending aorta. An ultrasonic flow probe is placed around the outflow conduit. Together with the probe's wiring, the pump motor cable exits the skin above the right iliac crest and attaches to the VAD's external controller system. The pump is designed to achieve 5 L/min against 100 mm Hg pressure, with a rotor speed of 10,000 rpm and power input of less than 10 W. The measured pump flow is displayed either on the external VAD controller or when connected on the clinical data acquisition system (CDAS) together with pump speed, power consumption, and current signals from the pump. Adjustments to the pump speed can be performed only when it is connected to the CDAS. For optimal mobilization of the patient and freedom to move, power can be delivered by two 12-V direct-current batteries for several hours.

Patients

Six male patients with end-stage left heart failure received a DeBakey VAD as an LVAD for bridging to transplantation. All

patients were listed for cardiac transplantation and showed signs of acute hemodynamic deterioration and end-organ dysfunction at the time of implantation (Table 1). All patients were placed on broad antibiotic prophylactic therapeutic regimens in the perioperative time. The study protocol was in compliance with the rules of the Ethical Review Board of Vienna University Hospital.

Immune Phenotype of Circulating T Cells and Expression of CD95/Fas and CD45RO on Peripheral T Cells

Immunologic investigations were done in 6 recipients of DeBakey nonpulsatile VADs in a sequential manner. Cell counts from patients were achieved by FACS (fluorescence-activated cell sorter) counter analysis. We used fluorochrome-labeled monoclonal antibodies to CD4, CD8, CD3, CD45RO (T-cell memory marker), and CD95 (Apo-1) in 2-color or 3-color immunofluorescence analyses, as previously described by Robins.¹⁵ Log fluorescence was measured by a FACScan 500 flow cytometer (Becton Dickinson Systems, San Jose, Calif).

Soluble Death-inducing Receptors Tumor Necrosis Factor-R1 and CD95

Serum samples were obtained from study populations and kept frozen. Circulating serum levels of soluble CD95 (sCD95) and soluble receptor of tumor necrosis factor (sTNF-R1) were measured in a commercial enzyme-linked immunosorbent assay (ELISA) by using polyclonal antibodies against human CD95 and TNF-R1 (Cytoscreen, Biosource International, Inc, Camerillo, Calif [sCD95], and R&D Systems Inc, Minneapolis, Minn [sTNF-R1]). The sensitivity of the ELISA is 20 pg/mL and 3 pg/mL, respectively. The amount of protein in each serum sample was calculated according to a standard curve of optical density values constructed for a known level of sCD95/sTNF-R1 protein.

T-cell Apoptosis In Vivo

Flow cytometry peripheral blood mononuclear cells (PBMCs) were analyzed with a flow cytometric apoptosis detection kit (Becton Dickinson Systems, Franklin Lakes, NJ). PBMCs 3×10^5 were stained with phycoerythrin-conjugated monoclonal antibodies to CD3 and then co-stained with 10 μ L fluorescein-isothiocyanate-conjugated annexin V (R&D Systems, Minneapolis, Minn) to detect phosphatidylserine expression on cells during early apo-

ptotic phases. Dead cells were excluded with 7-amino actinomycin D. The samples were analyzed by a FACStar 500 cytometer.

Statistical Analyses

The percentage of lymphocytes was deemed significantly altered when values differed mean $\pm$ 2 SD. Continuous variables such as protein content were analyzed by the Student *t* test.

Results

Immunophenotypic Analysis of T Cells

The percentage of circulating T lymphocytes and CD4/CD8 subsets did not alter significantly in the course of the implantation period in the study population (Figure 1, A).

Expression of CD95/Fas and CD45RO on Peripheral T Cells

Because specific and nonspecific triggering of T lymphocytes is inducing apoptosis *in vitro*, we investigated whether expression of CD95 (Apo-1) and levels of the T-cell memory marker CD45RO were upregulated. The results are depicted in Figure 1, B. The expression of CD95, a molecule associated with a pathway of cellular apoptosis, was increased to a similar degree in CD3+ T cells before and after implantation. Similar values were also verified by CD45RO, a marker indicating the T-cell memory population ($P < NS$).

Soluble CD95 and TNF-R1

Because translocation of phosphatidylserine as an early component of T-cell apoptosis occurs after ligation of CD95 and TNF engagement, we investigated whether related soluble shedding forms of CD95 and TNF-R1¹⁶ were present in the serum. As shown in Figure 1, C, the mean serum levels of sCD95 rose sharply after implantation of nonpulsatile VAD and decreased after 6 weeks to baseline values before implantation (before implantation, 3 weeks, 6 weeks [sCD95 1.32 ± 0.21 , 7.6 ± 4.2 , 0.9 ± 0.7 pg/mL; sTNF-R1 1.5 ± 0.23 , 10 ± 6.9 , 2.1 ± 0.8 ng/mL], $P < .01$ between all values).

