



studies. Major problems in experimental sepsis models derive from the time period allowed for development of septic encephalopathy.<sup>14</sup>

The aim of this study was to show that infusion of live bacteria into the portal vein, simulating an intraabdominal septic focus, leads to brain damage that is not caused by systemic hypotension or ischemia or hypoglycemia. Sagittal sinus pressure (SSP), cerebral perfusion pressure, cerebral oxygen status, cerebrospinal fluid (CSF) cytokine levels, and brain histology were investigated.

## Material and Methods

### Animals

The study was conducted in accordance with the guidelines established by the National Institutes of Health, Committee on the Care and Use of Laboratory Animals. Twenty mature, female Göttingen miniature microbiologically defined pigs from a purpose-built full-barrier specific pathogen-free breeding facility (Ellegaard Göttingen Minipigs, Dalmose, Denmark) weighing 28–38 kg were used in these experiments. Each pig was certified as free of disease by the veterinary surgeon prior to delivery. Pigs were free from parasites and any viral and bacterial infection before the start of the study. Pigs received no antibiotic treatment before or during the study. The animals were fasted 16 h before operation but had access to water *ad libitum*.

### Instrumentation

The animals were sedated by the intramuscular injection of ketamine (15 mg/kg), azaperone (2 mg/kg), and atropine (0.5 mg). An ear vein was cannulated. After intravenous administration of propofol (2 mg/kg) the pigs were intubated endotracheally and mechanically ventilated (fraction of inspired oxygen  $[F_{iO_2}] = 0.3$ ; partial pressure of carbon dioxide = 32–38 mmHg). Anesthesia was maintained by continuous infusion of propofol ( $8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and fentanyl ( $4 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) during the operation.

The urinary bladder was catheterized and the rate of urine flow was recorded. The left femoral artery was cannulated using an optical catheter (Oximetrix 3SO<sub>2</sub>/CO Computer, Abbott laboratories, Wiesbaden, Germany) for measurement of systemic mean arterial pressure (MAP), oxygen saturation, and withdrawal of blood samples. A catheter was inserted through the right external jugular vein for measurements of central venous pressure (CVP) and body temperature. After left side

thoracotomy, the pericardium was incised and a precalibrated ultrasonic transit time flow probe (diameter 16–20 mm; Transonic Systems, Ithaca, NY) was positioned nonconstrictively around the pulmonary artery above the ligamentum arteriosum for measurement of cardiac output. After midline laparotomy a catheter was inserted into the portal vein through a splenic vein for infusion of bacteria and the catheter was tunneled through subcutaneous tissue. After craniotomy occipital of the coronal suture (diameter 3 cm) the superior sagittal sinus was cannulated with a catheter (4-French) with tip location about 1 cm occipital of the puncture for measurements of SSP and for sagittal sinus blood sampling. A spinal catheter (5-French, Fa. Braun, Melsungen, Germany) was inserted between S1 and L5 or L4 and L4 (using radiographic control) for sampling of CSF. After instrumentation all parameters were recorded continuously. All animals were allowed to recover from instrumentation for at least 12 hours.

### Intensive Care Treatment

All pigs were sedated with propofol ( $6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) and fentanyl ( $2 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). This sedation regimen ensured that all pigs were able to cough during tracheal suction or to move in response to pain stimuli. Controlled mechanical ventilation (Elvira, GambroEngström Broma, Sweden) was adjusted to ensure normoxia (arterial partial pressure of oxygen  $[P_{aO_2}] \geq 100 \text{ mmHg}$ ) and normocapnia according to repeated blood gas analysis (Ciba Corning 865, Chiron Diagnostics, Giessen, Germany). The lungs were inflated manually two times every hour, and tracheal secretions were suctioned to avoid atelectasis. The animals were kept in left or right lateral position during the experiment. For decubitus prophylaxis the positioning was changed every 8 h.

Intravenous nutrition was established by 20% glucose according to metabolic monitoring (ElizaMC, Gambro Engström). Glucose infusion was stopped at plasma glucose levels above 150 mg/dl. No insulin was administered in this study.

Pigs were monitored for heart rate, MAP, CVP, SSP, cardiac output, and arterial oxygen saturation. Using heating pads, core temperature was kept at a minimum of 36.5°C. Hyperthermia was not treated. MAP was kept at a minimum of 70 mmHg using norepinephrine in steps of  $0.3 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , up to  $3.0 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and lactated Ringer's solution. A requirement of more than  $3.0 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  to keep MAP over 70 mmHg was defined as septic shock, which led to termination of the experiment. Baseline fluid input was set at

2 ml · kg<sup>-1</sup> · h<sup>-1</sup> crystalloid in all pigs. Supplementary infusion of lactated Ringer's solution was given if the urine flow dropped below 1.5 ml · kg<sup>-1</sup> · h<sup>-1</sup>, cardiac output dropped below 20% of values at day 1 before bacteria infusion, or MAP dropped below 70 mmHg although norepinephrine was increased 0.3 μg · kg<sup>-1</sup> · min<sup>-1</sup>. To evaluate fluid balance pigs were weighed before and after the experiment.

