Sepsis is the systemic inflammatory response associated with gram-negative, gram-positive, and fungal infections. An estimated 750,000 cases of severe sepsis occur each year in the U.S., causing 210,000 deaths annually (1). Once severe sepsis is recognized, development of cardiovascular failure is associated with high mortality rates that have been estimated to be 30% to 60% (2). The majority of septic patients die of refractory hypotension and cardiovascular collapse. Proposed mechanisms of sepsis-induced cardiovascular dysfunction include microvascular dysfunction, presence of activated leukocytes, and effects of various circulating and/or locally produced proinflammatory cytokines, such as tumor necrosis factor (TNF)-alpha and interleukin-1-beta (1,2).

In addition, recent evidence suggests that severity of myocardial dysfunction and eventual mortality could be correlated to mitochondrial dysfunction (3–7). Indeed, both human sepsis and experimental models of sepsis manifest evidence of mitochondrial ultrastructural damage and bioenergetic abnormalities that correlate with septic response severity (4–7). Unresolved issues include, however, the need to establish that mitochondrial dysfunction is causative rather than epiphenomenal and to determine how relevant these abnormalities are in terms of vital organ function and outcome.

Manifestations of mitochondrial dysfunction are typically reflected in ultrastructural damage and swelling, often associated with membrane potential collapse and permeability transition (3,8). Mitochondrial permeability transition has been associated with matrix swelling, uncoupling of the respiratory chain, efflux of Ca²⁺, membrane potential collapse, and release of small proteins such as cytochrome c (8,9). Mitochondrial permeability transition is thought to be mediated by the opening of specific high-conductance channels, whose molecular structure remains imperfectly known (10). Key structural components of these multiprotein complex channels are adenine nucleotide translocator in the inner mitochondrial membrane, cyclophilin D in the matrix, and the voltage-dependent anion channel in the outer mitochondrial membrane (11–13). Cyclosporine A (CsA), a potent immunosuppressive compound, inhibits mitochondrial permeability transition by binding to matrix cyclophilin D (14,15). Other inhibitors of permeability transition pore opening include non-immunosuppressive cyclosporine analogs, bongkrekic acid and anti-apoptotic...
B-cell leukemia (Bcl) proteins, such as Bcl-2 and Bcl-xL (10). The relevance of mitochondrial permeability transition inhibition by cyclosporine analogs for their cytoprotective effects is well documented in many cellular models (11–13). Mechanisms of action in vivo are more difficult to define, and accordingly the evidence is as yet less compelling in animal models of ischemia/reperfusion injury (14,15), trauma (16), and sepsis (6).

Hence, the specific objective of our study was to test whether inhibition of permeability transition achieved by means of immunosuppressive and non-immunosuppressive analogs of CsA and mitochondrial Bcl-2 protein overexpression would improve vital organ dysfunction and outcome in a mouse model of peritonitis chronic sepsis.

METHODS

Animals used. All experiments were conducted in accordance with the National and European Institutes of Health guidelines for the use of laboratory animals and were approved by the Lille University. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). In brief, 6- to 10-week-old female C57Bl/6 mice (Charles Rivers Laboratories, Lyon, France) and Bcl-2 transgenic mice were housed for 6 days before manipulation. Transgenic mice overexpressing human Bcl-2 (Bcl-2-22, CDTA Institut de Transgénose CNRS, Orléans, France) were generated on a C57Bl/6 background. All animals in the study were screened for the presence of Bcl-2 transgene by using polymerase chain reaction with tail deoxyribonucleic acid.

Sepsis model. Cecal ligation and puncture (CLP) was used to induce intra-abdominal peritonitis and sepsis as previously described (17). Under anesthesia (intraperitoneal [IP] ketamine 2.5 mg/kg and xylazine 0.25 mg/kg), the cecum was ligated with 4-0 silk suture immediately distal to the ileocecal valve, punctured once with a 21-gauge needle and gently squeezed to extrude some stool. The cecum was then replaced into the abdomen, which was closed in two layers, followed by a 1.0-ml subcutaneous injection of 0.9% saline. Sham-operated animals were treated identically, except the cecum was neither ligated nor punctured. Animals were maintained on 12-h light/dark cycles with free access to water.

