The Impact of Anti-Endotoxin Core Antibodies on Endotoxin and Cytokine Release and Ventilation Time After Cardiac Surgery

Markus Rothenburger, MD,* Rasjid Soeparwata, MD,* Mario C. Deng, MD, FACC, FESC,† Elmar Berendes, MD,‡ Christof Schmid, MD,* Tommy D. T. Tjan,* Markus J. Wilhelm, MD,* Michael Erren, MD,§ Dirk Böcker, MD,|| Hans Heinrich Scheld, MD*

Muenster, Germany and New York, New York

OBJECTIVES We hypothesized that a temporary cardiopulmonary bypass (CPB)-induced reduction of endotoxin antibody levels contributes to elevated endotoxin levels and the associated inflammatory consequences, with a significant influence on the postoperative ventilation time period.

BACKGROUND Cardiac surgery using CPB induces a systemic inflammatory response syndrome with an associated risk of increased postoperative morbidity and mortality.

METHODS A total of 100 consecutive patients undergoing elective coronary artery bypass graft surgery using CPB were prospectively investigated. Endotoxin core antibodies (immunoglobulin [Ig] M/IgG against lipid A and lipopolysaccharide), endotoxin, interleukin (IL)-1-beta, IL-6, IL-8 and tumor necrosis factor-alpha were measured serially from 24 h preoperatively until 72 h postoperatively.

RESULTS Eighty-five patients had no complications (group 1), whereas 15 patients required prolonged ventilation (group 2). In both groups, there was a decrease of all antibodies 5 min after CPB onset, compared with baseline values (p < 0.001), an increase of endotoxin and IL-8 peaking at 30 min postoperatively (p < 0.001) and an increase of IL-6 peaking 3 h postoperatively (p < 0.001). In group 2, preoperative antibody levels were lower (p < 0.01) — specifically, the decrease in IgM was significantly stronger and of longer duration (p < 0.002) — and levels of endotoxin (p < 0.001) and IL-8 (p < 0.001) were higher at 30 min postoperatively.

CONCLUSIONS We conclude that a CPB-associated temporary reduction of anti-endotoxin core antibody levels contributes to elevated endotoxin and IL-8 release. Furthermore, lower levels of IgM anti-endotoxin core antibodies were associated with a greater rise in endotoxin and IL-8, as well as prolonged respirator dependence. (J Am Coll Cardiol 2001;38:124–30) © 2001 by the American College of Cardiology

Cardiac surgery using cardiopulmonary bypass (CPB) frequently results in a systemic inflammatory response syndrome, including fever, and leukocytosis (1,2). The inflammatory response syndrome can lead to hemodynamic instability, respiratory insufficiency and multiorgan failure (1,2). The consequences of uncontrolled activation of the proinflammatory response is evident microscopically in different organs as diffuse microvascular thrombi lead to organ failure, especially of the lung (3). This may be induced by proinflammatory cascades with coagulopathy after an imbalance of hemostatic mechanisms and chemokine-induced alterations of leukocyte homing. Although the initiation of this sequence is coincident with CPB, the mechanisms remain to be elucidated.

Endotoxin is recognized to be a major stimulus for the development of the systemic inflammatory response syndrome (4). The lipopolysaccharide (LPS) component of the cell wall of gram-negative bacteria is believed to be responsible for the majority of the pathogenic effects of these organisms. Endotoxin causes an increase in tissue oxygen demand, myocardial dysfunction and complement activation and triggers contact activation, which may lead to a bleeding tendency and microvascular thrombosis, a major cause of organ failure (5). Exposure to endotoxin is known to occur in most patients undergoing surgery involving CPB (6), although the origin of the endotoxin remains a subject of great debate. Translocation secondary to gut hypoperfusion has long been considered as one possible source (7), and, with large fluid shifts and low perfusion pressures encountered during bypass surgery, it remains an attractive hypothesis.

Starting in early fetal life, humans possess a degree of endogenous endotoxin immunity conferred initially by maternal transfer and subsequently enhanced by exposure to endotoxin (8,9). Anti-endotoxin core antibodies of the different antibody classes are commonly present in substantial amounts in the sera of most healthy adults (8,9).
The only area of the endotoxin molecule that is conserved across a whole range of gram-negative organisms is the inner core region. Therefore, it can be assumed that antibodies in this area should be most cross-reactive and thus most protective (10).

