Superantigen-induced multiple organ dysfunction in a toxin-concentration-controlled and sequential parameter-monitored swine sepsis model

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Objective: In order to examine the biological activity of low-dose and continuously infused superantigen, and to establish a superantigen-induced multiple organ dysfunction animal model, several pathophysiological parameters were sequentially monitored in a toxin-concentration-controlled pig model.

Methods: Anesthetized, mechanically ventilated and Swan–Ganz thermodilution catheter-inserted pigs were treated with toxic shock syndrome toxin-1 (TSST-1) by infusion at 2 mg/kg/h for 5 h. Monitoring was performed for both the infusion period and a subsequent 1-h post-infusion period.

Results: The serum concentration of TSST-1 was controlled so as to elevate it to a level over 1000 pg/mL within 1 h of initiation of infusion, and then gradually increased further and reached a plateau of about 2500 pg/mL at 4 h after initiation. The animals showed a significant increase in cardiac output, the intrapulmonary arteriovenous shunt ratio, and infiltration of white blood cells into the lung. Although the observed increase in pulmonary vascular resistance was not statistically significant, it did correlate with the reduction in white blood cell counts.

KEYWORDS
Acute respiratory distress syndrome; Animal model; Multiple organ failure; Superantigen
Introduction

Sepsis is a principal cause of death in critical care units worldwide. In particular, Gram-positive sepsis accounts for 50% of serious sepsis cases. Despite considerable progress in understanding the central role of lipopolysaccharide (LPS) in the pathogenesis of Gram-negative septic shock, the pathogenesis of Gram-positive bacterial sepsis remains only poorly understood. Accumulating evidence suggests that the pathogenesis of Gram-positive sepsis is different from that of Gram-negative sepsis.

Unlike the case with Gram-negative sepsis, it is believed that endotoxin is not an essential mediator in the toxic shock syndrome (TSS) associated with Gram-positive bacterial infections. Although several bacterial products, such as peptidoglycan, lipoteichoic acid, and superantigens, have been postulated as pathogenic toxins responsible for Gram-positive sepsis, no conclusive evidence has yet been obtained.

Among these bacterial products, toxic shock syndrome toxin-1 (TSST-1), a superantigenic protein produced by Staphylococcus aureus, is the most extensively studied to date in terms of its pathogenic properties, and it has been studied in a variety of animal models. In animal studies, it has been shown that TSST-1 alone at relatively low doses can induce TSS-like symptoms indicative of circulatory and respiratory failure, but exerts lethal effects only at relatively large doses unless endotoxin is co-administered. Furthermore, TSST-1 has been shown to significantly enhance the lethal effects of endotoxin. These findings suggest that the primary role of TSST-1 in the pathogenesis of Gram-positive sepsis is not an induction of refractory systemic hypotension, but rather, the development of multiple organ dysfunction as the precipitating event to hypotension. Thus, if this suggestion were to be borne out, it would be more important to focus on the biological processes which trigger the multiple organ dysfunction seen prior to the ultimately lethal event in order to gain a better understanding of the pathogenesis of, and potential therapeutic intervention in, Gram-positive sepsis.

In this vein, it is noteworthy that apparent species differences can be observed in terms of the susceptibility to develop TSS-like symptoms upon artificial infection with Staphylococcus aureus or intravenous doses of TSST-1. Rabbits and pigs are highly susceptible species, while mice and monkeys do not exhibit any significant TSS-like symptoms. Accordingly, rabbits are the most widely used model animals for Gram-positive bacterial sepsis. Rabbits, however, are relatively small in size, and their circulating blood volume is scant. Thus, it is technically too difficult to perform repeated blood sampling from the same rabbits without having a negative effect on their physical well-being. A further negative feature is that special devices are required for proper time-course monitoring of the rabbits’ cardiac and respiratory functions.

