Administration of Anti-TNF Antibody Improves Left Ventricular Function in Septic Shock Patients*
Results of a Pilot Study
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In this pilot study, murine monoclonal anti-TNF antibody (2 mg/kg) was administered to ten patients within 24 h of septic shock which persisted after initial resuscitation with intravenous fluids and adrenergic agents. This treatment resulted in a reduction in heart rate (from 122±10 to 113±10 beats per minute at 4 h, p<0.01) associated with an increase in LVS1W (from 25.5±5.6 to 31.5±10.5 gm/s at 2 h, p<0.05), indicating an absence of change in cardiac filling pressures, an improvement in ventricular function. Arterial oxygenation improved concurrently in six patients. These changes, however, appeared transient. The improvement in cardiac function following anti-TNF antibody administration in patients is in keeping with recent experimental studies indicating the role of TNF in the myocardial depression characterizing septic shock.

(Chest 1992; 101:810-15)

Despite great scientific progress, septic shock remains a highly fatal disease so that new therapeutic options must be sought. Recent experimental and clinical studies have implicated TNF (TNF-alpha or cachectin), a macrophage-derived cytokine, as a potentially important mediator of septic shock. This has been based on three observations. First, TNF blood levels in severely septic patients have been related to the severity of the acute disease and the likelihood of death. Second, the administration of TNF in animals reproduces the hemodynamic, metabolic and pathologic findings associated with endotoxic shock. Therapeutic infusion of TNF in cancer patients also can result in fever, tachycardia, hypotension and increased energy substrate mobilization. Third, the administration of anti-TNF antibodies in animals can counteract the deleterious effects of endotoxin or improve survival from Gram-negative sepsis.

These latter observations also have stimulated the research for new therapeutic interventions in the management of the patient with septic shock. In initial experimental studies, the protective effects of anti-TNF antibodies were present only when the antibodies were administered prior to the induction of sepsis, thus limiting the potential value of this treatment in patients with septic shock. However, recent studies indicated that these antibodies could still be effective when administered at the same time or shortly after the septic insult. Importantly, the TNF release might be more transient in both these acute animal models and after endotoxin administration in human volunteers than in human septic shock. Recent clinical studies have indicated that plasma TNF levels could remain elevated for more than 24 h after the onset of septic shock. Hence, there could be a period during which anti-TNF antibody might still be effective.

A murine monoclonal antibody against TNF recently has become available for human use, and its administration in patients has no obvious side effects. Therefore, it is possible to test the hypothesis that anti-TNF antibody can reduce morbidity and mortality from septic shock. This will require a large multicenter, prospective, double-blinded, randomized clinical trial. Before embarking on such a large study, we conducted a pilot study on ten patients who were carefully monitored during the course of septic shock to investigate whether any consistent cardiovascular effects occurred in these patients in response to anti-
TNF antibody. To achieve relative hemodynamic stability prior to the antibody administration, we administered the anti-TNF antibody (CB0006, Celltech) after initial resuscitation but during the first 24 h of the shock state.

PATIENTS AND METHODS

Following approval by the ethical committee of the Erasme University Hospital, the study included ten patients with severe septic shock of less than 24 h duration. Patients who were less than 18 years or more than 75 years of age, who had received organ transplantation or in whom pregnancy could not be excluded, were not considered. Circulatory shock was defined by hypotension (systolic arterial pressure less than 90 mm Hg or a decrease in mean arterial pressure by more than 30 mm Hg from the usual value) associated with a reduction in urine output (less than 20 ml/h), an abnormal mental status and a circulating blood lactate level of at least 2 mEq/L. In addition, there was a suspected source of sepsis, a temperature greater than 38.5 or less than 36.0°C, and a white blood cell count more than 10 or less than 6 x 10^9/L. Written informed consent was obtained from a responsible relative.

Management of septic shock followed a routine standardized therapeutic protocol including intravenous colloids (4.5 percent albumin solution, Red Cross of Belgium) according to a fluid challenge protocol and infusion of dopamine to restore a systolic blood pressure of at least 90 mm Hg. If the vasopressor therapy had to be maintained despite fluid therapy until the central venous pressure increased to greater than 12 to 14 mm Hg, a PA catheter was inserted and fluid challenge was resumed until the PAOP reached 15 to 18 mm Hg. Dobutamine could be added to maintain cardiac index greater than 3 L/min/m^2. If the signs of shock and the requirements for vaspressors (dopamine) persisted after this initial resuscitation, anti-TNF antibody was administered. The clinical data of the ten patients are presented in Table 1. Three patients (Nos. 3, 8 and 10) had positive blood cultures. Adult respiratory distress syndrome was defined as severe hypoxemia (PaO\textsubscript{2} less than 70 mm Hg under a FiO\textsubscript{2} of 0.4) and bilateral pulmonary infiltrates on the roentgenogram, in the absence of left ventricular failure.

