CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

TIME-DEPENDENT CELLULAR POPULATION OF TEXTURED-SURFACE LEFT VENTRICULAR ASSIST DEVICES CONTRIBUTES TO THE DEVELOPMENT OF A BIPHASIC SYSTEMIC PROCOAGULANT RESPONSE

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Objective: Textured-surface left ventricular assist devices (LVAD) have been shown to enhance ventricular function and survival in patients with end-stage heart failure. Furthermore, we have described a procoagulant physiology in our LVAD population with sustained thrombin generation (elevated thrombin-antithrombin III complex and prothrombin fragment 1+2) and fibrinolysis (D-dimers), even up to 335 days after LVAD placement. To explain such sustained activation of coagulation, we speculated that the LVAD surface selectively adsorbed and promoted activation of circulating blood cells. **Methods:** In a prospective study of 20 patients with LVADs, we examined samples of peripheral blood as well as cells harvested from the surface of the LVADs at the time of their explantation for procoagulant proinflammatory markers. **Results:** Analysis of the cells populating the LVAD surface revealed the presence of pluripotent hematopoietic CD34⁺ cells, as well as cells bearing monocyte (CD14)/macrophage (CD68) markers, which also expressed procoagulant tissue factor. Reverse transcriptase-polymerase chain reaction confirmed cellular activation on the LVAD surface, revealing transcripts for interleukin 1 α , interleukin 2, and tumor necrosis factor α , in addition to vascular cell adhesion molecule-1 consistent with their capacity to continually recruit and activate circulating cells, thereby propagating their response. In the periphery, elevated levels of tissue factor were found in the plasma of patients with LVADs, along with enhanced procoagulant activity. **Conclusion:** These observations suggest that the LVAD surface selectively absorbs and activates circulating hematopoietic precursor and monocytic cells, thereby creating a sustained prothrombotic and potentially proinflammatory systemic environment. (J Thorac Cardiovasc Surg 1999;118:404-13)

Although the left ventricular assist device (LVAD) has emerged in the past decade as an efficacious treatment for end-stage heart disease, the understanding of host-device interactions is still in its infancy.¹⁻⁶ Compared with early-design LVADs, whose surfaces

were mainly of smooth contour, textured-surface LVADs were developed with a polyurethane diaphragm, intended to minimize the potential for thromboembolic complications by facilitating the formation of a tightly adherent "pseudointima" on the blood-contacting

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surface.⁷⁻¹² The goal of this strategy was to reduce the risk of development of thromboemboli by eliminating direct contact between the device and circulating blood.¹¹ The apparent success of this modification in LVAD design has been suggested by studies reporting a thromboembolic rate of less than 2% associated with use of this modified surface in patients receiving no systemic anticoagulation.¹²

The physiologic consequences of this biologic lining, however, are many and varied, owing to the nature and characteristics of those cells that adhere to the LVAD surface.¹³⁻¹⁶ We¹⁷ have previously described a clinical phenomenon of a “compensated coagulopathy” underlying the apparent autoanticoagulation in recipients of textured-surface LVADs and have attributed this finding to procoagulant stimuli elicited from the LVAD cell surface microenvironment. Our findings of increased thrombin generation and fibrinolysis have been further confirmed by those of Bibiokis and associates,¹⁸ who similarly demonstrated significant perioperative activation of coagulation in LVAD recipients when compared with control subjects undergoing coronary artery bypass operations.

Although these results have implicated host-device interactions as the nidus for this activation, no study to date has comprehensively characterized the specific nature of these interactions and their development and change over time. Although previous investigators have suggested that the activation of coagulation was due largely to the continuous contact of blood with the foreign LVAD surface,¹⁸ we propose that specific cells, which progressively adhere to the textured surface and become activated, may account for the observed compensated coagulopathy. Furthermore, the dynamic nature of this cell-surface microenvironment likely reflects changes in cellular phenotypes, themselves a function of ongoing host-device interactions.

We undertook the current study to evaluate further the time-dependent generation of the sustained procoagulant response that follows implantation of textured-surface LVADs. We hypothesized that changes in the cell-surface microenvironment over time would likely parallel clinically apparent systemic phenomena. We therefore prospectively evaluated clinical indicators of this procoagulant phenotype in LVAD recipients, as well as characterizing cellular aspects of their LVAD surface milieu.