Bennett-Guerrero et al. (11) described the association between preoperative anti-endotoxin immune status and morbidity in patients who had cardiac surgery. The authors demonstrated that low preoperative serum immunoglobulin (Ig) M anti-endotoxin core antibody concentrations were associated with adverse outcome after cardiac surgery, compliant with the concept that endotoxia may be an important cause of perioperative morbidity after cardiac surgery.

These data imply that anti-endotoxin core antibodies are important in preventing an endotoxin-induced overshoot of the immunologic host response, with cytokine release and adverse events (12). However, changes in antibody levels during CPB have not been examined.

The hypothesis tested in this study is that low preoperative endogenous anti-endotoxin core antibody levels in patients undergoing coronary artery bypass graft surgery (CABG) result in higher postoperative endotoxin and cytokine levels. A secondary objective was to relate these changes to the duration of respirator dependence.

METHODS

Patients. Study participants included patients undergoing elective CABG in our institution. Study inclusion criteria were: 1) age between 18 and 75 years; 2) preoperative left ventricular ejection fraction >0.45; 3) stable clinical condition; and 4) consent declaration to participate in the study, which was approved by the Ethics Committee (University of Muenster, Germany). Patients were characterized using the risk score reported by Estefanous et al. (13).

Clinical events. Group 1 was defined as those patients requiring a ventilation time <24 h (group 1A = ventilation time <12 h; group 1B = ventilation time 12 to 24 h). Patients requiring a ventilation time >24 h postoperatively were defined as the event group (group 2).

Weaning from the respirator was attempted some hours after arrival in intensive care unit if patients were hemodynamically stable. In patients with stable blood gases and low chest tube secretion, rapid extubation was accomplished by reducing the rate in a synchronized intermittent mandatory ventilation mode. All outcome measurements (extubation time) were performed by clinicians who had no knowledge of the patients’ antibody, endotoxin and cytokine levels.

Blood sampling. Blood was drawn at 11 time points. The blood sampling was done 24 h before surgery (time point [TP] 1), after induction of anesthesia (TP 2), after sternotomy (TP 3), 5 min after CPB onset (TP 4), after protamine reversal (TP 5), during skin closure (TP 6) and 30 min (TP 7), 3 h (TP 8), 24 h (TP 9), 48 h (TP 10) and 72 h postoperatively (TP 11).

The first and postoperative blood samples were collected by venipuncture (21-gauge butterfly needle); all other samples were taken using the arterial line.

Blood was collected into Vacutainer tubes containing buffered ethylenediaminetetraacetic acid (EDTA)-K3 (15%, 0.084 ml, 0.34 mol/l; Becton Dickinson) and into EndoTubes containing sodium heparin (120 IU, endotoxin-free, 4 ml; Chromogenix, Sweden) that were immediately placed on ice and processed within 30 min.

The EDTA plasma for interleukin (IL)-1-beta, IL-6 and tumor necrosis factor (TNF)-alpha determination was obtained by centrifugation at 4,000 × g, 4°C for 20 min. Sodium heparin-anticoagulated plasma for determination of endotoxin and anti-endotoxin core antibodies was obtained by centrifugation at 1,000 × g at room temperature for 10 min. Sodium heparin-anticoagulated plasma for determination of IL-8 was obtained by centrifugation at 4,000 × g at 4°C for 20 min. Aliquots of plasma were snap-frozen in liquid nitrogen and stored at −80°C until analyzed.

Blood cultures were obtained, on average, on the third postoperative day in patients with clinical evidence of infection (i.e., fever >39°C and radiologic or clinical signs of infection). In patients without clinical evidence of infection, no blood cultures were obtained.

All laboratory measurements were performed on coded samples so the investigators would have no knowledge of the patients’ identity and outcome.

Analysis of cytokines. Levels of IL-1-beta, TNF-alpha, IL-6 and IL-8 in the plasma were determined by means of a commercially available enzyme-linked immunosorbent assay (ELISA; R&D systems, Biermann, Bad Nauheim, Germany).