#### *Bacteria Preparation and Microbiology*

The *Escherichia coli* strain D 14604 (086a:K61) was obtained from the American Type Culture Collection (Rockville, MD). The freeze-dried culture was rehydrated by adding 0.4 ml of liquid medium. Bacteria were grown in American Type Culture Collection Culture Medium 3 (Nutrient Difco 0003; Biosciences, Sparks, MD) under static conditions at 37°C for 24 hours (in an Incubator Innova 4230, New Brunswick Scientific, NJ), concentrated 25-fold, and frozen at -80°C in 50% glycerol as 400-μl aliquots. Before infusion of live bacteria, the growth curve of the *E. coli* strain was measured in order to determine a correlation between the light absorbency of a suspension and the concentration of viable bacteria. A single aliquot was used to inoculate 200 ml of nutrient broth, which was grown for about 3 hours at 37°C with constant agitation and aeration. This suspension was used for serial dilution in nutrient broth (10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>), followed by plating on nutrient agar (Nutrient Broth Difco 0001). Plates were incubated overnight and bacteria were quantified by a viable colony count. After this procedure was performed several times, a good correlation was found between the absorbency of the resuspension and the viable cell count (absorbency of 0.3 = 1.9 × 10<sup>8</sup> colony forming units per milliliter). In a preliminary investigation we had evaluated the daily dose to induce infection in pigs (n = 5) to be 10<sup>7</sup> colony forming units per milliliter using the same preparation. According to the weight of the animals, 1.6–2.1 ml of the described suspension was taken and centrifuged for 10 minutes, at 10,000 revolutions/min and 4°C. The pellets were washed in sterile saline (NaCl 0.9%), resuspended in 100 ml of sterile saline, and immediately infused.

#### *Experimental Procedure*

At the first postoperative morning (about 12 h after completing instrumentation), animals were allocated randomly into a septic group (n = 11) or a control group (n = 10) receiving one infusion per day for 4 days of bacteria (suspended in 100 ml saline) or saline, respec-

tively, at a rate of 200 ml/h through the portal venous catheter. Samples from the femoral artery and the sagittal sinus were taken before and 2, 4, and 8 h after infusion of bacteria or saline. All hemodynamic parameters were recorded at the same time. Serum and CSF samples were taken at same time points for measurement of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Blood cultures were taken every day before infusion of bacteria or saline infusion. The pigs were killed in the evening of the fourth postoperative day with 20 mmol potassium chloride after increasing the propofol and fentanyl infusion rates.

#### *Serum Chemistry Analysis*

Blood gases, electrolytes, and glucose concentration were measured (Ciba Corning 865) using blood collected from the femoral artery and the sagittal sinus. Blood cytokine probes from femoral artery blood were centrifuged immediately at 3,500 revolutions/min and 4°C for 10 min and then deep frozen at -70°C. CSF cytokine probes were deep frozen promptly at -70°C. Under these storing conditions, cytokine levels are stable over several months. For measuring cytokine levels, the probes were thawed and subjected to a quantitative determination using enzyme-linked immunosorbent assay kits (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The detection limit of the TNF-α assay is 3 pg/ml, and that of the IL-6 assay is 18 pg/ml.

#### *Brain Fixation and Morphologic Analysis*

The brain was removed immediately after death and fixed in 4% formalin for 96 h. On fixation, the brain was inspected grossly and the cerebrum was cut by consecutive coronal section into slabs about 1-cm thick. Brain stem and cerebellum were cut in horizontal sections. Tissue specimens (1 cm<sup>2</sup>) from each hemisphere were taken from the frontal lobe including gray and white matter, the midbrain near by the third ventricle, the brain stem, and the cerebellum containing gray and white matter. The cerebrum and cerebellum specimens each included meningeal structures. These samples were dehydrated in xylol and embedded in paraffin (Gewebeeinbettungsautomat 1.411.00, PSI-Grünwald, Laudenbach, Germany). Paraffin blocks were cut into 5-μm thick slides using a microtome (Universalmikrotom 1140 Autocut, R. Jung, Heidelberg, Germany). Slides were stained with hematoxylin and eosin.

Each slide was inspected by a blinded neuropathologist for histopathologic signs of the four categories of damage: perivascular or interstitial edema, cytotoxic

edema or cell damage, inflammation, and hyperemia or hemorrhage. Pathologic findings in the last three categories were graded using four severity codes:<sup>15</sup> negative, weak, moderate, and strong. The specific histopathologic meaning of each severity code is described in the legends of the tables. The severity ratings of the four slides per region (structure) were averaged. These mean values for each of seven regions (structures) were added up to achieve a total score of each animal within the category (range 0–21 points).

#### Data Management and Statistical Evaluation

Systemic vascular resistance was calculated as  $([MAP - CVP]/\text{cardiac output})$  (in  $\text{dyne} \cdot \text{s}^{-1} \cdot \text{cm}^{-5}$ ). Cerebral perfusion pressure was calculated as  $(MAP - SSP)$  (in mmHg). Sagittal sinus fraction of oxygenated hemoglobin, arterial oxygen content, and sagittal sinus oxygen content were given by the blood gas analyzer. The difference between arterial and sagittal sinus oxygen content was calculated.

All parameters obtained consecutively (before and 2, 4, and 8 h after portal infusion) were subjected to two-way repeated-measures analysis of variance with the within-groups factor, time; the between-groups factor, group (sepsis or control); and their interaction (tables 1 and 2 present only the values before and 8 h after starting the portal infusion). Once time or group  $\times$  time proved to be significant ( $P < 0.05$ ), a repeated-measures analysis of variance was performed in each group separately. Once interaction effects (group  $\times$  time) proved to be significant ( $P < 0.05$ ), an effect of the deteriorating septic state on the variable was assumed.