Methods

Patients. Twenty patients with implantable Thermo Cardiosystems, Inc (TCI, Woburn, Mass) HeartMate LVADs were studied in accordance with the rules and regulations of

Table I. Demographic profile of patients having an LVAD

Patient (age [y], sex)	Diagnosis	Length of LVAD treatment (days)	Type of LVAD pump (TCI HeartMate)
1 (52, F)	Idiopathic CM	335	Pneumatic
2 (65, M)	Ischemic CM	46	Pneumatic
3 (51, M)	Ischemic CM	139	Pneumatic
4 (19, M)	Idiopathic CM	35	Electric
5 (61, M)	Ischemic CM	101	Electric
6 (17, M)	Idiopathic CM	65	Electric
7 (61, M)	Ischemic CM	100	Electric
8 (58, M)	Ischemic CM	70	Electric
9 (58, M)	Ischemic CM	68	Pneumatic
10 (48, M)	Ischemic CM	221	Electric
11 (68, M)	Ischemic CM	56	Pneumatic
12 (39, F)	Subacute MI	49	Pneumatic
13 (56, M)	Ischemic CM	9	Pneumatic
14 (51, F)	Subacute MI	7	Pneumatic
15 (65, M)	Ischemic CM	33	Electric
16 (59, F)	Ischemic CM	35	Pneumatic
17 (65, M)	Ischemic CM	33	Pneumatic
18 (52, M)	Idiopathic CM	25	Pneumatic
19 (51, M)	Ischemic CM	11	Pneumatic
20 (30, M)	Idiopathic CM	5	Pneumatic

CM, Cardiomyopathy; MI, myocardial infarction; M, male; F, female.

the institutional review board of Columbia University College of Physicians and Surgeons from June 1995 through February 1996 (Table I). These patients were evaluated prospectively from the time point immediately before implantation of the device, at 4 time points during the operation (at the time of bypass, immediately after implantation of the device, 30 minutes after implantation, and at sternal closure), and then weekly until explantation of the device because of transplantation (n = 18), explantation (n = 1), or death (n = 1).

Peripheral blood analysis

PROCOAGULANT PLASMA MARKERS. Peripheral blood was obtained by venipuncture at the time points indicated, and intraoperative blood was obtained through an indwelling arterial catheter. Complete blood count, prothrombin time, activated partial thromboplastin time, fibrinogen levels, and levels of fibrinogen (fibrin) degradation products were obtained from the clinical laboratory of Columbia University College of Physicians and Surgeons according to standard methods. Enzyme-linked immunosorbent assays (ELISAs) for thrombin-antithrombin III complex and prothrombin fragment 1+2 were obtained from Behring Diagnostics, Inc (Westwood, Mass), and ELISA kits for D-dimer and tissue factor were obtained from American Diagnostica (Greenwich, Conn). All ELISAs were performed according to the manufacturers' instructions.

TISSUE FACTOR ACTIVITY. For the evaluation of tissue factor activity in peripheral blood, assays were conducted as previously described.¹⁹ In brief, 18 mL of blood was drawn by

peripheral venipuncture into 3.8% sodium citrate (9:1 vol/vol), diluted with an equal volume of 25 mmol/L HEPES buffer in Hanks' balanced salt solution, pH 7.4 (Gibco BRL, Life Technologies, Inc, Rockville, Md), and layered onto a Histopaque-1077 gradient (Sigma Chemical Co, St Louis, Mo) for mononuclear cell isolation. Mononuclear cells from the original blood samples (2.5×10^6 cells/well) were then incubated in the presence or absence of lipopolysaccharide (10 μ g/mL) from *Escherichia coli* serotype 026:B6 (Sigma) in human plasma (20%) for 2 hours at 37°C. Procoagulant activity of lysed mononuclear cells was measured by a 1-step recalcification time. Cells were lysed by the addition of octyl β -D glucopyranoside (15 mmol/L; Calbiochem, San Diego, Calif) and HEPES (25 mmol/L) in Hanks' balanced salt solution (Gibco). The resulting suspension was incubated with pooled normal plasma (Sigma) at 37°C for 3 minutes, after which time CaCl_2 (0.001 mol/L) was added and the clotting time determined by visual detection of clot formation. Each sample was run in triplicate. Serial dilutions of recombinant native tissue factor (American Diagnostica, Greenwich, Conn) were used to generate a standard curve.