Animal treatments. Mice were treated immediately after surgery procedures. Sham and CLP C57Bl/6 wild-type mice were randomized to receive 1.0-ml subcutaneous injection of either: 1) vehicle (1% ethanol in saline); 2) 2, 10, and 100 mg/kg CsA (a potent immunosuppressive drug that also inhibits mitochondrial permeability transition); 3) 2 mg/kg tacrolimus FK506 (a potent immunosuppressive drug that has no effects on mitochondrial permeability transition); or 4) 2, 10, and 100 mg/kg N-methyl-4-isoleucine CsA (Novartis, Basel, Switzerland) NIM811 (a non-immunosuppressive drug that inhibits mitochondrial permeability transition) in vehicle. Sham and CLP Bcl-2 transgenic mice received 1.0-ml injection of vehicle.

Survival studies. Survival studies after CLP were repeated twice. An investigator blinded to the identity of the mice performed a 96-h follow-up both in wild-type and in Bcl-2 transgenic mice in two separate experiments with 12 mice per group.

Myocardial function. Isolated heart preparation was performed with a method modified for the mouse heart (18). Mice were anesthetized with sodium pentobarbital (65 mg/kg IP), and heparin (100 U) was injected intravenously. The thorax was opened. The heart was quickly excised, placed in ice-cold saline, and immediately mounted, via the ascending aorta, onto a perfusion apparatus. The heart was perfused with a nonrecirculating perfusate at a constant flow (1.5 ± 0.1 ml/min). Coronary flow and coronary perfusion pressure were measured with a transit-time ultrasound flowmeter (Transonic, Ithaca, New York) and pressure transducers (Baxter Healthcare Corp., Irvine, California). A metal hook was inserted into the apex of the heart to control and record tension and heart rate. Tension was measured with a UF1 dynamometer transducer (UF1 BS4, Harvard Apparatus, Les Ulis, France). Transducers were calibrated and connected to a ML118 bridge amplifier that fed into a Powerlab 8 SP high-performance data acquisition system (ADInstruments Ltd. by Physyp, Paris, France).

Heart mitochondrial function. MITOCNDRIAL RESPIRATORY STUDIES. Mouse heart was placed in isolation buffer A containing (in mmol/l): sucrose 300, TES 5, EGTA 0.2, pH 7.2 (4°C). The tissue was finely minced and homogenized by the use of a Kontes tissue grinder. After 800-g centrifugation for 5 min, supernatant was centrifuged at 8,800 g for 5 min. Mitochondrial pellet was resuspended in buffer A and centrifuged one more time at 8,800 g for 5 min. Protein concentration was determined according to the Bradford method. Purity and integrity of isolated mitochondria were assessed by measuring specific activities of nicotinamide adenine dinucleotide phosphate-cytochrome c reductase, as an endoplasmic reticulum marker enzyme, and cytochrome c oxidase, as an inner membrane marker enzyme (19).

For respiration studies, 200 μg/ml mitochondria were suspended in respiration medium MiRo5 (20). The following parameters of mitochondrial respiration (Oroboros, Innsbruck, Austria) were evaluated: state 4 respiration rate (oxygen...
uptake with glutamate 5 mmol/l malate 2 mmol/l in the absence of exogenous ADP; pmol oxygen/s/mg); state 3 respiration rate (oxygen uptake during ADP 1 mmol/l phosphorylation; pmol oxygen/s/mg); and respiratory control ratio (RCR): ratio of state 3 and state 4 oxygen uptake rates. In addition, we distinguish respiration with glutamate malate as flux through the respiratory chain from complex I to IV and respiration with $N,N',N''-\text{tetramethyl-p-phenylenediamine} \text{(TMPD)} \text{ (0.5 mmol/l) + ascorbate (2 mmol/l) (after rotenone complex I inhibition) as maximum flux through the isolated step of complex IV (20)}$.

