The Role of Tumor Necrosis Factor in Endotoxic Shock*

SABRINA M. HEIDEMANN, M.D.
and ASHOK P. SARNAIK, M.D.

Department of Pediatrics,
Children's Hospital of Michigan
Wayne State University School of Medicine,
Detroit, MI 48201.

ABSTRACT

Tumor necrosis factor (TNF) has been implicated in hemodynamic changes of endotoxic shock. The temporal relationship of hypotension and TNF release in endotoxemia was studied. Carotid arteries of five intubated rats were cannulated and Escherichia coli 0127:B8 lipopolysaccharide (LPS) was infused over 10 seconds. Arterial blood pressure (ABP), heart rate, and plasma TNF concentrations were measured at 0, 5, 15, 30, and 60 mins. Five to 15 mins after LPS, there was a marked decline in ABP (146 ± 23 vs 57 ± 5 mm Hg, p < 0.005), without a significant rise in TNF. The heart rate did not change. From 15 to 60 mins, there was a rise in TNF concentrations (523 ± 333 vs 5783 ± 629 pg/ml, p < 0.005) while the same degree of hypotension persisted. It is concluded that early hypotension after acute endotoxemia is not dependent on TNF alone. However, TNF may play a role in sustaining hypotension after endotoxemia.

Introduction

The mortality of patients with septic shock has remained unchanged over the last several decades in spite of improved antibiotics and surgical intervention. Recent studies have shown that inflammatory mediators play an important role in the development of septic and endotoxic shock. Endotoxin incites the production of tumor necrosis factor (TNF) which leads to the activation of a cascade of inflammatory mediators, which may result in circulatory collapse, multiple organ failure and death. Understanding of the causal and temporal relationship of the release of inflammatory mediators and pathophysiologic events is important in devising therapeutic strategies aimed at preventing or treating manifestations of endotoxemia. The objective of this study is to determine the relationship of TNF and the development of early shock in endotoxemic rats.

Methods

This study was approved by the animal care committee at William Beaumont
Hospital and performed in agreement with the guidelines from the National Institutes of Health for the care and use of animals in research.

**ANIMAL PREPARATION**

Five female Wistar rats* weighing (250 to 300 g) were anesthetized and sedated with 60 mg/kg intramuscular injections of ketamine hydrochloride, and 5 mg/kg xylazine, respectively. After paralysis with 2 mg/kg intramuscular vecuronium bromide, orotracheal intubation was performed with previously beveled 16G angiocatheters. The animals were oxygenated and ventilated using a Harvard rodent ventilator† with settings of 100 percent oxygen, tidal volume of 10 mL/kg, rate of 90 BPM, and an end expiratory pressure of zero. Rectal temperature was continuously monitored by a temperature probe‡ and maintained by an external heating source within 0.5°C of the baseline temperature. The right carotid artery was isolated by dissection, and cannulated with an 18G, 1.5 inch angiocatheter.

**PHYSIOLOGIC MEASUREMENTS**

Continuous electrocardiogram (ECG) monitoring and arterial pressure measurements were performed.§ Heart rate and blood pressure were recorded at times of 0, 5, 15, 30, and 60 minutes.

**EXPERIMENTAL PREPARATION**

A solution of *Escherichia coli* O:127 B8 lipopolysaccharide¶ was prepared by dissolving 10 mg endotoxin in 1 mL 0.9 percent sodium chloride. At time 0, 10 mg/kg endotoxin was infused into the carotid artery over 10 seconds.

**SPECIMEN COLLECTION**

Blood (0.5 mL) was removed for the measurement of TNF just prior to endotoxin administration, and at 5, 15, 30, and 60 minutes following the infusion of endotoxin. The blood was collected in a sterile clot tube for TNF. The samples were immediately centrifuged and the plasma was stored at −70°C until analysis. All samples were obtained through the arterial line and replaced with an equal amount of hetastarch.

**TERMINATION OF EXPERIMENT**

All animals were sacrificed by an arterial infusion of 120 mg/kg pentobarbitol at 60 minutes after endotoxin infusion.