LVAD cellular microenvironment analysis

HARVEST AND STUDIES ON CELLS FROM THE LVAD. At explantation, LVADs were opened with a device provided for this purpose by the manufacturer (TCI), and the surfaces immediately rinsed in sterile, cold phosphate-buffered saline solution (without magnesium or calcium; Gibco). A standard tissue culture-type cell scraper (Baxter, McGraw Park, Ill) was then used to gently detach cells adherent to the polyurethane diaphragm. Aliquots of harvested cells were immediately immersed in RPMI 1640 test medium, which was subsequently supplemented with either fetal calf serum (10%) or autologous serum (10%), as well as antibiotics, as described earlier. Aliquots of cells were also immersed in cold Trizol Reagent (Gibco), snap-frozen in liquid nitrogen, and stored at -80°C after homogenization for subsequent total RNA isolation.

Cells harvested from the LVAD were incubated for the indicated time in tissue culture wells either alone or in the presence of sterilized, endotoxin-free LVAD polyurethane diaphragm (10^7 cells/well). Experiments were performed in RPMI 1640 medium supplemented with either fetal calf serum (10%) or autologous serum (10%) collected at the time of explantation and separated from the patients' blood by centrifugation at 6000 rpm. Experiments were performed both in the presence and absence of lipopolysaccharide, 50 ng/mL.²⁰ Aliquots of cellular supernatants were removed immediately (0 time point), at 72 hours, at 1 week, and at 10, 14, and 21 days and then assayed for levels of tissue factor as described earlier.

IMMUNOHISTOCHEMISTRY. Cells were harvested as described earlier, resuspended in Dulbecco's modified Eagle's medium supplemented with fetal calf serum (10%), plated in 4-well plates (Nunc, Naperville, Ill), and placed in an incubator at 37°C. At 24 hours, cells were washed once with Hanks' balanced salt solution (Gibco) and resuspended in fresh medium as described earlier. After 48 to 72 hours of incubation, viable

cells were fixed in paraformaldehyde (2%) and solubilized with 0.1% Nonidet P-40 (Sigma) as indicated. After an overnight fixation at 4°C, slides were washed with phosphate-buffered saline solution and hydrogen peroxide (0.3%; Sigma). They were blocked with phosphate-buffered saline solution containing bovine serum albumin (1%) and serum (4%), species depending on the animal in which the secondary antibody was prepared, for 30 minutes at 37°C. Primary antibody was added at the appropriate dilution (as suggested by the manufacturer), followed by incubation for 1 hour at 37°C. Antibody for tissue factor (1:100) (mouse monoclonal antibody) was obtained from American Diagnostica. Antibodies to CD34 (1:10), CD14 (1:100), CD68 (1:100), smooth muscle actin (1:50), and nonimmune immunoglobulin G (1:100), all mouse monoclonal antibodies, were obtained from DAKO (Carpenteria, Calif). The fixed cells were then washed and incubated with appropriate secondary antibody (1:100 dilution) for 1 hour and developed for 5 minutes at room temperature in buffer containing aminoethylcarbamazole (Sigma), hydrogen peroxide (0.1%), and acetate buffer (0.50 mol/L; pH 5.0).

REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR). Cells were harvested directly from the LVAD surface at explantation, as described earlier. An aliquot was immediately placed in cold Trizol reagent (5 mL; Gibco) and homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) for 60 seconds before being snap frozen in liquid nitrogen for extraction of total cellular RNA at a later time. Homogenized samples were then incubated at room temperature for 5 minutes (to permit the total dissolution of the nucleoprotein complexes), and phase separation was achieved by incubation with chloroform followed by centrifugation at 12,000g for 30 minutes at 4°C. The RNA was then precipitated from the aqueous phase with isopropyl alcohol followed by another centrifugation at 12,000g for 30 minutes. The RNA was washed twice with cold ethanol (75%) and resuspended in diethyl pyrocarbonate-treated water to a concentration of 1 μ g/ μ L. RT-PCR was then performed according to previously described methods.²¹⁻²² Primers for β -actin, yielding an amplicon of 661 base pairs (bp), were obtained from Stratagene (La Jolla, Calif). For β -actin, thermocycling parameters were as follows: 5 minutes at 94°C, 5 minutes at 60°C; and 35 cycles of 1.5 minutes each at 72°C, 45 seconds at 94°C, 45 seconds at 60°C, with a final extension of 10 minutes at 72°C. Primers for tumor necrosis factor- α (TNF- α) (644 bp), interleukin 2 (IL-2) (605 bp), and interleukin 1 α (IL-1 α) (691 bp) were obtained from Clontech (Palo Alto, Calif). For the latter primers, thermocycling conditions were as follows: 40 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 2 minutes at 72°C, with a final extension of 7 minutes at 72°C. Primers for identification of transcripts for vascular cell adhesion molecule-1 (505 bp) were prepared as described.²³ Thermocycling conditions were run over 30 cycles after 5 seconds at 96°C, 15 seconds at 51°C, 60 seconds at 72°C, with a final extension of 7 minutes at 72°C.