Because they were not distributed normally, daily

mean norepinephrine infusion rates were analyzed statistically between groups using the Mann-Whitney U test ( $P < 0.05/5 = 0.01$ , to allow for five comparisons) and within each group using the Friedman test ( $P < 0.05/2 = 0.025$ , to allow for two comparisons). The score of the histopathologic alteration and gain in body weight at day 4 were statistically analyzed using the Mann-Whitney U test. All statistical analyses were performed using StatView for Windows 4.55 (Abacus Concepts, Berkeley, CA).

#### Results

Two septic pigs were killed before the fourth postoperative day because of hemodynamic, respiratory, or metabolic failure. One septic pig did not reach  $P_{aO_2}$  of 100 mmHg despite  $F_{iO_2}$  of 1.0 and developed lactate acidosis (20 mM). The second excluded pig needed more than  $3.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  norepinephrine to keep MAP at 70 mmHg. One control pig died of intracerebral bleeding at the end of the first postoperative day; another died of cardiac dysrhythmia; and a third developed a spontaneous infection on the second day. The data from these animals were discarded from further analysis. The data from 16 pigs (nine septic, seven control) were subjected to statistical evaluation.

All pigs treated with infusion of bacteria but no control animal showed signs of septicemia. After bacteria infusion all pigs developed continuously increasing body temperature from  $37.1 \pm 0.5$  to  $40.6 \pm 1.2^\circ\text{C}$ . In septic pigs, white blood cell counts decreased below  $4,000/\text{mm}^3$  or increased over  $12,000/\text{mm}^3$ .

The applied *E. coli* strain was identified in blood culture.

**Table 1. Hemodynamic Parameters**

Parameter	Group	1st Day		2nd Day	
		24 h	32 h	48 h	56 h
CO (l/min)	Control	$3.2 \pm 0.7$	$3.1 \pm 0.6$	$3.2 \pm 0.6$	$3.3 \pm 0.5$
	Sepsis	$2.9 \pm 0.41$	$3.8 \pm 0.7$	$3.5 \pm 0.6$	$4.1 \pm 0.9$
SVR ( $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5}$ )	Control	$1,669 \pm 429$	$1,757 \pm 336$	$1,882 \pm 443$	$1,587 \pm 189$
	Sepsis	$1,572 \pm 342$	$1,309 \pm 306$	$1,548 \pm 318$	$1,195 \pm 335$
CVP (mmHg)	Control	$7 \pm 3$	$7 \pm 3$	$5 \pm 2$	$6 \pm 2$
	Sepsis	$7 \pm 2$	$8 \pm 2$	$9 \pm 3$	$11 \pm 3$
SSP (mmHg)	Control	$6 \pm 2$	$7 \pm 3$	$7 \pm 2$	$7 \pm 2$
	Sepsis	$6 \pm 2$	$9 \pm 2$	$10 \pm 3$	$11 \pm 3$
CPP (mmHg)	Control	$69 \pm 9$	$67 \pm 10$	$71 \pm 10$	$64 \pm 6$
	Sepsis	$65 \pm 4$	$60 \pm 4$	$65 \pm 5$	$58 \pm 7$

Data are mean  $\pm$  SD.

CO = cardiac output; SVR = systemic vascular resistance; CVP = central venous pressure; SSP = sagittal sinus pressure; CPP = cerebral perfusion pressure.



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tures from four of nine septic pigs before the second and in all septic pigs before the third and fourth bacteria infusions. No bacteria were found in the control group. No bacteria were found in the CSF except in one pig of the sepsis group.

No pigs received any norepinephrine to keep MAP above 70 mmHg before bacterial infusion. During the following days control animals required norepinephrine in low rates without changes between days, presumably as a result of the side effects of the propofol-fentanyl infusion. In septic animals mean daily norepinephrine infusion rates had to be increased significantly. After bacteria infusion, norepinephrine infusion rates (median [25th percentile, 75th percentile]) were significantly higher in septic pigs compared with control pigs at each day (day 1: 0.021 [0.018, 0.033] *vs.* 0 [0, 0.009]  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; day 2: 0.057 [0.031, 0.118] *vs.* 0 [0, 0.015]; day 3: 0.182 [0.062, 0.570] *vs.* 0.013 [0, 0.048]; and day 4: 0.602 [0.149, 1.659] *vs.* 0.042 [0, 0.062]). In septic pigs cardiac output, CVP, and SSP were increasing within 8 h after the first bacteria infusion; SVR was decreasing continuously. Because MAP was kept constant, these changes resulted in a decrease in central perfusion pressure (table 1). A gain in body weight (mean [25th percentile, 75th percentile]) was observed in septic pigs only (2.5 [2.0, 3.3] *vs.* 0.5 [0.2, 0.8] kg;  $P < 0.05$ ).

All septic pigs developed respiratory failure. The quotient of the partial pressure of oxygen divided by  $\text{F}_{\text{I}\text{O}_2}$  in septic pigs decreased from  $535 \pm 39$  to  $273 \pm 66$ .  $\text{Pao}_2$ , however, was kept above 100 mmHg by adjusting mechanical ventilation and increasing  $\text{F}_{\text{I}\text{O}_2}$ . Arterial oxygen content diminished in the control and septic groups. In septic pigs, sagittal sinus oxygen saturation continuously

increased; the difference between arterial and sagittal sinus oxygen content decreased during the experiment in both groups (table 2).

Interleukin-6 was increased after the first infusion of bacteria and remained at elevated levels during the investigation period (fig. 1).  $\text{TNF-}\alpha$  was increased periodically 2–4 h after bacteria infusion and dropped to near baseline values overnight.  $\text{TNF-}\alpha$  levels in serum and CSF changed simultaneously (fig. 2). In control animals, there were no changes in serum and CSF levels of  $\text{TNF-}\alpha$  and IL-6.