Analysis of endotoxins. A commercially available chromogenic Limulus amebocyte lysate test for endotoxin measurement (Coatest Endotoxin, Kabi-Chromogenix, Mölndal, Sweden) was carried out.

All reagents (Kabi Vitrium, Stockholm, Sweden) and materials were endotoxin-free, and all preparation steps, except for photometry, were performed under sterile conditions. To minimize contamination, blood samples from healthy donors served as negative control samples.

Analysis of anti-endotoxin core antibodies. Semiquantitative ELISA for determination of IgM and IgG antibodies against lipid A and LPS were performed as previously described (14). The lower detection limits were 3.9 U/µl for
IgM against lipid A, 4.3 U/μl for IgM against LPS, 8 U/μl for IgG against lipid A and 8.1 U/μl for IgG against LPS.

During the first step, microtiter plates were coated with lipid A and LPS, separately, for 24 h (coating buffer: Na2CO3, NaHCO3, NaN3 in ad water; pH 9.6). The plasma of the patient samples was added and incubated for 1.5 h at room temperature. Thereafter, the plasma samples were washed out three times using phosphate-buffered saline (PBS) Tween wash solution (NaCl, Na2HPO4, NaN3, Tween 20) were added. After incubation for 16 h, a chromogenic substrate (p-nitrophenyl phosphate, 1 mg/ml) in substrate buffer (diethanolamin, MgCl2 × 6 H2O, NaN3; pH 9.8) was added. The samples were photometrically measured at 405 nm. The results are displayed as units/microliter, which is related to the photometric index derived from the assay methodology.

**Hemodilution.** The measured serum concentration of a biologically active compound is relevant for the response of the patient. However, to compare the intraoperative results with the preoperative and postoperative levels, it was necessary to correct the concentrations measured during bypass for hemodilution. Volume correction was performed for samples taken during and after CPB. Only corrected data are depicted and used for statistical analysis.

**Statistical analysis.** For statistical analysis, the Statistical Package for the Social Sciences (SPSS version 9.01, Chicago, Illinois) was used. After log transformation, a normal distribution of the variables was confirmed. A descriptive statistical analysis of cytokine, endotoxin and antibody levels was performed and presented as the mean value ± SD. Analysis of variance for repeated measures was performed according the Scheffé post hoc comparison. Preoperative antibody levels were used as covariates (independent variables) in the statistical analysis. The dependent variables (antibodies, endotoxins and cytokines) were included in the model as the change from baseline (preoperative values). A type I error <5% was considered significant.

**RESULTS**

**Clinical outcomes.** One-hundred patients undergoing elective CABG were included. The patient characteristics and perioperative data are presented in Tables 1 and 2. All consecutive patients received at least one internal mammary artery graft on the left anterior descending coronary artery. All patients survived the hospital stay, and no patient required re-explosion for bleeding after the operation. Eighty-five of 100 patients were extubated within 24 h (group 1A: n = 51; group 1B: n = 34). Fifteen patients required ventilation >24 h postoperatively (group 2) (Table 2). In group 2, blood cultures for bacterial agents were positive in 66% of cases. Blood culture sampling was done, on average, 48 ± 9 h postoperatively (*Staphylococcus aureus* [n = 2], coagulate-negative *staphylococcus* [n = 5] and

**Table 1. Patient Characteristics and Clinical Data**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 85)</th>
<th>Group 2 (n = 15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>63/22</td>
<td>11/4</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.5 (53.4–66.9)</td>
<td>64.3 (51.6–71.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Body surface (m²)</td>
<td>1.89 (1.78–1.95)</td>
<td>1.83 (1.71–1.92)</td>
<td>NS</td>
</tr>
<tr>
<td>Risk score*</td>
<td>0.5 (0–2)</td>
<td>1 (0.5–1.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative ejection fraction (%)</td>
<td>63 (43–69)</td>
<td>61 (45–70)</td>
<td>NS</td>
</tr>
<tr>
<td>Extent of vessel involvement (n)</td>
<td>2.5 (1.8–3)</td>
<td>2.5 (1.8–3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*See reference 13. Data are presented as the number of patients or median (interquartile range). NS = not significant.