MITOCHONDRIAL PERMEABILITY TRANSITION AND TRANSMEMBRANE POTENTIAL. These parameters were assessed following Ca$^{2+}$ overload as previously described (14,21). In

![Graph A](image1)

**Figure 1.** Survival rate of different groups of mice: survival rate of mice undergoing cecal ligation and puncture without treatment (CLP) was compared with that of animals treated with 2, 10, and 100 mg/kg cyclosporine A (A) or N-methyl-4-isoleucine cyclosporine (NIM811) (B). Mice overexpressing B cell leukemia (Bcl)-2 had a survival rate of 80% (C). Sample size is 12 in each group. Survival studies were conducted twice. *p < 0.05, sham versus treatment or transgenic CLP mice; †p < 0.05, CLP versus treatment or transgenic CLP mice, by Kaplan-Meier, log-rank.
brief, isolated mitochondria (1 mg/ml proteins) were suspended in buffer C (in mmol/l): sucrose 250; Tris-MOPS 10; glutamate-Tris 5; malate-Tris 2; Pi-Tris 1; EGTA-Tris 0.02; pH 7.4 at 25°C in a multiport measurement chamber (NOCHM-4, WPI, Aston, United Kingdom) equipped with Ca$^{2+}$, tetraphenylphosphonium (TPP$^+$)-selective microelectrodes, and reference electrodes (WPI), as previously described (14,21). First, mitochondria were gently stirred for 1.5 min in buffer C containing 1.5 μmol/l TPP$^+$ (Sigma, Saint Quentin Fallavier, France). At the end of the pre-incubation period, 10 μmol/l CaCl$_2$ administration was performed every 90 s with a micro syringe injector adapted to a Micro4 pump controller (UMPII and Micro4, WPI). Each 10-μmol/l CaCl$_2$ pulse was detected as a peak of extramitochondrial Ca$^{2+}$ concentration. The Ca$^{2+}$ is then very rapidly taken up by the mitochondria resulting in a return of extramitochondrial Ca$^{2+}$ concentration to near baseline level (13,14). The mitochondrial transmembrane potential was estimated by calculating the transmembrane distribution of TPP$^+$. Transmembrane potential $\Delta\psi_{tm}$ was calculated as $59\log(v/V) - 59\log(10^{E/59} - 1)$, where $v$ is matrix volume (1.1 μl/mg mitochondrial protein), $V$ is volume chamber (1 ml), and $E$ is voltage difference before and after permeability transition pore opening and expressed in mV (21).

**Lung injury.** Mice were anesthetized (sodium pentobarbital 65 mg/kg IP) for bronchoalveolar lavage fluid (BALF), lung tissue samples, and histological studies. In the first series of experiments, the lungs were harvested, weighed, and then dried to constant weight at 80°C over 48 h in an oven. To assess tissue edema, the wet/dry ratio was calculated. In the second series of experiments, BALF was harvested from the lung after tracheal intubation (percentage of fluid recovered: from 85% to 90%). Protein concentration in BALF was measured with the Bradford method. In the third series of experiments, lungs were harvested for myeloperoxidase (MPO) activity, which was used as an index of leukocyte infiltration. Briefly, after lung homogenate preparation, MPO activity was determined spectrophotometrically (650 nm) by measuring hydrogen peroxide-dependent oxidation of 3,3',5,5'-tetramethylbenzidine (22). In the last series of experiments, lungs were fixed, embedded in paraffin, and the sections were stained with hematoxylin and eosin. Two observers, unaware of the nature of the experiment, scored the lung injury under light microscopy from 0 (no damage) to 4 (maximum damage), according to the combined assessment of alveolar congestion, hemorrhage, edema, infiltration/aggregation of neutrophils in the airspace or vessel wall, thickness of the alveolar wall, and hyaline membrane formation.

**Plasma levels of nitrite/nitrate and immunoblotting.** Plasma levels of nitrite/nitrate, an indicator of nitric oxide synthesis, were measured by the Griess reaction, as previously described (22).

Isolated mitochondrial protein (50 μg) was run on a 12% sodium dodecyl (lauryl) sulfate-polyacrylamide gel (SDS-PAGE). The proteins in the gel were electrophoretically transferred to nitrocellulose membranes. After blocking, membranes were treated with a rabbit polyclonal anti Bcl-2 antibody (Santa Cruz Biotechnology, California). Membranes were incubated with horseradish peroxidase-conjugated sheep anti-rabbit immunoglobulin G (IgG) secondary antibody (Biorad, Marnes-la-Coquette, France). Membranes were then washed, and bound antibodies were detected by the use of ECL Plus kit (Amersham Biosciences Europe GmbH, Freiburg, Germany) (23).