In the control group, brains did not show any pathologic changes on visual inspection. Two pigs of the control group showed minor histologic alterations of brain tissue (e.g., beginning perivascular edema and hyperemia of meningeal vessels and single red blood cells were found in cortex tissue). No histologic brain damage was detected in any of the other control animals (fig. 3A). In all septic pigs damage to neurons was seen: The cytoplasm became markedly eosinophilic in sections stained with hematoxylin and eosin (fig. 3B) and contained finely granular dispersed substance of Nissl. The nucleus also was shrunken, often triangular, and darkly stained. Sometimes the cytoplasm was uniformly structureless and eosinophilic and the nucleus showed advanced degeneration with beginning disintegration of the nuclear membrane (fig. 4). In contrast, cerebellar cortex and especially the layer of the Purkinje cell did not show any histologic pathology in either control pigs (fig. 5A) or septic pigs (fig. 5B).

All pigs in the septic group showed moderate to severe perivascular edema (fig. 6A), especially in the white matter. The myelin sheaths often were swollen abnormally and vacuolated. They were less closely packed than in normal white matter, with the picture of spon-

Table 1. Continued

3rd Day		4th Day		Group $\times$ Time	Time
72 h	80 h	96 h	104 h		
3.4 $\pm$ 0.4	3.3 $\pm$ 0.7	3.4 $\pm$ 0.6	3.5 $\pm$ 0.7	NS	NS
3.8 $\pm$ 0.7	3.9 $\pm$ 0.8	3.9 $\pm$ 0.5	3.8 $\pm$ 0.62		0.002
1,552 $\pm$ 239	1,657 $\pm$ 623	1,746 $\pm$ 653	1,605 $\pm$ 494	0.0349	NS
1,281 $\pm$ 385	1,220 $\pm$ 342	1,164 $\pm$ 224	1,131 $\pm$ 216		<0.0001
10 $\pm$ 4	10 $\pm$ 4	9 $\pm$ 3	9 $\pm$ 3	0.0479	NS
12 $\pm$ 3	13 $\pm$ 2	17 $\pm$ 3	17 $\pm$ 3		<0.0001
8 $\pm$ 3	7 $\pm$ 2	7 $\pm$ 2	8 $\pm$ 3	<0.0001	0.0473
12 $\pm$ 2	13 $\pm$ 2	14 $\pm$ 2	16 $\pm$ 2		<0.0001
70 $\pm$ 14	66 $\pm$ 10	72 $\pm$ 10	70 $\pm$ 9	0.0297	0.0290
59 $\pm$ 8	58 $\pm$ 8	59 $\pm$ 6	55 $\pm$ 9		0.0008

**Table 2. Cerebral Oxygen Balance**

Parameter	Group	1st Day		2nd Day	
		24 h	32 h	48 h	56 h
CaO <sub>2</sub> (ml/dl)	Control	11.3 ± 1.8	11.4 ± 2.0	11.3 ± 1.5	10.4 ± 1.4
	Sepsis	11.7 ± 1.8	12.1 ± 2.2	12.4 ± 2.6	11.7 ± 2.5
Ssso <sub>2</sub> Hb (%)	Control	54 ± 5	57 ± 8	57 ± 5	60 ± 7
	Sepsis	60 ± 11	60 ± 8	63 ± 10	67 ± 8
C(a-ss)O <sub>2</sub> (ml/dl)	Control	6.8 ± 0.7	6.4 ± 1.7	6.5 ± 0.9	6.0 ± 1.1
	Sepsis	6.3 ± 2.2	6.4 ± 1.7	5.8 ± 1.3	5.0 ± 1.6

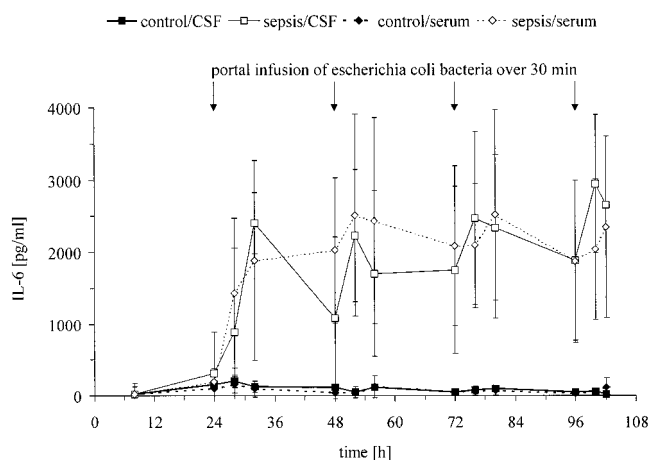
Data are mean ± SD.

CaO<sub>2</sub> = arterial oxygen content; Ssso<sub>2</sub>Hb = sagittal sinus fraction of oxygenated hemoglobin; Ca(a-ss)O<sub>2</sub> = difference between arterial and sagittal sinus oxygen content.