Fig 1. Enhanced tissue factor activity in peripheral blood monocytes of patients with LVADs. Peripheral blood mononuclear cells were isolated from LVAD patients ($n = 5$) or from normal control subjects ($n = 3$), and tissue factor activity assays were performed as described above, alone or in the presence of lipopolysaccharide (LPS) ($10 \mu\text{g/mL}$). Tumor factor activity was then assayed as described in triplicate and reported as the mean $\pm$ standard error. $*P < .05$ by analysis of variance.

Table II. Prospective analysis of hemostatic parameters obtained from patients with textured-surface LVADs

	Length of implantation				
	Preop	1 Day	1 Week	6 Weeks	51 Weeks
TAT ($\mu\text{g/L}$)	2.3 ± 0.8 (nl = 1.5 ± 0.3)	$98 \pm 14.3^*$	$10.4 \pm 4.8^*$	$49 \pm 13^*$	62
$F_{1,2}$ ($\mu\text{g/mL}$)	1.6 ± 0.7 (nl = 0.7 ± 0.1)	9.7 ± 2.1	$5.8 \pm 3.1^*$	$6.4 \pm 3.1^*$	7.2
D-dimers (ng/mL)	128 ± 30 (nl = 125 ± 12)	$525 \pm 60^*$	$2500 \pm 560^*$	$2000 \pm 780^*$	2215

TAT, Thrombin-antithrombin complex III; $F_{1,2}$, prothrombin fragment 1+2.

$^*P < .01$ compared with normal values by analysis of variance.

Results

Patient demographics. The mean age of the patients receiving LVAD support was 51.7 years (range, 17-65 years); 16 of the patients were male and 4 female. The cause of cardiac failure was most commonly ischemic cardiomyopathy ($n = 13$), myocardial infarction ($n = 2$), and idiopathic cardiomyopathy ($n = 5$). The mean time for LVAD treatment was 83 days (range, 5-335 days). None of the patients with an LVAD in this study received heparin, warfarin sodium, or other types of systemic anticoagulation. Patients were followed up prospectively from the time of LVAD insertion for up to 335 days. The clinical characteristics of these subjects are identified in Table I.

Peripheral blood analysis

PROCOAGULANT PLASMA MARKERS. Consistent with previous reports,^{17,18} as demonstrated in Table II, prospective evaluation revealed an initial perioperative rise in markers of thrombin generation as measured by

elevated levels of prothrombin fragment 1+2 and thrombin-antithrombin III complex. The data demonstrated an increase in each of these variables in the immediate perioperative period, which declined progressively by 1 week after the operation. Subsequently, indices of thrombin generation rose in a time-dependent manner, reaching an apparent maximum by 6 weeks. These values were then sustained through at least day 335 (as measured in the 1 patient who was followed up to this point after implantation). Similar results were observed for levels of D-dimers.

Elevated levels of soluble tissue factor were observed in the plasma of LVAD patients compared with values in the normal population when examined over the entire time course of observation ($5.4 \pm 0.3 \text{ pg/mL}$ vs $0.4 \pm .09 \text{ pg/mL}$, $P < .005$) (up to 335 days). Enhanced elaboration of tissue factor was first evident during the operation at the time of LVAD placement and remained elevated throughout the observation period (data not shown).

