A Circulating Myocardial Depressant Substance is Associated with Cardiac Dysfunction and Peripheral Hypoperfusion (Lactic Acidemia) in Patients with Septic Shock*

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Using spontaneously beating rat myocardial cells as an in vitro model of myocardial depression, recent studies demonstrated that septic shock patients' sera frequently contain a myocardial depressant substance (MDS) that is associated with a reversible decrease in left ventricular ejection fraction (LVEF). To further characterize MDS, 50 consecutive patients with possible septic shock were studied serially from shock onset until recovery or death. Thirty-four patients had criteria diagnostic of septic shock, and 16 had a nonseptic critical illness. Of the 34, 14 met strict criteria for circulating MDS, with a mean inhibition of 35 percent (range 20 percent to 62 percent). Compared with those patients not exhibiting significant MDS activity, the 14 MDS-positive patients had a lower mean minimal EF (28 percent vs 39 percent, p<0.01), a greater mean decrease in EF (22.1 percent vs 8.8 percent, p=0.002), a higher pulmonary artery wedge pressure (16.8 vs 11.9 mm Hg, p<0.01), greater LV dilatation (162 vs 115 ml/m², p=0.02), and a higher circulating mean peak lactic acid (6.9 vs 2.7 mmol/L, p<0.01). In the 14 MDS-positive patients, the in vitro myocardial cell depression had a negative correlation with the in vivo EF (r = -0.60, p<0.05). These findings suggest that a circulating MDS is a cause of the myocardial depression frequently accompanying human septic shock.

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MDS = myocardial depressant substance; EDC = end-diastolic counts; ESC = end-systolic counts; Bkgd = background counts; EDVI = end-diastolic volume index; SVR = systemic vascular resistance

Reversible myocardial depression has been demonstrated in human and canine septic shock.\textsuperscript{1-3} This myocardial depression occurs within the first several days of shock and is characterized by left ventricular (LV) dilatation and a decrease in LV ejection fraction (EF). In survivors, these profound changes are transient, and the EF usually returns to normal in seven to ten days.

The pathogenetic mechanism of this early, transient LV dilatation and decrease in EF is not known. This decrease in EF is not associated with a global reduction in coronary blood flow nor with an elevated myocardial lactic acid production in humans,\textsuperscript{4,5} so myocardial ischemia cannot be implicated as the cause of the myocardial depression. Previous data regarding a circulating cardiac depressant were derived almost exclusively from animal models of hemorrhagic shock,\textsuperscript{6} such models are thought to provide limited applicability to humans. A recent clinical study provided evidence that a circulating myocardial depressant substance (MDS) played an important pathophysiologic role in producing the reversible myocardial depression seen during septic shock in humans.\textsuperscript{7}

We used a modification of a previously described in vitro model of myocardial cell contractile performance to assay for the presence of MDS in serial blood samples.\textsuperscript{7-11} These samples were obtained from 50 consecutive (unselected) patients with possible septic shock admitted to our critical care unit as part of an ongoing prospective study of septic shock. Clinical, biochemical, and cardiac parameters that might distinguish patients with and without MDS were analyzed. The results of these analyses form the basis for this report.

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METHODS

Patient Population

From July 1985 to April 1987, 50 consecutive patients with possible septic shock (fever and hypotension) had serial studies of cardiovascular and metabolic function as part of an ongoing prospective study of human sepsis and septic shock in the critical care unit at the National Institutes of Health. "Possible" septic shock was defined as fever (38°C or higher) and hypotension (mean arterial pressure 60 mm Hg or less). The diagnosis of septic shock was confirmed in 34 of these 50 patients. "Confirmed" septic shock was defined using one of two sets of criteria: (1) fever, hypotension, and positive blood cultures (21 patients); or (2) fever, hypotension, and a localized culture-positive infection, but negative blood cultures, attributed either to the transient nature of bacteremia or to concomitant broad-spectrum antibiotic therapy (13 patients). The remaining 16 patients proved to be critically ill but did not have sepsis or septic shock. All subjects gave informed consent and the study protocol was approved by the institutional committee on human research.