Since miniature pigs have been shown to be sensitive to TSST-1, and superantigen-induced expansion is inducible in pig T-helper cells, and also that cytokine is inducible by superantigens from the peripheral blood mononuclear cells of normal pigs, conventional pigs were selected as the model animals, most especially because the frequent repeated blood sampling required for the time-course monitoring of hematological and biochemical changes would affect the animals’ physiological condition only to a negligible extent. In addition, pigs are sufficiently large that it is possible to use conventional clinical devices used for the monitoring of such parameters in human patients to monitor the animals’ cardiac and respiratory functions.

The present study reports a superantigen-induced multiple organ dysfunction model developed in conventional pigs by continuous infusion of TSST-1. The TSST-1 concentration in the blood was controlled during the investigation. Time-course changes in cardiac and respiratory function are described in relation to changes in hematologic and blood chemistry in these model animals.

Materials and methods

Toxins

Toxic shock syndrome toxin-1 (TSST-1) was chromatographically purified from the culture supernatants of the Staphylococcus aureus FRI1169 strain, as described by Igarashi et al. The purified prepara-
tion gave a single band on SDS gel electrophoresis with coomassie brilliant blue staining. The purified TSST-1 had undetectable levels of lipopolysaccharide (LPS) (limits of detection <5 pg/mg TSST-1), as judged by limulus assay.

TSST-1 was diluted to give a concentration of 10 μg/mL, with physiological saline supplemented with 5% swine auto-serum. The solution was filtered through a 0.22-μm filter before use in the infusion treatment of animals.

Animals

Ten conventional Landrace male pigs weighing 25—35 kg (age 2—3 months) were obtained from a local supplier. The anti-TSST-1 IgG antibody and anti-TSST-1 IgM antibody levels were below the detection limit, as judged by ELISA. LPS in the blood stream was also below the detection limit (<5 pg/mL). These animals were divided into two groups, and a statistical difference in mean body weight was not observed, 29.5 ± 4.6 (TSST-1 infused group) versus 28.6 ± 2.8 (control group).

Treatment of animals

The study protocol was reviewed and approved by the institutional animal research committee of both the Tokyo Medical College and Specialty Material Research Laboratories of Toray Industries, Inc.

Surgical procedure

Each animal was anesthetized by intramuscular administration of ketamine (17 mg/kg) and atropine sulfate (0.03 mg/kg). The animal was maintained under anesthesia by mechanical ventilation with an oxygen-enriched air mixture containing 1% isoflurane (FIO2 = 0.80) at a tidal volume of 10 mL/kg and a respiratory rate of 15 strokes/min by means of a Servo Model 900D ventilator (Siemens—Elema, Stockholm, Sweden). The animal was also maintained under conditions of a constant body fluid balance by continuous infusion of physiological saline at 3 mL/kg/h from the epiotic vein. The right carotid artery was cannulated for monitoring arterial blood pressure and heart rate (HR) and for the sampling of the arterial blood samples, which were subjected to blood gas analysis, hematology, and blood chemistry. One of the jugular veins was cannulated with a Swan—Ganz Opticath thermodilution catheter (Abbott Inc., Abbott Park, IL) for monitoring cardiac output. The oxygen saturation of mixed venous blood (SvO2) was measured with an Oxymetrax 3 (Abbott Inc., Redwood City, CA). The monitoring of the pulmonary arterial pressure, central venous pressure, and pulmonary capillary wedge pressure was carried out with a Model 54 S analyzer (Hewlett Packard, Palo Alto, CA), and the mixed venous blood samples were subjected to blood gas analysis. The infusion port of the thermodilution catheter was connected to an infusion line unit having two injection ports: one for continuous infusion of physiological saline at 3 mL/kg/h to maintain the fluid balance in the animal and the other for continuous infusion of TSST-1. After the connection was completed, the animal was maintained under continuous infusion of physiological saline via the thermodilution catheter rather than via the epiotic vein, and mechanical ventilation was carried out with an air mixture (FIO2 = 0.21) containing 1% isoflurane for recovery from the effects of the surgery.