**Caspase-like activities.** After incubation in assay buffer containing (in mmol/l) HEPES 50, NaCl 100, EDTA 1,
DTT 10, with CHAPS 0.1% and Glycerol 10%, and in (μg/ml) aprotinin 10, leupeptin 10, and pepstatin 10 at pH 7.32, homogenized hearts were lysed with a Kontes Glass. Proteins (200 μg) were diluted in 200 μl of assay buffer; then either N-acetyl-Asp-Glu-Val-Asp-AMC (7-amino-4-methylcoumarin) or N-acetyl-Leu-Glu-His-Asp-AMC (7-amino-4-methylcoumarin) (50 μmol/l; Biomol, Plymouth Meeting, Pennsylvania) was added. Sample fluorescence (excitation: 380 nm; emission 437 nm) was measured at 2 h (Spex Fluoromax, Is-à-Horiba, Longjumeaux, France) (23).

Statistical analysis. Results were analyzed with the SPSS for Windows software, version 11.0.1 (SPSS France, Paris-la-Défense, France). Data represent means ± SEM and were analyzed by analysis of variance procedures. When a significant difference was found, we identified specific differences between groups with a sequentially rejective Bonferroni procedure. After application of the Bonferroni correction, p < 0.05 was taken as a level of statistical significance. Survival was evaluated with Fisher exact test.

RESULTS

Sepsis model characteristics. In an independent series of experiments (n = 12 septic mice), we observed that wild-type mice began to die between 6 and 12 h after CLP surgery. Maximum mortality rate (75%) was observed 48 h after CLP and remained stable at 96 h. Mortality rate of 50% was achieved at 18 to 24 h. Consequently, physiological and biological studies were performed at this time point. At the time of sacrifice, all CLP mice exhibited clinical signs of sepsis, including severe hypoactivity and shivering. The amount of Bcl-2 in heart mitochondria of C57Bl/6 mice and Bcl-2 transgenic mice was assessed by Western blot analysis. The Bcl-2 mitochondrial protein levels of transgenic mice were three-fold higher than wild-type mice (data not shown).

Cyclosporine treatment and Bcl-2 overexpression improved survival in septic mice. Survival studies included 12 mice in each treatment group and were performed twice. To determine the effects of immunosuppressive and non-immunosuppressive analogs of cyclosporine, CsA, NIM811, and tacrolimus were injected in saline immediately after CLP surgery. Sham mice received equal doses of saline. As shown in Figures 1A and 1B, CsA (2 mg/kg) and NIM811 (2 mg/kg) fully protected mice against CLP-induced mortality. A consistent level of protection was obtained with CsA (10 mg/kg) and NIM811 (10 mg/kg), whereas a 100-mg/kg-dose regimen had deleterious effects on mortality rate in CLP mice (Figs. 1A and 1B). Tacrolimus FK 506 (a potent immunosuppressive drug that has no effects on mitochondrial permeability transition) (2 mg/kg) had deleterious effects on CLP-induced mortality (mortality rate < 8%; data not shown). Cyclosporine analogs had no effects on mortality rate in sham mice (Figs. 1A and 1B). In a separate series of experiments, CLP was initiated in Bcl-2 transgenic mice and wild-type mice as controls. The difference in survival between these two groups was apparent, because only 25% of the wild-type mice compared with 80% of the Bcl-2 transgenic mice were still alive 96 h after CLP (Fig. 1C).

Cyclosporine and Bcl-2 overexpression improved organ dysfunction in septic mice. Baseline heart contractile function was similar in wild-type and Bcl-2 transgenic mice (Figs. 2A and 2B). Cecal ligation and puncture induced significant myocardial contractile dysfunction in wild-type mice compared with wild-type sham mice. Treatments with cyclosporine analogs (CsA and NIM811; 2 mg/kg) reduced CLP-induced contractile dysfunction (Fig. 2A). The Bcl-2 transgenic mice were also protected against CLP-induced contractile dysfunction (Fig. 2B). Hearts from CLP-treated Bcl-2 transgenic mice (Fig. 2B) showed major increases in maximal, developed left ventricular pressure and dP/dt max compared with CLP wild-type mice.

Effects of cyclosporine analogs (CsA and NIM811; 2 mg/kg) in CLP mice were evaluated on BALF protein concentration and lung wet/dry weight ratio, MPO activity, and histopathologic grading (Table 1). Treatments with CsA and NIM811 (2 mg/kg) in sham had no effects on the aforementioned lung function parameters (data not shown). Cyclosporine A and NIM811 (2 mg/kg) largely prevented CLP-induced increases in BALF protein concentration, lung MPO activity, and histological injury score (Table 1).