**ANALYSIS OF INFLAMMATORY MEDIATORS**

Tumor necrosis factor activity was determined by a cytolytic assay using actinomycin D-treated mouse L929 cells. Mouse L929 cells¶ were maintained in Eagle's Minimum Essential Medium,** 10 percent bovine serum,†† 5000 IU/mL penicillin and 5000 μg/mL streptomycin. The cells were trypsinized, reconstituted with media, counted, placed in 24 well plates and incubated for 24 hours. Actinomycin D (2 μg/mL) was added to the cells and reincubated for 30 minutes. Mouse TNF‡‡ standards or plasma and ultrafiltrate samples were added 30 min following the addition of actinomycin D. The plates were incubated for an addi-
tional 18 to 20 hours, and the cells were then counted. A standard curve was determined by plotting the log of mouse TNF standards against the corresponding cell counts. Concentrations of TNF in plasma and ultrafiltrate samples were determined by interpolation of cell counts from the standard curve.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney test to analyze continuous variables. All values are reported as mean ± standard error of the mean.

Results

The blood pressure values and TNF concentrations at various time intervals are presented in figure 1. Five minutes after the administration of endotoxin, the blood pressure started to decline with the nadir reached at 15 minutes. The blood pressure at 15 minutes was significantly lower than that prior to administration of endotoxin (146 ± 23 vs 57 ± 5 mm Hg, p < 0.005). There was no significant change in the TNF concentrations at 5 and 15 minutes compared to the baseline values.

From 15 to 60 minutes, there was a marked increase in TNF concentration (523 ± 333 vs 5783 ± 629 ng/ml, p < 0.005) while the same degree of hypotension persisted. The heart rate remained unchanged throughout the experiment.

Discussion

Gram negative bacteria trigger the development of shock by the release of endotoxin. Endotoxin is bound to a carrier protein which is produced in the liver. The endotoxin-carrier protein complex induces monocytes and macrophages to produce and release TNF. Concentrations of TNF peak 90 to 120

Figure 1. Relationship of tumor necrosis factor (TNF) levels and blood pressure (BP) other administration of endotoxin. *p < 0.005, changes in BP from 5 to 15 minutes after endotoxin; #p < 0.005, changes in TNF levels, 15 to 60 minutes after endotoxin.
minutes after endotoxin release and become undetectable at 4 to 6 hours. Subsequently, the TNF gene is down-regulated, and the macrophages remain refractory to endotoxin for 12 to 24 hours.\(^3\)

Tumor necrosis factor has been implicated as the major mediator of hemodynamic alterations in endotoxic shock. Administration of TNF in rats has been shown to result in cardiovascular instability which is identical to that observed in endotoxemia.\(^4\) Plasma TNF concentrations are elevated at 15 and 30 minutes after the infusion of endotoxin.\(^5,6\) However, the onset of hypotension following endotoxemia has not been correlated with TNF concentrations.

In our study, the precipitous decline in blood pressure observed between 5 and 15 minutes was not temporally related to a significant rise in TNF. Thus, the initiation of hypotension after acute endotoxemia does not appear to be solely dependent on the concentration of TNF in blood. It may be argued that the early hypotension is mediated through TNF produced in the heart and peripheral vasculature. This is, however, unlikely since the heart and the blood vessels are not rich in monocytes, macrophages, or other TNF producing cells. It is also unlikely that although statistically insignificant, the small rise in plasma TNF levels at 5 and 15 minutes was responsible for early hypotension, since the marked rise in TNF observed subsequently was not associated with further decline in blood pressure.

The mechanism of initiation of hypotension in endotoxemia remains to be established. Several inflammatory mediators have been shown to cause hemodynamic alterations observed in septic shock. The concentration of thromboxane A\(_2\) (TxA\(_2\)) has been shown to rise within 10 minutes in rats receiving an intravenous injection of *Salmonella enteritidis*.\(^7\) The infusion of *E. Coli* 0111-B4 endotoxin resulted in a peak concentration of platelet activating factor (PAF) within 2 to 5 minutes of injection.\(^8\) The TNF has been shown to stimulate the release of both TxA\(_2\) and platelet-activating factor (PAF). It is possible that these mediators released in response to a small rise in TNF, along with the direct action of endotoxin, are responsible for initiating hypotension after endotoxemia. Better understanding of the mechanism of initiation of hypotension may lead to therapies to prevent circulatory shock, multiple organ failure, and death in endotoxic shock.

References