Reversal of shock, as defined by the absence of a requirement for vaspressors (eg, dopamine <5 µg/kg/min) and a decrease in blood lactate levels less than 2.5 mEq/L, occurred in five patients.

The anti-TNF antibody used (CB0006, Celltech) is a murine monoclonal antibody raised against recombinant human TNF. Vials containing 70 mg of antibody in 20 ml isotonic solution in 50 mEq sodium chloride buffered saline solution were stored at 4°C and rewarmed at ambient temperature for 30 min prior to administration.

Each patient was monitored with a PA catheter (Swan Ganz catheter 93A-431H-7F, Baxter Healthcare, Irvine, Cal) and an arterial catheter. For each patient, baseline hemodynamic, metabolic and immunologic measurements included recording of heart rate, arterial pressure and PA pressures, cardiac output and arterial and mixed venous blood gas levels, together with arterial lactate concentrations and cytokine levels. For each parameter, the values were taken from the average of two baseline measurements taken 15 min apart. The anti-TNF antibody was then slowly administered at a dose of 2 mg/kg over approximately 5 min. The same measurements were repeated after 1, 2, 4 and 8 h. The study protocol did not influence the routine management of the patient, except that changes in therapy (especially adrenergic therapy and ventilatory conditions) were avoided during the 8 h following the administration of anti-TNF antibody.

Measurements of heart rate and intravascular pressures were

**Table 1—Clinical Data of the Ten Patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Underlying Disease</th>
<th>Source</th>
<th>Microorganism</th>
<th>Time of Anti-TNF Antibody Admin*</th>
<th>Mechanical Ventilation</th>
<th>Adrenergic Agents (Type and Dose in µg/kg/m²)</th>
<th>Reversal of Shock (Days)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M 63</td>
<td>COPD/pneumonia/ ischemic heart disease</td>
<td>Lungs</td>
<td>Haemophilus influenzae</td>
<td>2 h</td>
<td>No</td>
<td>+ DOP 12</td>
<td>2</td>
<td>Died after 17 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>2 M 62</td>
<td>Infected aortic prosthesis</td>
<td>Aortic prosthesis</td>
<td>Atypical mycobacteria</td>
<td>6 h</td>
<td>No</td>
<td>+ DOP 14</td>
<td>1</td>
<td>Survived</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 M 69</td>
<td>Peritonitis (cecal perforation) endocarditis</td>
<td>Abdomen</td>
<td>Staphylococcus aureus</td>
<td>6 h</td>
<td>Yes</td>
<td>+ DOP 16</td>
<td>2</td>
<td>Died after 5 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>4 F 34</td>
<td>Chronic pancreatitis</td>
<td>Lungs</td>
<td>Escherichia coli</td>
<td>12 h</td>
<td>Yes</td>
<td>+ DOP 10</td>
<td>No</td>
<td>Died after 2 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>5 M 56</td>
<td>Cirrhosis/trauama</td>
<td>Lungs</td>
<td>Serratia mar.</td>
<td>12 h</td>
<td>Yes</td>
<td>+ DOP 15</td>
<td>No</td>
<td>Died after 7 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>6 F 68</td>
<td>Asthma/pneumonia</td>
<td>Lungs</td>
<td>H influenzae</td>
<td>15 h</td>
<td>No</td>
<td>+ DOP 20/DOP 10</td>
<td>5</td>
<td>Died after 12 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>7 M 75</td>
<td>Strangulated inguinal hernia/ aspiration pneumonia</td>
<td>Bowel</td>
<td>Unknown</td>
<td>16 h</td>
<td>Yes</td>
<td>+ DOP 10</td>
<td>2</td>
<td>Died after 8 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>8 M 65</td>
<td>Pneumonia</td>
<td>Lungs</td>
<td>S aureus</td>
<td>18 h</td>
<td>No</td>
<td>+ DOP 20/DOP 14</td>
<td>No</td>
<td>Died after 7 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>9 M 46</td>
<td>Peritonitis (perforated ulcer with abscess) lung cancer</td>
<td>Abdomen</td>
<td>Enterobacter cloacae</td>
<td>18 h</td>
<td>Yes</td>
<td>+ DOP 20/DOP 8</td>
<td>No</td>
<td>Died after 9 days</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>10 M 57</td>
<td>Esophagus resection for cancer</td>
<td>Mediastinum</td>
<td>S aureus</td>
<td>24 h</td>
<td>No</td>
<td>+ DOP 4/DOP 10</td>
<td>No</td>
<td>Died after 1 day</td>
<td>Survived</td>
<td></td>
</tr>
</tbody>
</table>