Fig 2. Elaboration of procoagulant tissue factor and enhanced procoagulant activity by cells derived from the LVAD surface. **A**, Cells from the LVAD surface placed onto representative sections of LVAD surface in tissue culture wells or tissue culture plastic in the presence of lipopolysaccharide (LPS) (10 $\mu\text{g/mL}$). Aliquots were harvested at 6 hours and assayed for tissue factor. Data are reported as percent above control (LVAD-derived cells cultured on tissue culture plastic in the presence or absence of lipopolysaccharide). *Black bars* represent LVAD-derived cells in the presence of LVAD material. *Open bars* represent LVAD cells in the presence of LVAD material + lipopolysaccharide (10 $\mu\text{g/mL}$). * $P < .01$ compared with controls by analysis of variance. ** $P < .001$ compared with controls by analysis of variance. **B**, Mononuclear cells from LVAD surface or from controls were isolated as above and exposed to lipopolysaccharide (10 $\mu\text{g/mL}$) for 6 hours on tissue culture plastic. Tissue factor activity assays performed as described above were then performed as described in triplicate and reported as the mean $\pm$ standard error. * $P < .05$ by analysis of variance. Data are reported as percent above control (peripheral derived mononuclear cells not exposed to lipopolysaccharide, 10 $\mu\text{g/mL}$). ** $P < .001$ compared with controls by analysis of variance.

TISSUE FACTOR ACTIVITY. Enhanced mononuclear tissue factor activity of peripheral blood was observed in cells derived from LVAD patients compared with those

from normal volunteers both in the absence and presence of lipopolysaccharide (Fig 1).

Analysis of LVAD cellular microenvironment

HARVEST AND STUDIES ON CELLS FROM THE LVAD. Harvest of cells from LVADs at the time of explantation and subsequent tissue culture demonstrated the capacity of these cells to secrete increased levels of tissue factor in the presence and absence of lipopolysaccharide (Fig 2, A). Furthermore, mononuclear cells isolated from the LVAD surface cells also demonstrated enhanced response to lipopolysaccharide compared with mononuclear cells derived from normal controls, suggesting the basal activation of these cells (Fig 2, B).

IMMUNOHISTOCHEMISTRY. In agreement with the work of Rafii and colleagues,²⁴ we identified a population of CD34⁺ cells that morphologically appeared elongated, variably described in the literature as spindle-type cells or dendritic cells, similar to hematopoietic stem cell precursors present in the normal bone marrow and to some extent in peripheral blood (from 0.01% to 0.1% of peripheral blood^{25,26} (Fig 3, panel A). Evidence for the presence of activated macrophages and monocytes was identified by the presence of CD68- and CD14-positive cells, respectively (Fig 3, panel B, left and right, respectively). Furthermore, the presence of increased immunostaining for tissue factor from cells derived from the LVAD surface (Fig 3, panel C) suggested enhanced procoagulant activity in this setting. In contrast, there was no staining with antibody to smooth muscle actin or nonimmune immunoglobulin G (Fig 3, panel D, left and right, respectively).

RT-PCR. Evidence for cellular activation on the LVAD surface was confirmed by evaluating the presence of transcripts for inflammatory mediators. RT-PCR of RNA prepared from the cells harvested from the LVAD surface demonstrated amplicons of the appropriate size for the proinflammatory cytokines, IL-1, IL-2, and TNF (Fig 4, A to C, respectively), as well as for proinflammatory cell-cell adhesion molecules, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 (Fig 4, D and E). RT-PCR for β -actin was used as the positive control (data not shown).

Discussion

Although the clinical phenotype of a compensated coagulopathy in recipients of textured-surface LVADs have been both previously described and attributed to complex host-device interactions at the blood-device interface, the precise events governing the development of this phenomenon have yet to be defined. We undertook the current study to evaluate the time-dependent generation of this sustained procoagulant response under the assumption that changes in the cell-surface

Fig 3. Immunohistochemical analysis of cells derived from the LVAD surface: cell culture. Cells were harvested from the LVAD surface and placed onto Nunc tissue culture dishes. After 48 to 72 hours in culture, cells were fixed in formalin (10%) and immunohistochemical analysis using specific antibodies to the following was performed as described above: *panel A*, anti-CD34-immunoglobulin G; *panel B (left)*, anti-CD68-immunoglobulin G; *panel B (right)*, anti-CD14-immunoglobulin G; *panel C*, anti-tissue factor-immunoglobulin G; *panel D (left)*, smooth muscle actin; *panel D (right)*, nonimmune immunoglobulin G (IgG). (Original magnification: $\times 100$.)

microenvironment over time would likely parallel clinically apparent systemic phenomena and, therefore, that alterations in the surface microenvironment would ultimately induce changes in the systemic macroenvironment. These findings led us to hypothesize about cellular events that may account for this procoagulant phenotype and associated “autoanticoagulation.” In particular, we undertook the current study to evaluate the *development* and presumed dynamic changes that underlie these events.