**Table 2. Operative and Postoperative Data**

<table>
<thead>
<tr>
<th></th>
<th>Group 1A</th>
<th>Group 1B</th>
<th>p Value (Group 1A vs. 1B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of CABGs</td>
<td>3.1 ± 1</td>
<td>3.1 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>70 ± 12</td>
<td>70 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Cross-clamp time (min)</td>
<td>41 ± 7</td>
<td>41 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>132 ± 16</td>
<td>132 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>Ventilation time (h)</td>
<td>9.8 ± 4.3</td>
<td>16 ± 5.6</td>
<td>0.048</td>
</tr>
<tr>
<td>Postoperative hospital time (days)</td>
<td>6.2 ± 2</td>
<td>6.5 ± 3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as the mean value ± SD.

CABGs = coronary artery bypass graft surgeries; CPB = cardiopulmonary bypass; NS = not significant.
Pseudomonas aeruginosa \([	ext{n} = 3]\). In group 1, no patient had clinical symptoms requiring blood cultures.

**Anti-endotoxin core antibody levels.** In the total group, a significant decrease of all anti-endotoxin core antibodies occurred at CPB onset \((p < 0.001; \text{Fig. 1})\). In subgroups 1A and 1B, antibodies of the IgM type were significantly decreased compared with baseline values at post-CPB TPs 4 to 8 \((p < 0.01)\). Group 2, patients had significantly lower preoperative antibody levels \((p < 0.001)\) and a more pronounced decrease of anti-endotoxin core antibodies at 72 h postoperatively compared with baseline values \((\text{IgM lipid A: } p < 0.02; \text{IgM Re[recombinant]}LPS: p < 0.05; \text{IgG lipid A: } p < 0.05; \text{IgG ReLPS: } p < 0.02). In group 2, antibodies of the IgM type were significantly decreased compared with baseline values at all post-CPB TPs \((p < 0.01; \text{Fig. 1})\).

**Endotoxin levels.** Preoperatively, no endotoxins were detectable. Thirty minutes after termination of CPB, an increase of endotoxin, compared with baseline values, occurred \((p < 0.001; \text{Fig. 2})\). A significant influence of the endotoxin peak levels at 30 min after CPB on postoperative ventilation time was found \((p < 0.001; \text{Table 3})\). In group 2, patients had a significantly greater rise of endotoxin levels at TPs 5 to 11 \((p < 0.001)\).

**Cytokine levels.** Preoperative levels of IL-8 were similar in all groups. We found a significant elevation of IL-8 in all groups 30 min after CPB \((p < 0.002; \text{group 1A: } p < 0.01; \text{group 1B: } p < 0.001; \text{Fig. 3})\). The IL-8 ranges at 30 min after CPB were significantly associated with postoperative ventilation time \((p < 0.001; \text{Table 3})\). A greater rise of IL-8 levels was significantly associated with lower preoperative antibodies in group 2 patients \((p < 0.01)\). Interleukin-8 increased 2 h before the IL-6 peak occurred \((p < 0.001)\). The preoperative IL-6 levels were low and not different between the two groups. Interleukin-6 increased postoper-
atively compared with baseline values in all groups, reaching its maximum at 3 h after CPB ($p < 0.001$). No significant difference between group 1 and 2 patients was observed. In all groups, a significant increase of TNF-alpha at the end of the operation, compared with baseline values, was determined ($p = 0.05$). Tumor necrosis factor-alpha did not differ between the groups. No significant change of IL-1-beta levels was observed.

**Ventilation time and blood culture results.** When comparing endotoxin and IL-8 levels of group 2 patients with positive blood cultures ($n = 10$) with those of patients with negative blood cultures ($n = 5$), no significant differences were found.

**DISCUSSION**

Patients undergoing CABG using CPB demonstrate a temporary decrease of endotoxin antibody levels, followed by an increase of endotoxin and IL-8. Patients requiring prolonged respirator therapy demonstrate lower preoperative IgM antibody levels, a more pronounced reduction in antibody levels intraoperatively and postoperatively and a significantly greater release of endotoxin and IL-8.