Hemodynamic Evaluation

Arterial pressure was monitored by an indwelling catheter in the radial or femoral artery. A flow-directed, balloon-tipped thermodilution pulmonary artery catheter was inserted into each patient. Serial measurements (every 6 to 8 h) were made of central venous pressure, pulmonary artery wedge pressure (measured at end-expiration), and cardiac output by the thermodilution technique. The following hemodynamic data were calculated according to standard formulas: cardiac index in L/min/m²; stroke volume index in ml/m²; and systemic vascular resistance in dynes/cm². The hemodynamic values reported here were obtained at initial line placement (immediately on entry of each patient into our septic shock study) and at the time of each radionuclide cineangiographic study.

The 50 patients had serial blood samples drawn according to the following protocol. Two samples were obtained within 24 h of the onset of the clinical septic shock syndrome, and one sample was obtained on each of days 2, 3, 4, and 5. Blood was allowed to clot and redden in glass test tubes without anticoagulant for approximately 45 min, it was spun at 1,000 rpm for 10 min, and serum was removed and stored at −70°C. MDS activity resisted rapid thawing to room temperature and freezing at −70°C for at least 3 months.

Radionuclide Cineangiography

Initial and serial ECG-gated radionuclide cineangiographic studies were performed on all patients at the bedside using techniques that have been described in detail previously. Patients received an injection of stannous pyrophosphate, and 30 min later they each received 0.3 mCi/kg 99mTc to accomplish in vivo labeling of erythrocytes. These radionuclide scans were obtained using a portable Picker camera according to the following previously described protocol. The initial scan was performed within the first 24 to 48 h of patient entry into the septic shock protocol. A follow-up scan was obtained at two to four days after entry into the study. A recovery scan was defined as a scan performed at least five days (usually five to eight days) after clinical recovery from the acute illness, or a scan demonstrating recovery to a patient's presepsis EF. Radionuclide LVEF was calculated as (EDC - ESC)/(EDC - Bkgd), where EDC = end-diastolic counts, ESC = end-systolic counts, and Bkgd = background counts. The end-diastolic volume index (EDVI) was calculated from simultaneously obtained hemodynamic studies and EF, using the formula EDVI = stroke volume index (from thermodilution cardiac output) divided by EF (from radionuclide cineangiography).

Therapeutic Protocol

Each patient was treated according to a uniform therapeutic protocol by the same group of critical care physicians. The major therapeutic goal was to maintain a mean arterial pressure of at least 60 mm Hg. Initially, all patients received IV fluids to maintain a mean pulmonary artery wedge pressure of 15 mm Hg. Dopamine was then added if the patient remained hypotensive. If the patient required >20 μg/kg/min of dopamine, norepinephrine was added, and the dopamine was tapered to 2 μg/kg/min in an attempt to preserve renal perfusion. All patients received broad-spectrum antibiotic coverage. Respiratory support was given as needed to maintain a normal pH and an arterial oxygen saturation >90 percent. Metabolic parameters were checked frequently, and abnormalities, especially of potassium, phosphate, calcium, and magnesium, were corrected promptly.

In Vitro Model of Myocardial Cell Contractile Performance

A modification of a previously described in vitro myocardial cell contractility system employed was used to assay for MDS activity. Briefly, spontaneously beating newborn rat heart cell cultures were established as follows. Hearts from two-day-old rats were pooled and minced into small blocks of tissue. The cells were disaggregated with 0.05 percent trypsin in modified Hanks solution without Ca²⁺ or Mg²⁺. The myocardial cells were suspended in culture media consisting of the following: potassium-free balanced salt solution (Dulbecco's BSS-K) 85 percent; Medium 199 (Grand Island Biological Company) 25 percent, supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 10 mM glutamine; and 10 percent newborn calf serum, heat-inactivated at 56°C for 30 min. The cells were then plated at a cell suspension of 5 × 10⁶ cells/ml (2 ml per Petri dish). Cells were incubated at 37°C in a 100 percent humidified atmosphere of 95 percent O₂ and 5 percent CO₂. Growth medium was changed initially at 72 to 96 h and every 48 h thereafter. Latex microspheres (4.1 μ in diameter; Polysciences, Inc) were added with the first change of growth medium. Spontaneously beating cells were used in the assay five to nine days following inoculation of cells into the Petri dish.