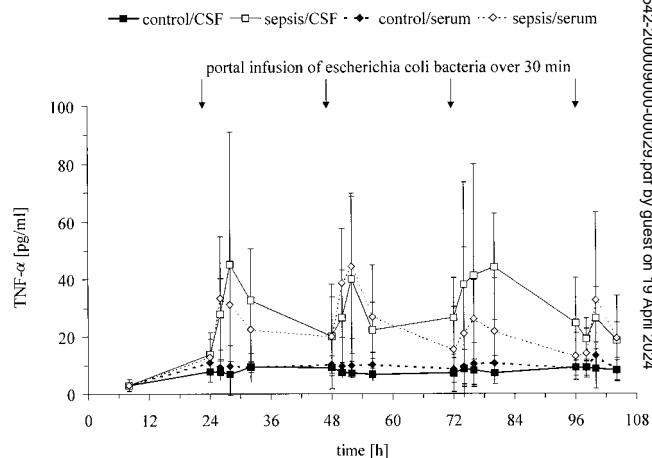
giform degeneration. This was accompanied by astrocytic hyperplasia. Perivascular macrophage-like cells, especially in periventricular locations, were found. The oligodendrocytes within the affected white matter showed no structural abnormalities on light microscopy. In two subjects in the septic group a desquamation of the ependyma cells was seen in combination with a periventricular edema. In the gray matter a swelling of astrocytes could be observed. The cerebral vessels of white and gray matter showed strong hyperemia, and brain purpura was found in most animals. In some slices polymorphonuclear neutrophils accumulated in the brain tissue around cerebral vessels. In the brain of one pig, microabscesses were found (fig. 6B). Results from all septic pigs are shown in tables 3-5.

## Discussion

In this study a porcine model of sepsis-induced brain damage was established. Sepsis syndrome was induced by infusion of live bacteria into the portal vein, simulating the effects of peritonitis. Infusion of bacteria was followed by the development of severe sepsis in all pigs according to clinical criteria as defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference:<sup>16</sup> body temperature more than 38°C, heart rate more than 90 beats/min, and white blood cell count more than 12,000/mm<sup>3</sup> or less than 4,000/mm<sup>3</sup>. All septic pigs needed high-dosage vasopressor support with norepinephrine to keep MAP at 70 mmHg. Cardiac output increased and systemic vascular



**Fig. 1.** Interleukin-6 (IL-6) levels in serum and cerebrospinal fluid (CSF) during 4 days of bacteria infusion. In septic animals IL-6 serum and CSF levels increased significantly during the course of intensive care compared with control animals (group × time:  $P < 0.0006$  each). CSF and serum levels did not differ significantly. The increases in IL-6 serum and CSF levels were significant in septic pigs ( $P < 0.0001$ ). Values are the mean ± SD.



**Fig. 2.** Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels in serum and cerebrospinal fluid (CSF) during 4 days of bacteria infusion. In septic animals TNF- $\alpha$  serum and CSF levels increased significantly during the course of intensive care compared with control animals (group × time:  $P < 0.0007$ ). CSF and serum levels did not differ significantly. The increases in TNF- $\alpha$  serum and CSF levels were significant in septic pigs ( $P < 0.0001$ ). Values are the mean ± SD.

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Table 2. Continued

3rd Day		4th Day		Group × Time	Time
72 h	80 h	96 h	104 h		
9.9 ± 1.3	8.5 ± 0.9	9.5 ± 1.4	9.3 ± 1.3	NS	<0.0001
11.4 ± 2.8	10.4 ± 2.2	9.7 ± 1.8	8.9 ± 1.9		<0.0001
57 ± 6	54 ± 7	55 ± 5	53 ± 5	<0.0001	NS
70 ± 3	71 ± 7	70 ± 5	75 ± 9		<0.0001
5.7 ± 1.6	5.3 ± 1.3	5.7 ± 1.5	5.9 ± 1.4	0.0014	<0.0001
4.5 ± 1.5	3.9 ± 1.4	3.7 ± 0.9	2.8 ± 1.3		0.0052

resistance decreased. Respiratory failure, common in gram-negative sepsis,<sup>17</sup> also was seen. In addition, the deteriorating course of sepsis is confirmed by the continuous increase in IL-6, a good marker of activation of the cytokine cascade.<sup>12,18-21</sup>

The brains of septic pigs showed a mixed pattern of pathologic histology with completely intact neurons next to neurons with signs of cell death and various stages of cellular decay indicating a progressive course of nerve cell lesions. These histologic findings correspond to those seen in patients who develop septic encephalopathy after the onset of gram-negative septicemia.<sup>22,23</sup> The percentage of irreversibly injured neurons with karyolysis, pyknosis, intensive cytoplasmic eosinophilia, loss of structure, and fragmentation confirms severe brain damage.

There are different factors that might be responsible for cellular brain damage in sepsis: ischemia or hypoxia,<sup>5,9</sup> but also external stimuli such as mild heat,<sup>24</sup> toxic

agents such as TNF- $\alpha$ ,<sup>25,26</sup> or direct infection of the brain.<sup>23,27</sup>

Systemic hypoxia can be excluded in our model, because Pao<sub>2</sub> was kept above 100 mmHg during the whole experiment. Although arterial oxygen content decreased because of multiple blood sampling during the experiment there were no significant differences between groups. Ischemia as a result of impaired global cerebral perfusion is also unlikely. Septic pigs showed decreases in central perfusion pressure resulting from increased SSP at constant MAP. A decrease in global cerebral blood flow should lead to a decrease in sagittal sinus fraction of oxygenated hemoglobin and an increased difference between arterial and sagittal sinus oxygen content (assuming constant cerebral metabolism). The observed increase in sagittal sinus fraction of oxygenated hemoglobin and decrease in the difference between arterial and sagittal sinus oxygen content in septic pigs, however, suggest that perfusion was adequate.