Cardiac and respiratory monitoring indicated that all animals undergoing the surgical operation described above recovered from the effects of surgery within 30 min of completion. Thus, the normal baseline levels of the parameters were recorded at 30 min after completion of surgery: HR = 100 ± 15 beats/min, mean arterial pressure (MAP) = 90 ± 10 mmHg, and arterial oxygen partial pressure (PaO2) = 90 ± 10 torr [12 ± 1.3 kPa], and SvO2 = 70 ± 10%.

After the baseline values of the cardiac and respiratory function parameters were recorded, animals were divided into two groups, the toxin infusion group and the control group. In the toxin infusion group, TSST-1 solution (10 μg/mL) was infused and in the control group, saline was infused both at 0.2 mL/kg/h for 5 h through the infusion tube of the thermodilution catheter. The cardiac and respiratory function parameters were monitored continuously for 6 h from the initiation of the TSST-1 continuous infusion. After 6 h, the arterial catheter and thermodilution catheter were withdrawn, the wounds were closed surgically, and the animals were sacrificed with a lethal dose of sodium pentobarbital.

Measurements

Serum TSST-1 levels were determined by enzyme-linked immunosorbent assay (ELISA) with a detection limit of 10 pg/mL, as previously reported.26 Serum anti-TSST-1 IgG or IgM antibodies were determined by ELISA. Serum was diluted 1000-fold to avoid non-specific reactions and then applied to a TSST-1 immobilized ELISA plate. Then anti-TSST-1 antibodies were detected by anti-swine IgG (KPL Inc., Gaithersburg, MD) or IgM (Serotec Ltd, Oxford, United Kingdom) antibodies. LPS was measured with a commercially available limulus assay kit (Wako...
Pure Chemicals, Osaka, Japan). Cytokines were quantitated with a colorimetric ELISA kit (Endogen Inc., Woburn, MA). Blood gas analysis was performed on an ABL-520 blood gas analyzer (Radiometer, Copenhagen, Denmark), and the measured values were standardized for body temperature. Hematological parameters and blood chemistry parameters were determined by standard methods at Tokyo Medical School, Hachioji Medical Center.

**Histopathology**

For histopathological examination, two pigs (one TSST-1 infused pig and one control pig) were subjected to euthanasia and necropsy 6 h after the initiation of the TSST-1 (or saline) infusion. The lungs were removed from the body of each animal. The tissues were cut into small sections, fixed in 10% neutral buffered formalin, and embedded in paraffin. Thin sections of the tissues were prepared and stained with hematoxylin and eosin for microscopic examination.

**Statistical analysis**

All data are presented as the mean ± standard deviation of five animals in each group. For each parameter, statistical significance of the difference between the baseline value and the value at 6 h after the initiation of the TSST-1 infusion was tested by paired t-test, and statistical significance of the difference between the TSST-1 infused group and the control group was tested by Student’s t-test using the parameters at 6 h. In both comparisons, significant differences were tested at a significance level of 0.05. Correlations between the parameters were tested by regression analysis at a significance level of \( p < 0.05 \).

**Results**

When conventional pigs were treated with TSST-1 alone by continuous infusion at 2 \( \mu \text{g/kg/h} \) for 5 h, no deaths were observed either during the 5-h TSST-1 infusion period or during the 1-h post-infusion observation period. In addition, none of the animals exhibited thrombocytopenia, rash, diarrhea, or vomiting.

Serum TSST-1 rapidly increased in these animals during the initial 1-h period of the TSST-1 infusion and appeared to reach a plateau of 2639 ± 846 pg/mL at 4 h after initiation (Figure 1A). Serum TSST-1 decreased on completion of the infusion, and the level was determined to be 1244 ± 419 pg/mL at 1 h after completion.

Serum gamma interferon (IFN-γ) became detectable at 2 h after initiation and reached a maximum level of 217 ± 135 pg/mL at completion of the infusion (Figure 1B). Serum IFN-γ, however, did not exhibit any significant decline at 1 h after completion.