The extent of myocardial cell shortening during contraction was assayed by a modification of a previously described technique using latex microspheres and a video tracking system. The latex microspheres move in concert with the contracting cell and are tracked with a closed-loop video tracking system. The amplitude or extent of myocardial cell shortening is a function of the distance traveled by the latex microsphere during each contraction of the myocardial cell.

The procedure can be summarized as follows. Spontaneously beating rat myocardial cells grown in a Petri dish were placed on a dissecting microscope stage. The growth medium was removed from the Petri dish supernatant and replaced by control physiologic medium employing 20 percent heat-inactivated newborn calf serum (20 percent serum, 58 percent Dulbecco's BBS-K, and 22 percent Medium 199). The cells were allowed to equilibrate for 15 min. Baseline values for extent of myocardial cell shortening (contraction) were obtained. Then 2 ml of 20 percent patient serum (control or test) plus physiologic medium was placed on the cells and allowed to equilibrate for 15 min. The percentage of depression of myocardial cell contractility was calculated as decrease in extent of shortening with test serum divided by extent of shortening with control serum. A positive assay for MDS was defined as 20 percent or greater depression of myocardial cell contractility (vs control) that was consistent and reproducible on two or more occasions. Any sample that failed to depress contractility 20 percent or more was considered negative. This 20 percent cutoff was chosen because it appears to provide good discrimination between normal control or critically ill control serum and patients with septic shock who have MDS activity.
Myocardial Cell Assay Reproducibility

A previous publication has described a revised method of assaying the extent and velocity of mammalian (rat) myocardial cell contraction in vitro. The revised method used a closed-loop electronic tracking system to quantify the distance traveled by a latex bead affixed to the myocardial cell membrane. This system represented very accurately the distance and velocity per unit time (velocity) of the myocardial cell membrane during contraction and relaxation.

With the revised system, it became apparent that the age of the myocardial cells grown in this primary cell culture system had an important relationship to the sensitivity of the myocardial cell response to the depressant stimulus. Simply stated, the sensitivity of the myocardial cells both to drugs (eg, verapamil) and to serum changes as the cells mature. In the early stages of development (days 4 to 6), the cells are sensitive to a variety of stimuli. As the cells mature (days 7 to 9), they become resistant to these same stimuli. This change in maturation of the heart cells is associated with a decrease in the intrinsic beating rate of these heart cells. We have been careful to choose culture dishes with the following characteristics: a regular rhythm; vigorous contractility; and cells that have not grown to confluence.

Using cells with these characteristics, the assay for myocardial depressant activity is conducted as follows. Young cells (days 4 to 6) are useful for screening patient samples. During this period, the cells are sensitive to relatively low concentrations of pharmacologic agents (eg, verapamil), and the beating myocytes are depressed by sera with relatively weak depressant capability. If negative results (ie, failure to depress the myocardial cells at least 20%) are obtained with a patient's serum using these younger sensitive cells, such a serum would be deemed "MDS negative." The older cells (days 7 to 9) are less sensitive and therefore more specific for MDS activity. If a patient's serum was positive on early sensitive cells, the assay was always repeated on older, less sensitive (more specific) myocytes. If positive on these cells, a patient was considered a definite positive. Each patient judged "MDS positive" had to depress older myocardial cells (seven to nine days in culture) on at least two of four occasions, including two different Petri dishes of myocardial cells in culture.

Ultrafiltration Experiments

Sera from selected critically ill, nonseptic patients, septic patients with and without MDS activity, and normal human volunteers were passed through Amicon filters (Amicon Corp), which allows passage of molecules with weights below 10,000 daltons (Amicon PM10) or below 30,000 daltons (Amicon PM30). Assays were performed on both the filtrates and the supernatants. The characteristics of the myocardial cells and the definition of a positive MDS assay used for these serum fractions were identical to those used for whole sera.

Statistics

Comparisons between patient groups were performed using paired or unpaired Student's t tests, as appropriate. Where appropriate, results were also analyzed using nonparametric Wilcoxon rank-sum test (Mann-Whitney). Spearman rank coefficients were calculated to evaluate the relationships between different independent variables. For categorical variables, groups were compared by χ² analysis. A p value of <0.05 was regarded as statistically significant.