*Time of anti-TNF antibody administration since onset of shock (in hours).
†DOP = dopamine; DOB = dobutamine.
obtained at end-expiration and stored electronically using an IBM computer. Cardiac output was measured by the thermodilution technique (computer 9500 A, Baxter Healthcare), using three to five injections of 10 ml of cold (≤1°C) DSW and a closed system (CO set, Baxter Healthcare). Arterial and mixed venous blood gas levels were determined with an automated analyzer and hemoglobin saturations were measured by an oximeter (Radiometer, Copenhagen, Denmark). Blood lactate levels were determined enzymatically (Hitachi analyzer, Japan). Derived parameters were calculated using standard formulas.

Cytokine determinations included TNF alpha, IL-1 beta, IL-2, IL-6 and IF-G. Serum concentrations of TNF, IL-1 and IF-G were determined using IRMA (IRE, Medgenix, Fleurus, Belgium). Briefly, the IRMA is based on coated-tube separation and the oligoclonal system, in which several monoclonal antibodies directed against distinct epitopes of TNF, IL-1 or IF-G are used. Standards of recombinant TNF and IL-1 were used at concentrations of 0 to 5 ng/ml. Standards of recombinant IF-G were used at concentrations of 0 to 90 IU/ml; 0.2 ml of standards and samples and 0.05 ml of anti-TNF, anti-IL-1 or anti-IF-G were mixed and incubated for 20 h at room temperature. With the contents aspirated, the tubes were washed with 2 ml Tween 20 and counted for 1 min in a gamma scintillation counter.

Serum concentrations of IL-2 were measured by RIA (IRE, Medgenix). Standards consisting of recombinant IL-2 were used at concentrations of 0 to 10 ng/ml; 0.1 ml of standards and samples mixed with 0.1 ml of rabbit antiserum were incubated at 24 h at 4°C. After adding 0.1 ml of IL-2, the tubes were incubated an additional 4 h. Anti-rabbit gamma-globulin antiserum mixed with polyethylene glycol was added to precipitate the 1:1 IL-2 antibody complex. After centrifugation, bound radioactivity was determined over 5 min in a gamma scintillation counter. Inter-assay variation was less than 15 percent.

Serum IL-6 activity was assayed using an IL-6-dependent mouse hybridoma TTD1 cultivated in flat-bottomed microtiter plates containing 2,000 cells per well in the presence of serial dilutions of supernatant. After four days of culture, the number of surviving cells was determined by a colorimetric assay for hexose amidase, as previously described. Hybridoma growth factor/IL-6 activity was expressed in units per milliliter defined as the dilution giving half maximal proliferation of TTD1 cells. One unit corresponds to approximately 5 pg/ml of IL-6; TTD1 cells do not respond to TNF,