Neither endotoxin nor tumor necrosis factor alpha (TNF-α) were detected in the arterial blood from any of the animals at any time point during the 5-h infusion period or the subsequent 1-h observation period.

When animals were treated with physiological saline in place of TSST-1, neither toxins nor cytokines were detected in the serum at any time point during the 5-h infusion period or the subsequent 1-h observation period. In these animals, no significant changes were detected in any of the hematological and biochemical parameters, except for a slight increase of blood urea nitrogen, as described below (Figures 2–5, open circle).

**Cardiac function**

The changes in cardiac function parameters in pigs treated with continuous infusion of TSST-1 are pre-
Figure 2  Time-course changes in cardiac parameters during the 5-h infusion of toxic shock syndrome toxin-1 (TSST-1) and subsequent 1-h post-infusion observation period. (●): TSST-1 infused group, (○): control group. HR: heart rate; CO: cardiac output; SV: stroke volume; MAP: mean arterial pressure. Data are expressed as mean ± SD. *: p < 0.05 (TSST-1 infused group versus control group at 6 h by Student t-test); **: p < 0.05 (0 h versus 6 h in TSST-1 infused group by paired t-test).

Figure 3  Time-course changes in respiratory parameters during the 5-h infusion of toxic shock syndrome toxin-1 (TSST-1) and subsequent 1-h post-infusion observation period. (●): TSST-1 infused group, (○): control group. Qs/Qt: intrapulmonary arteriovenous shunt ratio; DO2: oxygen delivery; PaO2: arterial oxygen pressure; SaO2: arterial oxygen saturation; VO2: systemic oxygen consumption. Data are expressed as mean ± SD. *: p < 0.05 (TSST-1 infused group versus control group at 6 h by Student t-test); **: p < 0.05 (0 h versus 6 h in TSST-1 infused group by paired t-test).
significantly higher than the baseline values or the control group. No other parameters, such as body temperature, pulmonary arterial pressure, pulmonary capillary wedge pressure, central venous pressure, left ventricle stroke work, right ventricle stroke work, pulmonary vascular resistance, or systemic vascular resistance exhibited any significant changes (data not shown).

Figure 4  Time-course changes in oxygen metabolism parameters during the 5-h infusion of toxic shock syndrome toxin-1 (TSST-1) and subsequent 1-h post-infusion observation period. (●): TSST-1 infused group, (○): control group. pH: arterial blood pH; BE: base excess; HCO₃⁻: bicarbonate ion concentration; AKBR: arterial ketone body ratio. Data are expressed as mean ± SD. **: p < 0.05 (0 h versus 6 h in TSST-1 infused group by paired t-test).

Figure 5  Time-course changes in biochemical and hematological parameters during the 5-h infusion of toxic shock syndrome toxin-1 (TSST-1) and subsequent 1-h post-infusion observation period. (●): TSST-1 infused group, (○): control group. TP: total protein; BUN: blood urea nitrogen; RBC: red blood cell count; Ht: hematocrit; Hb: hemoglobin; WBC/Ht: hematocrit value standardized white blood cell count. *: p < 0.05 (TSST-1 infused group versus control group at 6 h by Student t-test), **: p < 0.05 (0 h versus 6 h in TSST-1 infused group by paired t-test).
Respiratory function

The changes in the respiratory function parameters in the animals that received TSST-1 are shown in Figure 3. The intrapulmonary arteriovenous shunt ratio (Qs/Qt), and oxygen delivery (DO2) had significantly increased in the animals at 1 h after completion of the infusion, and statistically significant differences between the TSST-1 infused group and the control group were also observed. In addition, arterial oxygen partial pressure (PaO2), arterial oxygen saturation (SaO2) and systemic oxygen consumption (VO2) all exhibited significant changes at 1 h after completion of the TSST-1 infusion when the values were compared with the baseline; however, the differences between the TSST-1 infused and control groups at the time point of 6 h were not statistically significant. No significant changes were observed in arterial carbon dioxide pressure, systemic oxygen extraction ratio, respiratory index, mixed venous blood oxygen saturation or alveolar arterial oxygen difference (data not shown).