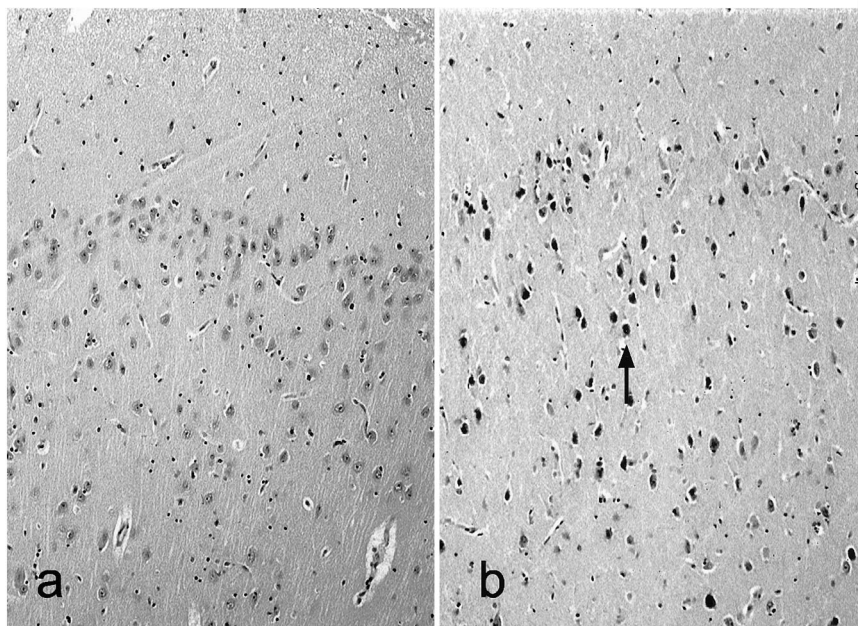
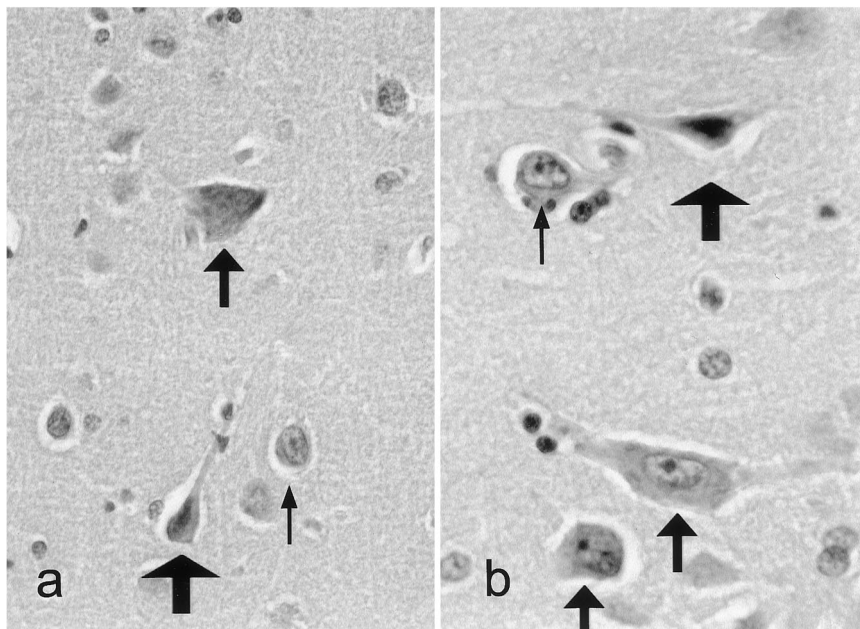


Fig. 3. Cortex of a control (no. 08) and a septic pig (no. 09) pig. (A) Normal cortex with normal pyramidal cells in a control pig. (B) In the cortex of a septic pig, the cytoplasm of the pyramidal cells (arrow) is markedly eosinophilic. The nucleus is shrunken, often triangular, and darkly stained. Hematoxylin and eosin staining, magnification × 220.



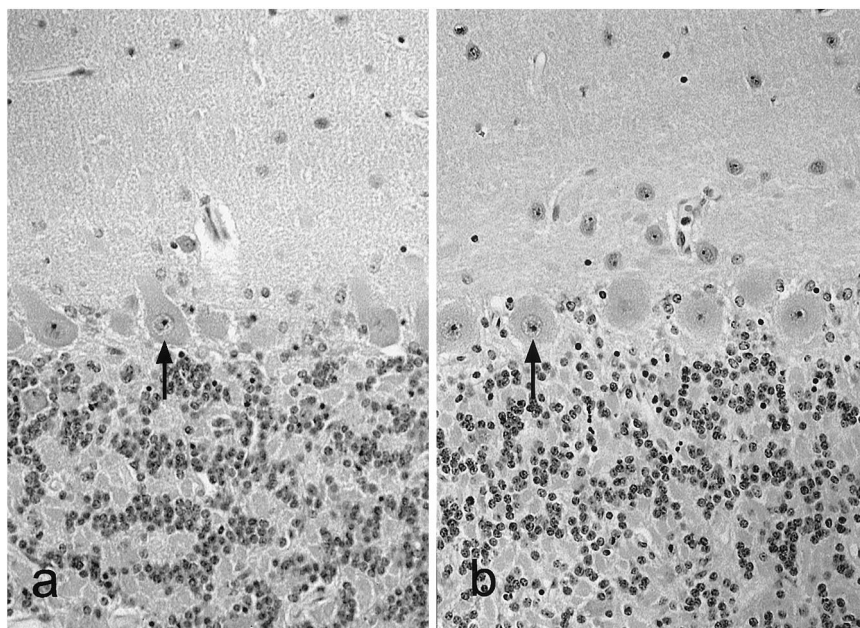


**Fig. 4.** Different stages of nerve cell damage in the cerebral cortex of a septic pig: Reversible damaged neurons (e.g., medium arrows) with clumped substance of Nissl (chromatolysis) are next to undamaged (e.g., small arrows) and irreversibly damaged (e.g., large arrow) neurons. The cell body of irreversible damaged neurons is shrunken and displays intensely eosinophilic cytoplasm; the nucleus is pyknotic and lacks discernible nucleolus. The perineuronal spaces are exceptionally prominent. Hematoxylin and eosin staining; magnification  $\times 888$  (A), 1,320 (B).