Results

Patient Characteristics and Classification

Fifty patients with a mean age of 49 years (range, 13 to 71) were analyzed in this study. Thirty-four of these 50 patients had definite septic shock, and 16 were critically ill but not septic. One of these 16 nonseptic patients had severe cardiogenic shock (LVEF of 5 percent) and had a positive assay for MDS; the other 15 had negative assays. Since the study protocol was designed to compare septic shock patients with and without myocardial depressant activity, these 16 nonseptic patients were not included when comparing MDS positive vs MDS negative patients.

The 34 septic shock patients had a mean age of 48 years (range, 13 to 70). Eighteen of the patients were males and 16 were females. Seventeen of these patients had leukemia or lymphoma, 12 had solid tumors, two had acquired immunodeficiency syndrome, one had hepatitis, one had ataxia-telangiectasia, and one had aplastic anemia. Twenty-one of the 34 patients had positive blood cultures, two patients had abdominal abscesses, and the remaining 11 had localized infections with positive bacterial cultures or Gram stains that were judged diagnostic of infection. These 11 patients were all receiving broad-spectrum antibiotics during the time of their episodes of fever and hypotension, and the concomitant antibiotic therapy was thought to account (in part) for the negative blood cultures.

Fourteen of these 34 patients had positive assays for MDS (see above, described as a −20 percent or greater decrease in the extent of myocardial cell shortening comparing test with control sera) in one or more serially obtained blood samples. This depressant activity usually could be demonstrated in sera obtained during the early phase of septic shock (ie, days 1 and 2); it was absent from sera obtained on day 3 or later. Twenty of the 34 patients had negative MDS assays of all serum samples obtained. The time between the onset of septic shock (onset defined as rigor, fever, or hypotension) and enrollment into the study protocol was equivalent in the patients with MDS (7.1 ± 1.2 hs) and patients without MDS (6.2 ± 2.2 hs). The distribution of underlying diseases did not differ between the two groups.

Of the 34 septic shock patients, seven died. Five of these seven (71 percent) had MDS. In comparison, only nine of the 27 survivors (33 percent) had MDS (0.05 < p < 0.10).

Two of the 50 patients enrolled in this study had cardiogenic shock, with low cardiac indices (<2 L/min/m²) and high systemic vascular resistances (>2,000 dynes·s·cm⁻⁵). One of these patients had fungemia (blood cultures positive for Cryptococcus), with no enzymatic or ECG evidence of acute myocardial damage, and is included among the 14 patients with septic shock and positive MDS. The other patient had a myocardial infarction, was classified as critically ill but not septic, and was also positive for MDS.
Cardiovascular Function

The serial changes in mean EF, as determined by radionuclide cineangiography, are analyzed in Figure 1. The group of 14 patients with MDS had an initial (day 0 to 1) mean EF of 40 percent that fell to 28 percent at the follow-up scan (days 2 to 4). This second mean EF is lower than the initial EF (p<0.05). The recovery (seven to ten days) mean EF of 51 percent is higher than the second EF (p<0.001).

The group of 20 patients without MDS had an initial mean EF of 42 percent, a follow-up EF of 39 percent, and a recovery EF of 46 percent. None of these serial changes is statistically significant. The patients with MDS have a lower follow-up (days 2 to 4) mean EF than the patients without MDS (28 percent vs 39 percent, p<0.05). These statistical relationships hold using either paired or unpaired Student's t tests.

Radionuclide cineangiographic results of the MDS-positive and MDS-negative patients are compared further in Figures 2A and 2B. The lowest EF obtained during the critical care unit stay in patients with MDS and without MDS are shown in Figure 2A. The lowest mean EF is 28.0 in the patients with MDS and 39.3 in the patients without MDS (p=0.01). Figure 2B illustrates the maximum change in EF that occurred for each patient. The mean difference from initial to lowest EF was 22.1 percent among patients with MDS and 8.8 percent among patients without MDS (p=0.002).