Oxygen metabolism

The serum lactate concentration was significantly increased at 1 h after completion of the TSST-1 infusion when compared with the baseline level (Figure 4). Other than this increase, all of the parameters concerning oxygen metabolism, arterial blood pH (pH), base excess (BE), bicarbonate ion concentration (HCO3-) and arterial ketone body ratio (AKBR), were found to be decreased at 1 h after completion of the TSST-1 infusion. However, the differences between the TSST-1 infused and control groups were not statistically significant.

Blood chemistry

Among the blood chemistry parameters examined, a slight but statistically significant increase was noted for the total protein concentration (TP) at 1 h after completion of the TSST-1 infusion when compared with the baseline level (Figure 5). However, the difference between the TSST-1 infused and control groups was not statistically significant. In addition, a moderate but statistically significant increase was observed in both the TSST-1 infused and control groups in the blood urea nitrogen (BUN) concentration. Also, BUN concentrations at the 6 h time point were statistically different between the TSST-1 infused and control groups. However, the creatinine level exhibited no significant elevation in either group (data not shown). None of the other parameters, including alanine aminotransferase, aspartate aminotransferase, glucose, creatine phosphokinase, amylase, or electrolytes exhibited any significant changes in level (data not shown).

Hematology

Significant increases were noted in red blood cell count (RBC), hematocrit (Ht), and hemoglobin concentration (Hb) in the animals at 1 h after completion of the TSST-1 infusion when compared with the baseline level (Figure 5). In contrast, the white blood cell count was observably, but not significantly, decreased in these animals. Statistical significance, however, was detected for the white blood cell count when the values were standardized with the hematocrit values (WBC/Ht) (Figure 5). However, the difference of WBC/Ht between the TSST-1 infused and control groups was not statistically significant at the 6 h time point. In addition, the decrease in white blood cell count (ΔWBC) did significantly correlate with the increase in pulmonary vascular resistance (ΔPVR) (Figure 6). The platelet count did not exhibit any significant change (data not shown).

Histopathology

Histopathological comparison between the TSST-1 infused pig (Figure 7A) and the control pig (Figure 7B) revealed five characteristic changes. First there was an increased amount of neutrophil infiltration into the intra-alveolar septa (yellow arrow), and second, mononuclear cell infiltration into the intra-alveolar septa (green arrow). The third visible change is hemorrhage (red blood cells in the alveolae, black arrow), and the fourth is macrophage infiltration into the alveolae (white arrow). The fifth change is the thickening of the intra-alveolar septa. In control pigs, only a small number of neutrophils were found in the intra-alveolar septa and thickening of the intra-alveolar septa was not observed.
Discussion

For investigations into the pathogenesis of Gram-positive bacterial sepsis, a variety of animal models have been utilized which monitor death as the primary endpoint. Because of their high susceptibility to TSST-1 toxicity, rabbits have been the most widely used model animals. In rabbits, the induction of lethal hypotension requires relatively large doses of TSST-1 unless endotoxin is co-administered. Similar results have been reported in a recent study in miniature pigs. These results suggest that the immediate role of TSST-1 in the pathogenesis of Gram-positive bacterial sepsis is to induce cardiac and respiratory failure rather than primary refractory systemic hypotension. Accordingly, we have focused on the initial stage of the biological process induced by continuous infusion of TSST-1. In the present study, we observed animals for a 6-h period: the 5-h infusion period and a subsequent 1-h follow-up observation period. This is because a preliminary experiment had revealed that significant changes were detectable in certain parameters 1-h post-infusion.

In order to examine the biochemical process leading to multiple organ dysfunction, it is necessary to simultaneously monitor several parameters in a time-course manner at once. Such parameters include cardiac and respiratory function, hematology, blood chemistry, serum toxin levels, and serum cytokine levels. Carrying out such simultaneous monitoring without affecting physiological conditions, however, is extremely difficult with small laboratory animals, such as rabbits, because of the limited quantity of circulating blood. Accordingly, no time-course data have been forthcoming in these model animals, especially in terms of a correlation of the serum TSST-1 levels and changes in these parameters.