Norepinephrine was used to maintain MAP at 70 mmHg, because it does not decrease global cerebral blood flow in endotoxin shock.<sup>28</sup> After disruption of the blood-brain barrier even increases in global cerebral blood flow have been reported.<sup>29</sup> Regions with insufficient oxygen supply, however, might have been present.

Recent experiments suggest that the level of IL-6 is increased during ischemic brain injury and is, in addition,

a crucial factor of neuroprotection, although its origin and its mechanism of action remain unclear.<sup>30,31</sup> The persistent high levels of IL-6 in the CSF of our septic pigs may reflect the severe histologic damage caused by local ischemia. The layer of Purkinje cells in the cerebellum of pigs, however, has been demonstrated to be the structure mainly affected during ischemia,<sup>32</sup> because these cells are most sensitive to lack of oxygen. Because

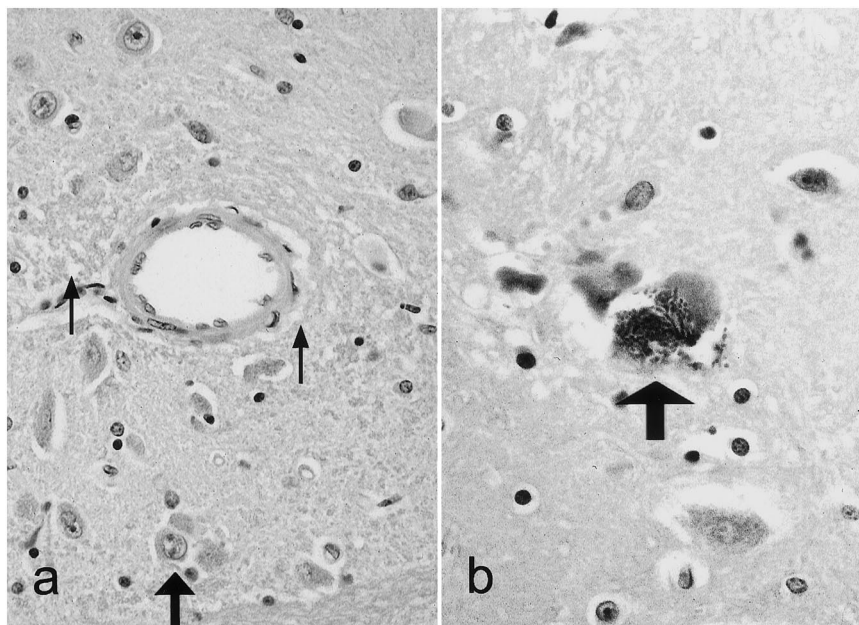


**Fig. 5.** Cerebellar cortex of a control pig (no. 08) and a septic pig (no. 04). Cerebellar cortex is composed of three layers: the eosinophilic molecular layer, the layer of Purkinje cells, and the densely populated granular cell layer. The large Purkinje cells (e.g., arrows) are in a normal state with lightly eosinophilic cytoplasm and a large central nucleus with a single prominent nucleolus in slices of control pigs (A) as well as septic pigs (B). Hematoxylin and eosin staining, magnification  $\times 440$ .



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Fig. 6. Perivascular (small arrows) and cytotoxic (medium arrow) edema (no. 16; *A*) and microabscess in the cerebral cortex (large arrow; no. 11; *B*) in a septic pig. Hematoxylin and eosin staining, magnification  $\times 440$  (*A*),  $880$  (*B*).



Purkinje cells also were not affected in septic pigs, we conclude that cerebral damage in our model is not caused by cerebral ischemia or hypoxia.

Brain temperature above  $44^{\circ}\text{C}$  is known to induce necrosis.<sup>24</sup> We did not attempt to reduce fever in this model. Only one pig developed body temperature of  $42^{\circ}\text{C}$ . Although mild hypothermia is considered to be neuroprotective,<sup>33</sup> data are missing about additive noxious effects of hyperthermia below  $44^{\circ}\text{C}$ . We believe it is unlikely that hyperthermia ( $< 42^{\circ}\text{C}$ ) alone caused the observed cellular brain damage.

Many of the deleterious effects of bacterial infections leading to end-organ failure are caused by combined

actions of toxins and cytokines.  $\text{TNF-}\alpha$  is one of the primary mediators in infectious diseases. In a murine model, intracerebral  $\text{TNF-}\alpha$  has been shown to be related to septic encephalopathy and lead to death within a few days.<sup>25</sup> In the central nervous system an active transport system<sup>34</sup> as well as a passive diffusion for  $\text{TNF-}\alpha$  have been described.<sup>35</sup> In addition,  $\text{TNF-}\alpha$  production by astrocytes and microglia can be induced by bacteremia.<sup>36,37</sup> Regardless of the source, high levels of  $\text{TNF-}\alpha$  were detected in the CSF of the septic pigs in this study. Although this has not been explored fully, recent investigations have shown some aspects of the underlying pathophysiologic mechanism of  $\text{TNF-}\alpha$ -induced cell