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>+1 h</th>
<th>+2 h</th>
<th>+4 h</th>
<th>+8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>37.8±1.1</td>
<td>37.8±1.1</td>
<td>37.9±1.1</td>
<td>37.9±1.0</td>
<td>37.8±1.0</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>60±10</td>
<td>73±10</td>
<td>73±8</td>
<td>73±9</td>
<td>71±11</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
<td>24.5±6.3</td>
<td>24.6±8.1</td>
<td>24.1±6.8</td>
<td>25.4±5.3</td>
<td>23.8±6.1</td>
</tr>
<tr>
<td>PO2, mm Hg</td>
<td>13.1±2.8</td>
<td>13.4±3.4</td>
<td>12.5±2.1</td>
<td>13.8±2.6</td>
<td>11.9±5.0</td>
</tr>
<tr>
<td>Pao2, mm Hg</td>
<td>11.0±4.6</td>
<td>11.3±4.0</td>
<td>10.5±3.3</td>
<td>12.0±2.8</td>
<td>10.2±4.1</td>
</tr>
<tr>
<td>Cl, L/min/m²</td>
<td>4.3±0.9</td>
<td>4.3±0.9</td>
<td>4.4±1.0</td>
<td>4.1±1.0</td>
<td>4.3±1.2</td>
</tr>
<tr>
<td>SI, m³/m²</td>
<td>35.8±8.7</td>
<td>37.4±8.9</td>
<td>38.2±9.9</td>
<td>36.4±10.0</td>
<td>37.3±12.6</td>
</tr>
<tr>
<td>SVRI, dynes·cm⁻⁵·m⁻²</td>
<td>1,169±442</td>
<td>1,193±351</td>
<td>1,207±322</td>
<td>1,283±377</td>
<td>1,211±317</td>
</tr>
<tr>
<td>PVRI, dynes·cm⁻⁵·m⁻²</td>
<td>246±122</td>
<td>228±143</td>
<td>236±141</td>
<td>258±159</td>
<td>249±125</td>
</tr>
<tr>
<td>RVSWI, g/m²</td>
<td>6.1±1.5</td>
<td>6.3±2.2</td>
<td>6.7±2.2</td>
<td>6.2±1.3</td>
<td>6.5±2.6</td>
</tr>
<tr>
<td>PaO₂/FiO₂, mm Hg</td>
<td>174±45</td>
<td>179±110</td>
<td>174±89</td>
<td>193±96</td>
<td>157±94</td>
</tr>
<tr>
<td>Do₂, ml/min/m²</td>
<td>587±151</td>
<td>590±151</td>
<td>590±179</td>
<td>548±157</td>
<td>562±185</td>
</tr>
<tr>
<td>VO₂, ml/min/m²</td>
<td>151±33</td>
<td>147±34</td>
<td>153±32</td>
<td>143±37</td>
<td>151±27</td>
</tr>
<tr>
<td>Lactate, mEq/L</td>
<td>2.8±1.1</td>
<td>2.8±1.2</td>
<td>2.9±0.8</td>
<td>3.0±1.2</td>
<td>2.9±0.9</td>
</tr>
<tr>
<td>TNF, pg/ml</td>
<td>55±45</td>
<td>48±39</td>
<td>62±60</td>
<td>53±77</td>
<td>80±79</td>
</tr>
<tr>
<td>IL-1, pg/ml</td>
<td>27±12</td>
<td>29±14</td>
<td>32±10</td>
<td>32±13</td>
<td>26±15</td>
</tr>
<tr>
<td>IL-2, U/ml</td>
<td>0.26±0.49</td>
<td>0.30±0.71</td>
<td>0.21±0.36</td>
<td>0.14±0.31</td>
<td>0.07±0.22</td>
</tr>
<tr>
<td>IL-6, U/ml</td>
<td>4,164±3,532</td>
<td>3,334±4,423</td>
<td>3,823±5,904</td>
<td>2,062±4,016</td>
<td>1,614±2,927</td>
</tr>
<tr>
<td>IF-G, U/ml</td>
<td>1.7±4.7</td>
<td>2.0±5.6</td>
<td>2.0±5.6</td>
<td>2.2±6.3</td>
<td>2.1±6.3</td>
</tr>
</tbody>
</table>

**Figure 1.** Time course of the heart rate during the first 8 h following administration of anti-TNF antibody. (mean ± SD). *Astertak = p<0.05.

IL-1, IL-2, IF-alpha, IF-beta, IF-G, nor to any of the known colony-stimulating factors other than IL-6. The biologic activity of IL-6 samples was completely neutralized by adding monospecific rabbit polyclonal anti-recombinant human IL-6 antibodies to the test samples. The inter-assay variability (±30 percent) was corrected by the use of an internal standard. Each sample was tested at least four times. To eliminate inhibitory effects present in undiluted sera and to determine the lower limit of detection of the assay, IL-6 activity in sera was measured without and after addition of 100 units of recombinant IL-6.

Statistical evaluation included an analysis of variance for repeated measurements and for posthoc comparison a Newmann-Keuls test. A F value corresponding to a p value of less than 0.05 was considered statistically significant. Data are reported as mean ± SD.

**RESULTS**

Anti-TNF antibody administration was well tolerated in all patients and no decrease in arterial pressure or any other sign of intolerance was noted. This administration was followed by a reduction in heart
rate (from 122 ± 10 to 113 ± 10 beats per minute at 4 h, p<0.01 [Fig 1]) which was associated with trends toward increases in stroke volume and arterial pressure (Table 2), but this did not reach statistical
significance. These changes were associated with a 19 percent increase in LVSWI (from 26.5 ± 5.6 to 31.5 ± 10.5 g/m² at 2 h, p<0.05 [Fig 2]). Cardiac filling pressures remained unchanged.

The PaO₂/FIO₂ ratio transiently increased in six of the ten patients between 4 and 8 h following anti-TNF antibody administration (Fig 3).

The TNF was recovered in the blood of all patients with levels ranging from 5 to 135 pg/ml and remained detectable during the study (Table 2). The IL-6 levels were also elevated. However, only low levels of IL-1, IL-2 and IF-N were detected throughout the study (Table 2).