Table 1 compares laboratory, hemodynamic, and radionuclide cineangiographic characteristics of septic shock patients with and without MDS. The following laboratory parameters were found to be equivalent between the two groups of patients: creatinine, bilirubin, prothrombin time, mixed venous oxygen tension, and leukocyte count. Peak lactic acid levels during septic shock were significantly higher in patients with MDS. Of the hemodynamic parameters evaluated, EDVI and PAWP were significantly higher in patients with MDS. The EDVI was calculated at follow-up scan (two to four days); the PAWP was measured at the time of the initial hemodynamic studies.
Table 1—Characteristics of 14 Patients with MDS and 20 Patients without MDS*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MDS Positive (N = 14)</th>
<th>MDS Negative (N = 20)</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td>Laboratory†</td>
<td></td>
<td></td>
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<tr>
<td>Lactate, mmol/L</td>
<td>6.9 ± 1.7‡</td>
<td>2.7 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>2.1 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Prothrombin time, s</td>
<td>15.4 ± 1.3</td>
<td>15.3 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Mixed venous Qs, mm Hg</td>
<td>37.6 ± 1.6</td>
<td>37.3 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Leukocytes, x10⁹/mm³</td>
<td>23.0 ± 1.7</td>
<td>7.4 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Bilirubin, mg/dl</td>
<td>4.5 ± 2.1</td>
<td>2.5 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Hemodynamics‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, min⁻¹</td>
<td>127.1 ± 4.4</td>
<td>121.2 ± 5.0</td>
<td>NS</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>64.0 ± 3.0</td>
<td>70.6 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac index, L/min/m²</td>
<td>6.3 ± 1.1</td>
<td>5.2 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>PAWP, mm Hg</td>
<td>16.8 ± 1.7</td>
<td>11.9 ± 0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SVI, ml/m²</td>
<td>41.7 ± 4.5</td>
<td>43.5 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>EDVI, ml/m³</td>
<td>162.2 ± 15.8</td>
<td>118.2 ± 9.8</td>
<td>=0.02</td>
</tr>
<tr>
<td>SVR, dynes-s/cm²</td>
<td>580 ± 140</td>
<td>855 ± 107</td>
<td>NS</td>
</tr>
<tr>
<td>Norepinephrine, µg/min</td>
<td>25.8 ± 11.8</td>
<td>13.3 ± 10.8</td>
<td>NS</td>
</tr>
<tr>
<td>Dopamine, µg/kg/min</td>
<td>12.9 ± 2.7</td>
<td>7.7 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Radionuclide cineangiograms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial EF</td>
<td>39.7 ± 4.5</td>
<td>41.6 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Follow-up EF</td>
<td>28.0 ± 3.4</td>
<td>39.3 ± 2.7</td>
<td>=0.01</td>
</tr>
<tr>
<td>Recovery EF</td>
<td>50.8 ± 5.6</td>
<td>46.2 ± 3.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*See abbreviations at beginning of text.
†Laboratory values were determined several times daily for each patient. Values given are the mean of the highest determinations.
‡All values are expressed as mean ± SEM.
§All hemodynamic values except for EDVI were determined at the onset of the septic shock syndrome. EDVI was determined at the time of the initial follow-up scan (2 to 4 days after the onset of septic shock; see Table 2).

Analysis of Factors that Can Influence Ejection Fraction

Although EF is used as a measure of cardiac performance, it can be altered by changes in preload, afterload, and heart rate. The PAWP and EDVI can be used as measures of LV preload. Both were significantly higher in patients with MDS, even though the mean EF was lower in these patients (Table 1). Thus, preload cannot account for the differences in EF between the groups with and without MDS.

Afterload can be represented by systemic vascular resistance. As shown in Table 1, both systemic vascular resistance and heart rate were similar in the MDS-positive and MDS-negative groups. Thus, neither of these cardiac parameters can account for the EF differences between the two patient groups.

To evaluate further the cardiac parameters that could affect EF, the serial determinations of EF, systemic vascular resistance (afterload), EDVI (preload), and norepinephrine dose (vasopressor therapy) are listed in Table 2. All of the serial systemic vascular resistance changes between initial and subsequent values were statistically insignificant in both groups of patients; thus, afterload changes could not account for

Table 2—Serial Relationships Among Preload, Afterload, Norepinephrine Dose, and Ejection Fraction in Patients with and without MDS*