Table 3. Cytotoxic Edema and Cell Damage

Structure	Cell Degeneration	Pig Number									
		2	4	7	9	10	11	16	19	20	
Neurons (gray)	Swollen	+	++	++	+++	++	+++	++	++	+	
	Eosinophilic	-	-	++	+++	-	+++	-	+	+	
Astrocytes (gray)	Swollen	-	++	-	-	+++	-	-	++	+	
	Axons (white)	-	-	+	++	-	++	-	-	+	
Ependym plexus	Desquamation	+++	-	-	-	-	-	-	++	-	
Purkinje cells	Swollen	-	-	-	-	-	-	-	-	-	
	Eosinophilic	-	-	-	-	-	-	-	-	-	
Score		4	4	5	8	5	8	2	7	5	

No damaged cells per high-magnification ( $\times 440$ ) field (-); less than 5 damaged cells per high-magnification ( $\times 440$ ) field (+); 5–10 damaged cells per high-magnification ( $\times 440$ ) field (++); more than 10 affected cells per high-magnification ( $\times 440$ ) field (+++). No cytotoxic edema or damaged cells were found in brains of control pigs. Score (possible maximum: 21) of these alterations (median [25th percentile; 75th percentile]) was 5 [4; 7] in septic animals versus 0 [0; 0] in control animals ( $P < 0.05$ ).

**Table 4. Inflammatory Signs**

Region	Pig Number								
	2	4	7	9	10	11	16	19	20
Cerebrum, gray matter	+	-	-	++	++	++	-	-	++
Cerebrum, white matter	++	-	-	-	++	+++	-	-	+
Mid brain, periventricular	-	+	-	++	++	-	-	+	-
Cerebellum, gray matter	+	-	-	++	++	++	-	-	++
Cerebellum, white matter	+	-	-	-	+	+	-	-	+
Brain stem	-	-	-	-	-	-	-	-	-
Meningeal structures	-	-	-	-	-	-	-	-	+
Score	5	1	0	6	9	8	0	1	7

No inflammatory signs (-); macrophage infiltration (+); infiltration of polymorph nuclear cells or reactive microglia (++); microabscesses (+++). No inflammatory signs were found in brains of control pigs. Score (possible maximum: 21) of the histopathologic alteration (median [25th percentile; 75th percentile] was 5 [1; 7] in septic animals versus 0 [0; 0] in control animals ( $P < 0.05$ ).

damage. TNF- $\alpha$  reduces cerebral oxygen uptake, increases intracranial pressure,<sup>38</sup> induces diffuse intravascular coagulopathy, and increases capillary permeability resulting in cerebral edema.<sup>39-41</sup>

Cerebral edema is defined as an increase in brain volume caused by an increased tissue water content.<sup>42</sup> This definition, however, does not differentiate between intra- and extracellular location of water. The type of cerebral edema encountered most frequently in clinical practice is a vasogenic edema: An incompetent blood-brain barrier permits extravasation of plasma-like fluid into the extracellular space. The edema fluid tends to be primarily increased in the white matter. In our model, interstitial edema with beginning spongiform degeneration of the white matter was found in the histologic brain slices of each individual septic pig.

In addition, a cytotoxic edema was observed in brain slices of the septic pigs. The morphologic correlate was a cytoplasmic hydropic swelling of astrocytes or neu-

rons. TNF- $\alpha$  is a potent cytotoxic agent in neuronal tissue.<sup>43,44</sup> All septic pigs in this model developed cytotoxic edema and elevated TNF- $\alpha$  levels in the CSF coincidentally.

In the brain of one pig, microabscesses were found. These microabscesses indicate direct infection of the brain, which does not meet the definition of septic encephalopathy in principle.<sup>8</sup> In deceased patients with septic encephalopathy, however, microabscesses are an occasional autopsy finding.<sup>23,27</sup>

Instrumentation and intensive care treatment themselves affect the physical conditions. No alteration in vital parameters, no signs of infection, and no pathologic histology, however, were observed in control animals. According to the protocol MAP and glucose concentrations were controlled. It is therefore unlikely that brain pathology developed as a result of dehydration, hypoglycemia, or hypotension.

In conclusion, we established a reproducible porcine

**Table 5. Cerebral Hyperemia and Hemorrhage**

Region	Pig Number								
	2	4	7	9	10	11	16	19	20
Cerebrum, gray matter	+	+	+	+	-	-	+	-	-
Cerebrum, white matter	+	+	+	+	-	-	+	-	-
Mid brain, periventricular	-	+++	++	-	-	-	-	-	-
Cerebellum, gray matter	+	+	+	+	-	-	+	-	-
Cerebellum, white matter	+	+	+	+	-	-	+	-	-
Brain stem	-	+++	+++	+++	-	-	+++	++	+++
Meningeal structures	-	-	+	-	-	-	+	-	++
Score	4	10	10	7	0	0	8	0	5

No pathologic findings (-); hyperemia in cerebral vessels (+); diapedetic bleeding (++), focal hemorrhage (+++). No inflammatory signs were found in brains of control pigs. Score (possible maximum: 21) of these alterations (median [25th percentile; 75th percentile] was 5 [0; 9] in septic animals versus 0 [0; 2] in control animals ( $P < 0.05$ ).

model of chronic sepsis. Abdominal sepsis induced severe histologic brain damage that was not related to systemic hypoxia or ischemia or to direct central nervous system infection. High CSF levels of TNF- $\alpha$  were related to an inflammatory process in the brain resulting in cerebral edema and death of neurons. Pathologic histology and elevated TNF- $\alpha$  levels in the CSF suggest the development of septic encephalopathy.

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