**Discussion**

Release of TNF from macrophages can contribute to the development of the hemodynamic and the metabolic alterations characterizing septic shock, and TNF antibodies could, therefore, be of potential therapeutic value. Anti-TNF antibodies have been shown to afford protection in various animal models of septic shock. In some of these studies, they were effective only when administered prior to the septic challenge. In others, efficacy was retained when the antibodies were administered 30 min after the infusion of live bacteria into baboons or in a less abrupt type of infection induced by Pseudomonas bacteria in neutropenic rats. It could be hoped therefore that the administration of anti-TNF antibodies could still be effective once the septic shock already is established. In short-term animal experiments, the release of TNF in the blood is transient, lasting not more than a few hours and is similar to that seen when endotoxin is administered to human volunteers. In contrast to these experimental conditions, TNF release could be more prolonged in patients with septic shock.

Indeed, recent studies on patients with septic shock have indicated that blood TNF levels could remain elevated for 24 h, 48 h to 72 h, and even ten days. Moreover, the TNF levels remained more elevated in those patients who were more likely to die, suggesting that an ongoing immunologic activation could be involved in this fatal course. These observations, however, must be mitigated by the documented phenomenon of tolerance of TNF, which suggests that these persisting elevated levels might not maintain the same biologic effects. Nevertheless, there could be a critical period during which anti-TNF antibodies could be effective in limiting the complications of the severe sepsis.

The primary goal of the present study was to evaluate the acute effects of an anti-TNF antibody in a limited group of patients with well-documented septic shock. Since the study design was thus restricted by the small number of patients and the lack of a control group, the data must be considered as preliminary. The interesting finding was that administration of anti-TNF antibody resulted in a significant increase in LVSW. This strongly suggested, in the absence of change in cardiac filling pressures, an improvement in myocardial function. Various studies have demonstrated a decrease in myocardial contractility in septic shock, even when cardiac output was not decreased. The release of myocardial depressant substances probably is implicated in this phenomenon. Natanson et al observed that TNF administration in dogs resulted in a left ventricular depression associated with a reduction in left ventricular ejection fraction. A recent experimental study in dogs indicates that TNF can reduce Emax, a relatively good index of
myocardial contractility. Other cytokines acting synergistically with TNF also might be involved in the myocardial depression. These findings support the implication that TNF is a myocardial depressant substance in septic shock, either directly or by triggering the release of other substances. Recently Levine et al reported that TNF levels are commonly elevated in patients with heart failure and especially in those with more advanced heart failure as reflected by a stronger activation of the renin-angiotensin system and a greater impairment of renal function. A possible role of TNF in the myocardial impairment of these patients is an intriguing hypothesis.

Tumor necrosis factor also has been implicated in the development of acute respiratory failure. First, its experimental administration in animals results in a permeability type of pulmonary edema. Second, an alteration of the respiratory function has been observed in patients treated with TNF. Third, the bronchoalveolar lavage fluid of patients with acute respiratory failure has revealed the presence of TNF. Although the present study did not focus on patients with respiratory failure, the administration of anti-TNF antibody appeared to improve oxygenation of six of the ten patients studied. These changes were, however, transient.

The TNF concentrations in the serum remained elevated following the anti-TNF antibody administration. The anti-TNF antibody activity against human TNF at the administered dose of 2 mg/kg has been established (data on file, Celltech), but the IRMA used was likely also to detect the TNF:anti-TNF antibody complexes.

In view of the early release of TNF in septic shock and the lack of effect of anti-TNF antibody when not administered very early in experimental studies, it may be important to administer the antibodies as early as possible during the course of the septic shock. In the present study, we desired first to collect complete hemodynamic data and second to achieve relative hemodynamic stability before administration of the anti-TNF antibody. Hence, we included patients with protracted septic shock who required invasive hemodynamic monitoring after initial resuscitation. In these severe conditions, the reversal of shock in only five of the ten patients could be expected but is inconclusive in the absence of control data.

The second goal of our pilot study was to rule out the development of side effects during the administration of the murine antibody in patients with septic shock. The lack of adverse reactions in these patients and in another preliminary study indicates that the anti-TNF antibody administration is relatively safe in septic shock patients.

ACKNOWLEDGMENTS: The authors are thankful to Denis O'Shaughnessy, Ph.D., and Simon Harris, Ph.D., from Celltech Limited (Slough, United Kingdom) for their valuable support, to Jean Content, M.D., Ph.D. (Parteu Institute of Brabant, Belgium), for the determination of IL-6 levels, and to Daniel De Backer, M.D., and Alain Kenter, M.D., for their help in the data collection.

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