**Anti-endotoxin core antibodies and CPB.** Anti-endotoxin core antibodies show an initial depletion in sepsis, as well as acute pancreatitis, predicting the development of multiorgan failure (15). However, results in cardiac surgery using CPB are conflicting. Andersen et al. (6) could not find any changes in the plasma levels of anti-endotoxin core antibodies after CPB. Bennett-Guerrero et al. (11) found low preoperative IgM endotoxin antibody levels to be associated with adverse outcome after cardiac surgery, independent of other established risk factors. Hamilton-Davies et al. (3) reported a relationship between preoperative endotoxin immune status, gut perfusion and outcome after valve replacement surgery. They found an increased risk of postoperative complications in cases of low preoperative IgG and IgM endotoxin core antibody levels. The investigators concluded that low preoperative endotoxin core antibody levels are related to a poor outcome after cardiac surgery.

In support of these results, we found lower preoperative...
levels of anti-endotoxin core antibodies, specifically of the IgM type, in patients with prolonged respirator therapy. However, all preoperative antibody levels were within the normal range. Furthermore, we found a more pronounced depletion of all IgM antibodies at CPB onset in the event group. In this group, all antibodies remained decreased throughout the observation period. These results suggest that an antibody deficiency with a temporary reduction in immune competence is accompanied by prolonged respirator dependence after cardiac surgery. The mechanisms of intraoperative antibody depletion may include adherence to CPB coating, consumption and decreased production.

**Endotoxin and CPB.** In current concepts, high levels of circulating endotoxins during CPB have been assumed to be caused by splanchnic congestion and translocation of bacteria from the intestinal mucosa into the circulation (16,17). The early depletion of all anti-endotoxin core antibodies during CPB may cause an imbalance between antigen (endotoxin) and antibodies, resulting in increased levels of endotoxin, with consecutive activation of B cells, T cells and leukocytes. This may provide an alternative explanation for endotoxin elevation. It has been reported that high endotoxin levels are recognized to be a major stimulus of the systemic inflammatory response syndrome and, subsequently, multiorgan failure (3). We found increased endotoxin levels peaking 30 min after CPB. A strong association between endotoxin peak levels at 30 min after CPB and postoperative ventilation time was determined.

**Proinflammatory cytokines and CPB.** The higher elevation of IL-8 in the event group may provide a link between endotoxin and clinical events. Various studies demonstrated increased IL-8 levels in patients undergoing CPB (12,18–20). Because IL-8 is known as a selective chemoattractant for neutrophils, which play an important role in reperfusion injury, an increasing number of studies have focused on this cytokine. Interleukin-8 is involved in cell injury after neutrophil activation, pathogenesis of adult respiratory distress syndrome, multiorgan failure and left ventricular wall motion abnormalities (12,21,22).

Our results suggest that release of IL-8 follows a dysbalance of the endotoxin/endotoxin antibody ratio. Elevation of IL-8 is associated with consecutive neutrophil activation after CPB and may play an important role in the pathogenesis of the prolonged respirator dependence after cardiac surgery.

Release of IL-6 after cardiopulmonary bypass was reported in numerous studies (1,19,23). Consistent with previously reported data, in the present study, a significant increase 3 h after CPB termination in both groups was observed. However, in our study, IL-6 had no discriminatory power regarding the ventilation time after cardiac surgery.

**Conclusions.** Our data suggest a key role for a CPB-induced temporary reduction of anti-endotoxin core antibodies and a consecutive increase of endotoxin and IL-8, associated with prolonged respirator dependence. Low pre-
operative anti-endotoxin core antibody levels are associated with prolonged respirator dependence after cardiac surgery, independent of other established risk factors. The data provide a rationale for the study of immunoglobulin substitution, which may help reduce ventilator time, hospital stay and cost of treatment.

Acknowledgment
We gratefully acknowledge the statistical advice of Dr. Joachim Heinecke, Institute for Medical Informatics and Biomathematics of the University of Muenster, Germany.

Reprint requests and correspondence: Dr. Markus Rothenburger, Department of Cardiothoracic Surgery, University of Muenster, Albert Schweitzer Strasse 33, 48129 Muenster, Germany. E-mail: markus.rothenburger@thgms.uni-muenster.de.

REFERENCES