Previous investigators have suggested that the procoagulant response of LVAD recipients may largely

reflect a generalized perioperative inflammatory reaction.¹⁸ Our data, however, indicate that the initial perioperative peak in markers of procoagulant activity resolves by postoperative day 7 and thus may represent overt indices of thrombin generation and fibrinolysis that are acute and procedure related. We propose that this initial rise may indeed reflect systemic responses to a variety of procoagulant stimuli, including the operation itself, in addition to contact between blood components and foreign surfaces associated with the cardiopulmonary bypass circuit and the LVAD.^{27,28}

Others have further suggested that these perioperative

Fig 4. Activated cells are present on the LVAD surface: RT-PCR was performed from material directly removed from the LVAD surface at the time of explantation as described above using specific primers. Positive amplicons for the following were identified: A, Interleukin 1 α (*IL-1*); B, interleukin 2 (*IL-2*); C, tumor necrosis factor α (*TNF- α*); D, vascular cell adhesion molecule-1 (*VCAM-1*); E, intercellular adhesion molecule-1 (*ICAM-1*). Markers are indicated on the right-hand side of each panel.

procoagulant abnormalities may persist throughout the period of LVAD support. In contrast, by prospectively evaluating time-dependent changes in this prothrombotic phenotype, we have demonstrated that activation of coagulation and fibrinolysis in patients supported with an LVAD is biphasic. An initial perioperative increase in markers of activation of coagulation and fibrinolysis is followed by a progressive decline in these parameters by day 7 after LVAD placement. Subsequently, a steady increase in hemostatic markers occurs, with peak levels observed by day 35 of treatment. Beyond this period, sustained thrombin generation and fibrinolysis were found in patients having an LVAD.

We propose that this biphasic phenomenon reflects 2 separate procoagulant stimuli. The initial rise in procoagulant markers likely reflects a generalized perioperative response. The later, more gradual rise, however, may result from progressive population of the LVAD surface with cells whose adherence and subsequent activation account for a localized procoagulant microenvironment that ultimately can generate a progressive, sustained systemic effect.

Interestingly, this effect, manifested in elevated plasma levels of tissue factor, was paralleled by increased tissue factor surface expression on cells harvested from the LVAD surface. Furthermore, this was correlated not only by the ability of these cells to secrete tissue factor, but also with their functional procoagulant activity. Taken together, these findings supported the contention that the progressive population of the LVAD surface by monocytic cells that themselves maintained procoagulant activity accounted for the systemic prothrombotic profile.

Characterization of the cells populating the LVAD surface by immunohistochemistry to localize this response revealed evidence of pluripotent hematopoietic

stem cells (CD34), as well as monocytes (CD14) and activated macrophages (CD68). All of these cells demonstrated cell-surface expression of tissue factor. Although these findings were suggestive of the integral role of the LVAD cell surface in the activated dynamic microenvironment we had hypothesized, we chose to further evaluate these cells by RT-PCR for confirmation of cellular activation.

The RT-PCR findings of increased proinflammatory cytokine and adhesion molecule expression suggested 2 important phenomena. First, the finding of increased proinflammatory cytokines (*IL-1*, *TNF- α*) support our contention that these cells maintain the ability to propagate the proinflammatory/procoagulant microenvironment both locally and systemically. Furthermore, the findings of enhanced proinflammatory adhesion molecule expression (vascular cell and intercellular adhesion molecules) additionally provides a mechanism for triggering the further recruitment of inflammatory/immune effector cells, thus propagating cellular activation on the LVAD.

Taken together, these data suggest that considerable interactions between the LVAD surface cells and the host are occurring throughout the time course of LVAD implantation. We postulate that the mononuclear cells, platelets, and pluripotent stem cells initially trapped by the polyurethane surface—not the titanium housing or Dacron grafts—become activated, thus leading to the generation of a local proinflammatory/procoagulant state responsible for triggering and subsequently sustaining the systemic activation of the coagulation and fibrinolytic cascades. Furthermore, inflammatory cells present on the LVAD surface appear to demonstrate production of proinflammatory cytokines and expression of adhesion molecules that promote cell activation, as well as facilitate recruitment of other cell types from the

circulating blood, thereby sustaining immune alterations.²⁹ Our observation that monocyte-derived macrophages seeded on the LVAD surface generate tissue factor, a cell-associated and released form, suggests a direct mechanism for activation of coagulation. This is consistent with the presence of tissue factor antigen in the plasma of patients with LVADs and suggests a mechanism for disseminating the locally intense inflammatory and procoagulant stimuli in the LVAD milieu.