Hence, pigs were selected as the model animals since pigs have been shown to be sensitive to TSST-1 toxicity and are also of sufficient size to withstand the multiple sampling required. In the present study, conventional pigs were treated with TSST-1 alone by continuous infusion in an effort to develop a model for the superantigen-induced multiple organ dysfunction observed in patients with Gram-positive bacterial sepsis. A thermodilution catheter was inserted into the jugular vein to allow continuous monitoring of cardiac function parameters and also mixed venous blood sampling. A normal catheter was also inserted into the carotid artery for blood pressure monitoring and a 15-mL blood sampler for blood gas, hematology, and blood chemistry measurements. All parameters were determined at baseline and at 1-h intervals for 6 h after the initiation of the TSST-1 infusion.

As expected, repeated blood sampling by itself did not significantly affect the animals when they were treated with physiological saline in place of TSST-1 (Figures 2–5).

The dose selection was based on a dose-finding study performed prior to the present study (data not shown). As previously reported by our laboratory and others, serum superantigen levels are found in the range of 1000–5000 pg/mL in most patients afflicted with Gram-positive bacterial sepsis. The dose-finding study revealed that continuous infusion of TSST-1 at 2 μg/kg/h could give serum TSST-1 levels in a range similar to that reported in TSS patients. In fact, in this study, continuous infusion at this dose resulted in a rapid increase in serum TSST-1 during the initial 1-h period of the TSST-1 infusion and then the serum TSST-1 levels reached a range similar to that reported with TSS patients at 4 h after the initiation of the infusion (Figure 1A).
Since conventional pigs were used, endotoxin translocated from the gut flora might have been detectable in the circulating blood. No detectable levels of serum endotoxin, however, were demonstrated in the animals at any of the time points examined in this study (Figure 1A). Thus, it is considered that endotoxin plays only a negligible role, if any, in this model.

In the animals treated with TSST-1, serum IFN-γ increased while serum TNF-α remained undetectable throughout the observation period. These results are apparently inconsistent with the hypothesis postulated by Miethke et al.\textsuperscript{29} that an elevation of TNF-α occurs at a very early stage and triggers the subsequent cytokine storm and lethal hypotension.

In the present study, post-infusion observation was performed for only 1 h. Accordingly, neither deaths nor TSS-like symptoms, such as diarrhea, vomiting, erythema, hypotension, or thrombocytopenia, were observed in the animals treated with TSST-1. These results are consistent with the report by Bulanda and coworkers.\textsuperscript{20}

To evaluate the sensitivity of pigs to TSST-1, LPS lethality enhancement capacity\textsuperscript{19} was examined in the TSST-1 infused pigs. LPS was infused 1 h after TSST-1 infusion terminated, at a concentration of 10 \textmu g/kg/h for 1 h. This LPS concentration was of non-lethal level; however, when pigs were pre-treated with TSST-1 at the same dose in the experiment, all animals died within 24 h (data not shown). Furthermore, TNF-α in the blood became elevated after the LPS infusion. These results indicate that pigs were sensitive enough to respond to TSST-1 even at this low infusion dose, and as expected, do respond with a burst of TNF-α.