<table>
<thead>
<tr>
<th>Days</th>
<th>EF, %</th>
<th>SVR, dynes-s/cm²</th>
<th>EDVI, ml/m³</th>
<th>Norepinephrine Dose, µg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 1</td>
<td>39.7 ± 4.5†</td>
<td>580 ± 140</td>
<td>138 ± 16</td>
<td>25.8 ± 11.8</td>
</tr>
<tr>
<td>2 to 4</td>
<td>28.0 ± 3.4</td>
<td>826 ± 104</td>
<td>162 ± 16</td>
<td>1.9 ± 1.2</td>
</tr>
<tr>
<td>5 to 8</td>
<td>50.8 ± 3.6</td>
<td>750 ± 65</td>
<td>98 ± 9</td>
<td>0.0</td>
</tr>
<tr>
<td>MDS Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 1</td>
<td>41.6 ± 2.4</td>
<td>755 ± 107</td>
<td>115 ± 10</td>
<td>13.3 ± 10.8</td>
</tr>
<tr>
<td>2 to 4</td>
<td>39.3 ± 2.7</td>
<td>899 ± 92</td>
<td>118 ± 9</td>
<td>0.0</td>
</tr>
<tr>
<td>5 to 8</td>
<td>46.2 ± 3.1</td>
<td>800 ± 210</td>
<td>113 ± 16</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*See abbreviations at beginning of text.
†All values are expressed as mean ± SEM. See text for statistical explanation of these serial values.
the large decrease in mean EF in MDS positive patients. The increase in EDVI from days 0 to 1 to days 2 to 4 in the MDS-positive patients could not account for the decreased EF, because increased preload should induce an increase (not a decrease) in EF. The decrease in norepinephrine dose from days 0 to 1 to days 2 to 4 in MDS positive patients would be expected to decrease afterload and increase EF; however EF decreased in MDS-positive patients during this interval. (Please see a previously published reference for discussion of this issue.)

Thus, the comparison of MDS-positive and MDS-negative groups (Table 1) and the analysis of serial parameters in these two groups (Table 2) both failed to identify another reason for the decreases in EF that occurred in the MDS-positive patients, supporting the hypothesis that MDS was the cause of these decreases.

Correlation of Ejection Fraction with Extent of Myocardial Cell Shortening

Figure 3 illustrates the relationship between the radionuclide determined in vivo EF (days 2 to 4) and the in vitro percentage of change in extent of myocardial cell shortening in the 14 septic shock patients with MDS. As mentioned above, a positive assay for MDS was defined as at least a 20 percent in vitro depression of the extent of myocardial cell shortening. The degree of in vitro depression ranged from 20 percent to 62 percent, with a mean of 35 percent. The EFs ranged from 7 percent to 52 percent, with a mean of 28 percent. Using Spearman rank coefficients, there was a significant correlation between these two variables (r = -0.60, p < 0.05).

Ultrafiltration Experiments

The results of ultrafiltration experiments on sera from critically ill nonseptic patients, septic patients with and without MDS activity, and normal human volunteers are summarized in Table 3. MDS activity did not pass through 10,000 or 30,000 dalton Amicon filters. No MDS activity was found in either the filtrate or the supernatant in critically ill nonseptic patients or in normal human volunteers. In patients positive for MDS, MDS activity was found only in supernatants containing molecules above 10,000 and 30,000 daltons. Serum with MDS activity maintained its activity despite multiple freezings at -70°C and repetitive thawings.

**DISCUSSION**

This study extends our understanding of cardiovascular dysfunction and the role of a circulating MDS in the pathogenesis of human septic shock. In this study, the presence of circulating myocardial depressant activity was temporally associated with cardiovascular abnormalities—specifically, reversible myocardial depression and ventricular dilatation, changes that characterize human septic shock. When compared with MDS negative patients, septic shock patients achieving strict criteria for the presence of a circulating MDS

**Table 3—Results of Ultrafiltration Experiments in Septic Shock Patients with and without MDS,**

*Critically Ill Nonseptic Patients, and Normal Human Volunteers*

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>10,000 Daltons</th>
<th>30,000 Daltons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filtrate</td>
<td>Supernatant</td>
</tr>
<tr>
<td>MDS positive</td>
<td>0/6</td>
<td>6/6</td>
</tr>
<tr>
<td>MDS negative</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Critically ill, nonseptic</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Normal humans</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