We believe, therefore, that the biology of cells on the LVAD surface resembles a "two-hit" phenomenon, in which the initial placement of the device first results in recruitment of adherent cells (initially CD34+ pluripotent hematopoietic cells and monocytes). Subsequently, these cells undergo differentiation and activation with the capacity to recruit other cells (dendritic-type cells and lymphocytes), thereby sustaining and expanding the local host response. The extent to which the cells populating the LVAD surface actually contribute to this environment is not absolutely determined, however, underlying the major limitation of these studies. It is possible that the device itself promotes coagulation and inflammation independently of these cells, or that the contribution of these cells is relatively minor. Nonetheless, the implantable LVAD clearly emerges as an immune-inflammatory organ, modulating multiple effector systems including coagulation and immune mechanisms with important physiologic implications for its host.

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Discussion

Dr D. Glenn Pennington (*Winston-Salem, NC*). Dr Spanier and her colleagues are to be congratulated for their persistence in more precisely describing the wide ranging interactions of this textured LVAD surface, which has demonstrated a low level of clinically apparent thrombogenicity in clinical trials. They and others previously described a compensated coagulopathy characterized by procoagulant, proinflammatory effects, a continuum of the systemic inflammatory response syndrome discussed by Kenneth Taylor earlier at this meeting.

In the current studies they attribute this sustained response to the selective absorption and activation of dendritic-type and monocytic cells by the textured surface. They have demonstrated elevated peripheral levels of prothrombin fragments, thrombin-antithrombin III complexes, and D-dimers, as well as increased tissue factor activity. Cells scraped from the LVAD surface demonstrated activation by the presence of cytokines and adhesion molecules, and there was strong evidence of immunoregulatory dysfunction characterized by T- and B-cell markers.

In the abstract they suggested that these findings would importantly affect their ability to perform heart transplantation in these patients and perhaps lead to an increased incidence of infection. However, I fail to find in the manuscript or the presentation any clear correlation of these events in the 20 patients described. Therefore, my questions relate primarily to clinical correlates of these events.

1. The first question relates to infection. Was the onset of the second phase, approximately 35 days, substantially affected by the development of a systemic infection in a patient? How would, or perhaps, how did LVAD endocarditis affect the delicately balanced coagulation state? Finally, was there a late increase in infection?

2. Were there instances of late bleeding related to this autoanticoagulation state?

3. Did any patients receive anticoagulants, perhaps for left ventricular thrombus formation? I know you would not use anticoagulants for the device. If they were receiving antico-

agulants, what were the anticoagulant effects on this cellular absorption activity?

4. How many of the long-term devices had to be replaced because of cellular activity on the surface?

5. How do these data correlate with previously published descriptions of endothelial-type cells on this textured surface? Presumably if the surface becomes more endothelialized with time, might not these proinflammatory effects diminish?

6. Are you aware of any similar studies or phenomena in patients who have been treated with smooth-surface devices with which we might compare your results?

7. Finally, do you have plans for therapeutic interventions based on these studies?

Dr Spanier. Thank you, Dr Pennington, for all of those questions. In this presentation, we have described the initial studies that were done at Columbia, which focused on the observed procoagulant phenotype in our LVAD population. Furthermore, we have defined both the proinflammatory and immunoregulatory dysfunction that we have found in our patients with LVADs, both of which have important impact on their clinical management.

The clinical implications of this described procoagulant phenotype or compensated coagulopathy have the advantage of a systemic auto-anticoagulation that allowed these patients to have been maintained without any systemic anticoagulation. However, this compensated coagulopathy, in which there is a delicate balance between thrombin generation and fibrinolysis, presents a potential for any proinflammatory stress to tip this balance toward potential disaster. Either an infection or a proinflammatory stress may be the culprit. The patients are perhaps most vulnerable at the time of LVAD explantation, when they undergo the further stress of cardiopulmonary bypass for their explant and, ideally, cardiac transplantation. At this time, this delicate balance certainly presents the potential for both thrombosis or hemorrhagic complications. The serious impact that these complications can have on these patients has been our main impetus to understand this phenomenon better, so that we can modulate these effects.