Constant infusion of TSST-1-induced three major dysfunctions in pigs: (a) a cardiac disorder characterized by an increase of HR and CO (Figure 2), (b) a respiratory disorder characterized by an increase in Qs/Qt and DO\textsubscript{2} (Figure 3), and (c) infiltration of white blood cells into the intra-alveolar septa, and these findings were significantly different between the TSST-1 infused and control groups. Furthermore, TSST-1-induced five minor changes in pigs: (a) a reduction of SV that suggests a cardiac disorder (Figure 2), (b) a decrease of PaO\textsubscript{2} and SaO\textsubscript{2} that suggests a respiratory disorder (Figure 3), (c) an increase in the arterial lactate concentration (Figure 4), AKBR (Figure 4), and VO\textsubscript{2} (Figure 3) that suggest oxygen metabolism disorders, (d) a decrease in white blood cell (WBC) counts that, standardized with the hematocrit values, suggests inflammation, and (e) an increase in the hematocrit values which suggests capillary leakage. All of these parameters were significantly different when compared with the baseline level at 1 h after completion of the TSST-1 infusion, although the differences between the TSST-1 infused group and the control group were not statistically significant.

The increase in arterial lactate resulted in metabolic acidosis. For compensation of this high-oxygen consumption, it was assumed that CO increased in an effort to increase oxygen delivery in the animals (Figure 2), and the oxygen extraction ratio remained constant. The increased cardiac output (high-output state) was probably a result of tachycardia, although myocardial depression and capillary leakage were suggested from the decrease in SV (Figure 2) and the increase in hematocrit (Figure 5). A high-output state might also be a result of hypoxia, as evidenced by the increase in Qs/Qt (Figure 3). These results suggest respiratory dysfunction characterized by inefficient gas exchange.

The serum IFN-γ level was observed to increase in the animals (Figure 1B). Since IFN-γ is known to activate T cells, especially Th1 helper T cells, the activation of leukocytes might be involved in the state of inefficient gas exchange. This was supported by histopathological findings that mononuclear cells and an increased number of neutrophils had infiltrated into lung tissue (Figure 7). When the white blood cell count was standardized with the hematocrit values (WBC/Ht), it was found to have significantly decreased after TSST-1 infusion (Figure 5). Furthermore, decreases in the WBC count observed in individual animals for the entire 6-h observation period had a statistically significant correlation with an increase in pulmonary vascular resistance during the same period (Figure 6). These results indicate that the activated WBC, which infiltrated into the lung, insulted the tissue (this being observed as hemorrhage and a thickening of the intra-alveolar septa) and may have triggered the respiratory disorder. It is certainly likely to have contributed to it. Arad et al.\textsuperscript{30} have also reported the activation of Th1 helper Tcells with superantigen. However, the specific relationship between certain activated species of immune cells and species of superantigens is still controversial. Herz et al.\textsuperscript{31} have reported that an airway exposure model of a superantigen, staphylococcal enterotoxin B, resulted in an increase in serum interleukin 4, a cytokine produced by Th2 type Tcells, but not in a production of IFN-γ. In the near future, when we are able to obtain the necessary anti-swine antigen antibodies, more details of the mechanism will be elucidated by immunostaining and/or flowcytometry.

In addition to the changes described above, blood chemistry revealed a mild but significant increase in blood urea nitrogen (BUN) in the animals (Figure 5). However, while an elevated BUN was also observed...
in control animals, the BUN concentration at the 6 h time point was significantly low in control animals, suggesting an early stage of renal failure. TSST-1 has previously been reported to exert some degree of renal toxicity in rabbits.18

In conclusion, we have developed a swine sepsis model to help evaluate the pathogenic mechanisms of TSST-1-induced multiple organ dysfunction. The serum TSST-1 level is readily controllable in this model. In addition, simultaneous and continuous monitoring of cardiac and respiratory function parameters, together with repeated blood sampling, can readily be performed. Thus, this model provides the first tool capable of simultaneously examining both the serum toxin levels and other parameters in a time-course related manner.

As described above, myocardial depression, tachycardia, acute lung injury, capillary leakage, and renal impairment were all observed in the model. These symptoms are well known in human toxic shock syndrome.4,21 Neither a high dose of TSST-1 nor an infusion of protracted duration was required for the induction of organ failure. Thus, this study provides an animal model for Gram-positive bacterial sepsis without lethal hypotension. This model should prove to be of considerable value not only in studies geared to a better understanding of pathogenesis, but also for the development of therapeutic interventions of the early septic state without attendant shock.

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