*MDS = Myocardial depressant substance.*
had a statistically greater decrease in LVEF and a lower minimum EF. Further, this in vitro myocardial depression was correlated significantly with the extent of myocardial cell shortening observed using an in vitro model of myocardial cell performance. This correlation suggests that the degree of in vitro myocardial cell depression reflects the pathophysiologic events responsible for the in vitro myocardial depression. These findings confirm and extend the observations made in a previous set of studies.7

The present study provides new data regarding the incidence of circulating MDS in patients with septic shock. As part of an ongoing prospective study of human septic shock, 50 patients admitted to our critical care unit were suspected of having septic shock based on the presence of fever and hypotension. Of the 50 patients, 16 turned out not to have sepsis; of these only one patient, with severe cardiogenic shock, met our criteria for circulating MDS. The remaining 34 patients subsequently met microbiologic criteria confirming the diagnosis of shock secondary to sepsis. Of the 34 confirmed septic shock patients, 14 (41 percent) had a circulating MDS.

With positive in vitro depression defined at \(-20\) percent, the MDS positive patients clearly have depressant activity, but some patients with \(0\) to \(-20\) percent depression may also have significant circulating biologic depressant activity. Biologic assays frequently lack precise discriminating capability. A highly significant correlation does exist between the in vitro myocardial cell assay and the in vitro EF in the 14 MDS-positive patients. To analyze the sensitivity of the existing biologic assay will require isolation and purification of the MDS molecule(s) and development of a radioimmunoassay or an enzyme-linked immunosorbert assay (ELISA).

Previous studies by our group4 and others5 have demonstrated clearly that the myocardial depression of human septic shock is not associated with reduction in coronary blood flow nor with net myocardial lactic acid production. Thus, it is very unlikely that myocardial ischemia causes the myocardial dysfunction of human septic shock.

A previous study excluded the possibility that myocardial depression resulted from electrolyte abnormalities or from circulating pharmacologic agents.7,13 The methods used to exclude electrolyte abnormalities or pharmacologic agents as causes of myocardial cell depression7 can be summarized as follows: (1) control groups receiving identical medications and having similar electrolyte derangements failed to show any myocardial cell depression; (2) measurements of the electrolyte solution bathing the myocardial cells reveal the same concentrations of electrolytes in the depressed and nondepressed cultures; (3) the assay is performed with 10 percent or 20 percent test serum added to a buffered electrolyte solution; this latter solution dilutes and corrects all electrolyte abnormalities; (4) as judged by Amicon filtration and gel filtration, MDS activity occurred at a molecular weight of greater than 2,000 daltons7 and probably greater than 10,000 daltons. Thus, the molecular weight of MDS was in a range inconsistent with any electrolyte molecule or pharmacologic agent.

We have evaluated extensively a number of other cardiovascular factors that might have contributed to the decrease in EF that occurred in the patients with MDS. The patients with MDS had significantly higher preload (both initially and at two to four days, measuring preload by both pressure and volume methods) than patients without MDS. This difference in end-diastolic pressure and volume suggests that MDS also may be associated with a diastolic compliance abnormality of the ventricle, a finding that has been described previously in a canine sepsis model6 and in humans with septic shock.4 There were no differences noted between MDS positive and negative groups with respect to afterload, heart rate, or the quantity of exogenous catecholamines infused therapeutically during the shock syndrome. Thus, these cardiovascular parameters cannot account for the myocardial dysfunction, and the most likely explanation for this reversible in vitro myocardial depression is the occurrence of a molecule(s) that can directly affect cardiac performance. Using a spontaneously beating rat myocardial cell model in vitro, the present study demonstrated myocardial depressant activity that correlates both temporally and quantitatively with this myocardial depression.