To answer your questions specifically: With regard to infections, we at Columbia have described the clinical phenomenon of elevated anti-HLA antibodies, both class I and class II, that develop progressively in these patients over the time course of LVAD implantation. We found that specifically the type 1 antibodies, which are those that are reflected in the panel reactive antibody, do not seem to be correlated with blood transfusions, as was previously thought. However, they do seem to be correlated with the time and duration of LVAD implantation, suggesting that there is a host-device interaction causing the production of these antibodies.

We also find that these patients seem to harbor T-cell defects, which may make them more susceptible to opportunistic infections. We are studying these patients in the hope of understanding these problems and modulating the surface such that we can protect the patients while allowing them to maintain the favorable state of autoanticoagulation.

I think that this also addresses your second question, which

concerned the late bleeding complications that develop with this compensated coagulopathy. The patients are all at risk for late bleeding complications, especially during transplantation, when they receive aprotinin as an antifibrinolytic agent to attenuate this response.

At our institution, we have started using aspirin as the anticoagulant in these patients. Our specific reason for doing this, and I think this answers your last question, is that aspirin acts in high doses as a nuclear factor κ B inhibitor. We believe that the mechanism of cellular activation at the LVAD surface is dependent on nuclear factor κ B. Inhibition of nuclear factor κ B with aspirin, therefore, may attenuate these responses. Interestingly, in initial clinical trials in patients who have been treated with aspirin starting in the immediate postimplantation period, we found that these patients maintain the systemic autoanticoagulation, but their immunoregulatory dysfunction, specifically the production of anti-HLA antibodies, seems to be attenuated with aspirin. By defining and understanding these host-device interactions on the LVAD surface, therefore, we hope we will be able to maintain the favorable state of autoanticoagulation while modulating the more dangerous outcomes, such as the potential for thrombin generation, thrombosis, and hemorrhage. We also hope to control the immunoregulatory dysfunction that may make transplantation difficult in these patients because of elevated panel reactive antibody, which also may impact on post-transplantation outcome.

Dr Roland Hetzer (*Berlin, Germany*). The findings from this study are in close agreement with our results as to the inflammatory response with the LVAD that were presented by my coworker, Dr Loebe, at the recent meeting of the American Society for Artificial Internal Organs (ASAIO). Our results showed that this response is very similar in the Novacor (Baxter Healthcare Corp, Novacor Div, Oakland, Calif), as well as in the TCI devices and in our Berlin Heart system (Mediport, Berlin, Germany), and may be less related to the type of device than to the degree of multiorgan failure and blood trauma. The findings also underline the previous experience that it is mandatory to overcome the inflammatory reaction before good results at transplantation can be achieved. I have 3 questions.

1. Do you conclude that the highly sensitized patients with TCI LVADs require special treatment when undergoing transplantation?

2. Can you exclude that the stimulation of inflammatory response may be related to the quite significant rate of opportunistic infections that you have been reporting?

3. Would you agree that the low incidence of thromboembolic complications as observed with the clinical use of the TCI LVAD may be related to the high fibrinolytic activity of the inner surface of the device rather than neointima?

Dr Spanier. Thank you for your comments and for bringing out the fact that this phenomenon has been described, although at different levels, in all types of the devices that are implanted, whether smooth or textured surface. This fact highlights the importance of understanding not just the textured surface but the overall host-device interactions with the implantable LVAD, as well as implantable heart replacements.

In answer to your question regarding highly sensitized patients: A great deal of information was presented at the recent International Society for Heart and Lung Transplant meeting about treatment of the highly sensitized patients. This included not just patients with a textured-surface LVAD, but smooth-surface LVADs as well. The highly sensitized patient does need special peritransplantation treatment. Intravenous immunoglobulin treatment and photopheresis have been suggested as efficacious measures.

Concerning the stimulation of the inflammatory response at the LVAD surface, we believe that it is not just the reaction at the textured surface, but that it is the inflammatory response to the implantation of the device that we must better understand.

Your third question addresses the low incidence of thromboembolic complications in the TCI LVAD. We believe that the autoanticoagulation with the TCI LVAD is probably most significantly related to macrophages that populate the textured-surface LVAD and express procoagulant tissue factor, thereby initiating the compensated coagulopathy. That is likely what distinguishes the textured surface from the other devices and allows this autoanticoagulation in that setting. This phenomenon does not appear to be present in smooth-surface devices.