Spontaneous T-cell Apoptosis In Vivo

In further experiments we addressed whether the elevated state of T-cell activation is associated with an increased percentage of peripheral T cells undergoing apoptosis. The results depicted in Figure 1, B, show that, before implantation, CD3+ T cells had increased binding to annexin V ($18\% \pm 8.5\%$), increased until the fourth week ($41.4\% \pm 9.3\%$), and leveled down by week 7 ($10.8\% \pm 3\%$). In vivo dead cell exclusion was performed in all samples. All samples contained less than 0.5% 7-amino actinomycin D positive cells.

Discussion

In this report we have shown that patients after implantation of nonpulsatile VAD present a biphasic immunologic reac-

Figure 1. Changes in cell phenotype, cell activation, and antigen shading during 10 weeks after implantation of the DeBaKey pump. Freshly isolated PBMC from 6 patients were stained with monoclonal antibody anti-CD3, CD4, CD8 (panel A) or with anti-CD3 and CD45RO, and/or annexin V (panel B) and subjected to FACS analysis. Soluble CD95 (pg/mL) and sTNFR1 (ng/mL) were measured in sera by ELISA, and the results are depicted in panel C. Data are presented as mean $\pm$ SE. Statistical significance: * $P < .01$.

tivity as seen with the immediate rise in soluble death-inducing receptors TNF-R1 and CD95 with no alteration in the percentage of T cells and CD4/8 ratio in sequential fashion. Moreover, a significant decrease of annexin V binding, indicating T cells undergoing early phases of apoptosis, was verified after a mean implantation of 7 weeks. These results support that initially there is a similar immunoaction present as shown in a cross-sectional fashion in recipients of TCI (Thoratec Corporation (Pleasanton, Calif) and Novacor (Baxter Healthcare Corp, Novacor Div, Oakland, Calif) VADs.⁹ Previous hypotheses were attributing chronic interleukin 6 activation with T-cell suppression and a shift from a Th1 to Th2¹⁷ with consecutive induction of B-cell hyperactivity as possible explanations for decreased T-cell immunity. It was furthermore reported that VAD implantation was also associated with formation of HLA class I and II antibodies.¹⁸ Recent observations have demonstrated that patients with pulsatile VADs demonstrate an aberrant T-cell activation with CD4 T cells being selectively susceptible toward activation-induced cell death and, moreover, demonstrated inversed CD4/8 ratio and increased Th2 cytokine IL-10 (interleukin 10).¹⁹ In addition to these observations, we²⁰ have demonstrated recently that patients who are undergoing hemodialysis demonstrate immunoactivation similar to that of patients who have received a pulsatile LVAD. In both entities prolonged blood/immune system polymer interaction is prevalent. As patients with an LVAD are not receiving any immunosuppression, we previously deduced that long-term exposure to an antigen, for example, the LVAD surface (polyurethane) or other polymers such as silicone, polytetrafluoroethylene, or Dacron, is able to trigger a state of susceptibility toward programmed cell death^{21,22} and thus is able to induce a defect in in vitro experiments. We were able to demonstrate that commonly used polymers are able to act as superantigens, hence are inducing the upregulation of nuclear factor of activated T cells and selective Fas ligand enhancement, as detected by immunoblot analysis. This was further verified by induction of caspase 8 and 3 upregulation in naive PBMCs exposed to biomaterial; this indicates that a proapoptotic pathway is initiated by a chemical that is leaching of the biopolymer. Moreover, the increased likelihood of PBMCs of patients requiring dialysis to undergo activated induced cell death is further supported by recent publications that demonstrated the presence of plastic softeners in such patients in vivo and their potential immunosuppressive capacity in vitro.^{23,24} With this in mind, one might speculate that a similar mechanism might be partially responsible for immunoactivation in patients treated with pulsatile VADs, devices that are constructed with large polyurethane surfaces. If our interpretation of the data is correct, the small size and steel containment of the mini-axial flow pump might have increased biocompatibility because of increased material

inertness and possibly more physiologic flow characteristics. This, however, remains to be determined and warrants separate in vivo and vitro studies. Since mechanical support is under investigation as a definitive treatment for heart failure, clinical evaluations have been initiated. Moskowitz and associates²⁵ identified that infection is the main cause of death in patients receiving pulsatile VADs, hence demonstrating no survival benefit of the VAD group in the PreREMATCH study versus conventional medical treatment. Since nonpulsatile VADs are becoming standard therapy,²⁶ our lessons learned from our clinical experience are the following: (1) Nonpulsatile VADs induce a biphasic immunologic response, as seen by a steep rise of soluble death-inducing receptors CD95 and sTNF-R1 with increased annexin V binding to CD3 leukocytes; (2) percentage of lymphocytes and CD4/8 ratio do not alter in the study cohort; (3) a significant decrease of mean annexin V binding to T lymphocytes occurs after 7 weeks in patients with the DeBakey VAD; and (4) no blood-borne infections were observed in the first 10 weeks in the patient cohort. In conclusion our immunologic results indicate that patients demonstrate a biphasic apoptosis-specific activation pathway that is prevalent initially and decreases within 7 weeks. We learned that prophylactic antibiotic/mycotic drug therapy is able to rule out systemic infections within the first weeks of VAD installation. These results suggest that this new technology has less immunologic consequence and ideally will invigorate trials including nonpulsatile VADs as definitive therapy.

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