In addition to cardiovascular function, this study investigates a possible association between MDS positivity and a variety of laboratory parameters and measures of organ function. The only laboratory parameter that distinguished between patients with and without MDS was a significantly higher mean peak lactic acid level in the group with MDS. Several possible pathophysiologic mechanisms that might explain the association of MDS with higher levels of lactic acid are: (1) a more pronounced decrease in cardiac output might occur relative to tissue needs in the group of patients with MDS. This would cause greater tissue hypoperfusion and thus higher lactic acid levels. Our data do not support this hypothesis, since mean values for cardiac outputs and mixed venous oxygen contents were not different between MDS positive and negative groups. (2) MDS might cause more pronounced shunting of blood peripherally, thereby elevating lactic acid. Our data do not support this hypothesis either, since mean mixed venous oxygen content was similar in the two groups. (3) The degree of tissue hypoperfusion (due to a number of possible mechanisms) might be more severe
in patients with MDS, thereby increasing lactic acid levels. Our data show that a trend toward more severe shock exists in the MDS positive group: they were less likely to have fluid-responsive septic shock (three of 14 vs 11 of 20, 0.05<p<0.10), they required higher levels (though not significantly higher) of exogenous catecholamines (dopamine and levaterenol), and they had a trend toward a higher mortality (five of 14 vs two of 20, 0.05<p<0.10). The association between MDS and elevated lactic acid suggests that a significant peripheral vascular abnormality is linked to circulating MDS in human septic shock. Further studies are needed to define the mechanism of the elevated lactic acid level in MDS positive patients with septic shock.

The in vitro myocardial cell contractility assay employed in this study represented a modification of a previously described cell culture system.7–9 The addition of the latex microspheres improved the reliability of the system. Using this revised method, the amount of depression can be quantified reproducibly. Since the sensitivity of the cell culture system changes as the cells mature, it is imperative to choose cultures at the same level of maturation each time an assay is performed. With the initial system that did not use latex beads,7 small changes in the relationship of myocardial cell edge to the microscope optics caused technical difficulties. These resulted in long delays while finding a cell in which the cell edge and the optical tracking system were very stable. However, the latex bead system9 is not altered by small changes in the relation of the myocardial cell to the microscope optics because the computer program tracking system continuously recenters the gate over the bead. Thus, this revised method decreases the technical difficulties and enhances the reproducibility and reliability of this in vitro bioassay of cardiac cell performance.

Understanding the changes in the maturation of the heart cells grown in tissue culture, coupled with the improved reliability of the latex bead video tracking system, allowed us to systematically evaluate serial ultrafiltration experiments in sera from a variety of patient groups. The criteria for MDS activity in these experiments were identical to those of the initial experiments performed on the 50 patients enrolled into the septic shock protocol to determine the incidence of patients with circulating MDS. The MDS activity was consistent in the supernatant fraction of the serum samples, suggesting that the molecular weight of the MDS activity is greater than 10,000 daltons and may be greater than 30,000 daltons.

This activity may be a somewhat different molecular weight than depressant molecules that have been previously described.6 This difference may be due to the fact that the myocyte system in the present study is capable of assessing in vitro myocardial cell performance using unmodified serum. Previous studies analyzing the properties of cardiodepressant substances have used plasma ultrafiltrates of cat hemorrhagic shock6 or rat endotoxic shock10 models that have limited applicability to the myocardial depression seen in human septic shock. The difference might also be attributable to the occurrence in human septic shock of more than one myocardial depressant molecule, with differing molecular weights. Another possibility is that several active, identical low molecular weight molecules can combine to form a higher molecular weight macromolecule that also has depressant activity. This could lead investigators to conclude that there are several active MDS molecules of different molecular weights, while in reality only a single active molecule existed, and combinations of several identical subunits were leading to a variety of molecular weights.

The potential diagnostic and therapeutic ramifications of MDS are in their preliminary stages. The data from this study and previous work1–7,9 strongly suggest that MDS depresses EF in vivo. It is not clear whether MDS directly increases EDVI or whether this ventricular dilatation is a compensatory mechanism stretching myocytes to increase preload, thus offsetting the depressed systolic function. Inhibition or neutralization of MDS might restore a normal EF, but this hypothesis awaits experimental verification in animal models. In a canine model which closely simulates the hemodynamic profile of human sepsis,3 plasmapheresis, which removes almost all noncellular blood elements, has not reversed myocardial depression or lethality.16

In the present study, we have confirmed the existence of MDS in the blood of at least 41 percent of patients with septic shock. This substance was detected early in the course of septic shock, and we quantitatively and temporally correlated MDS to concomitant in vivo evidence of myocardial dysfunction, demonstrated by a depression of the EF and increased ventricular dilatation. Further studies are needed to evaluate the structural characteristics of MDS and to define biochemically its precise role in the complex pathophysiology of human septic shock.

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