Cyclosporine and Bcl-2 overexpression improved mitochondrial function in septic heart. First, high purity and outer membrane integrity of isolated mitochondria were measured as undetectable activity levels of, respectively, NADPH-cytochrome c reductase, a specific reticulum

<table>
<thead>
<tr>
<th>Table 1. Lung Injury Parameters in Sham and CLP Septic Mice</th>
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<tr>
<td>BALF* protein (μg/ml)</td>
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<tr>
<td>Lung wet/dry ratio</td>
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<tr>
<td>Lung MPO (OD550)</td>
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<tr>
<td>Histology lung injury score</td>
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Score (0–4), on the basis of edema, hemorrhage, thickening of the alveolar wall, and infiltration of inflammatory cells into alveolar spaces. Sample size is 6 in each group. Results were analyzed with one-way analysis of variance and Bonferroni’s multiple comparison post hoc adjustment. *Bronchoalveolar lavage fluid. †p = 0.08 versus sham mice (Bonferroni adjusted; six comparisons). ‡p = 0.008 versus CLP mice (Bonferroni adjusted; six comparisons).

BALF = bronchoalveolar lavage fluid; CLP = cecal ligation and puncture; CsA = cyclosporine A; MPO = homogenate myeloperoxidase activity; NIM811 = N-methyl-4-isoleucine cyclosporine; OD = optical density.
marker enzyme, and cytochrome c oxidase, an inner membrane marker enzyme.

In C57Bl/6 wild-type CLP mice, RCR (increases in state 4 and reduction in state 3) at 18 h was reduced with no changes in maximum flux through the isolated step of complex IV dependent respiration (Table 2). In CLP mice, cyclosporine analog treatments and Bcl-2 overexpression resulted in improvement of RCR. Cyclosporine A reduced state 4 respiration and partially increased state 3 respiration, with no changes in maximum oxygen flux. The NIM811 treatment and Bcl-2 overexpression resulted in major increases in state 3 respiration, state 4 respiration, and maximum oxygen flux. In sham mice, cyclosporine analog-treated sham mice, and Bcl-2 transgenic sham mice, no changes in respiration parameters were observed 18 h after surgery (data not shown). In the cyclosporine analog-treated sham mice and Bcl-2 transgenic sham mice, mitochondrial membrane potential averaged $-220 \text{ mV}$ (data not shown).

In CLP mice, mitochondrial membrane potential was unstable and averaged $-190 \text{ mV}$ (Table 2). Cyclosporine A or NIM811 CLP mice and CLP Bcl-2 transgenic mice had stable mitochondrial membrane potential, which averaged $-210 \text{ mV}$ (Table 2).

Representative examples of a mitochondrial permeability transition recording are displayed in Figure 3. The amount of Ca$^{2+}$ added in the cuvette that was required to open the pore averaged 200 $\mu$mol/l in the sham mice, which corresponded to 200 nmol/mg mitochondrial protein (Fig. 4). A similar amount of Ca$^{2+}$ was required to open the pore in cyclosporine analog-treated sham and Bcl-2 transgenic sham mice (data not shown). The Ca$^{2+}$ overload was significantly reduced in CLP mice, averaging 60 $\mu$mol/l (Fig. 4). In CsA-treated or NIM811 CLP-treated mice and in CLP Bcl-2 transgenic mice, the Ca$^{2+}$ overload required

**Table 2.** Respiration Parameters and Membrane Potential of Isolated Heart Mitochondria

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CLP</th>
<th>CLP CsA</th>
<th>CLP NIM811</th>
<th>CLP BCL-2</th>
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<tbody>
<tr>
<td>State 4</td>
<td>37 ± 3</td>
<td>48 ± 2*</td>
<td>36 ± 3†</td>
<td>69 ± 8†</td>
<td>86 ± 13†</td>
</tr>
<tr>
<td>State 3</td>
<td>470 ± 77</td>
<td>299 ± 70*</td>
<td>434 ± 29†</td>
<td>555 ± 34†</td>
<td>719 ± 20†</td>
</tr>
<tr>
<td>RCR</td>
<td>9.7 ± 0.7</td>
<td>6.25 ± 0.3*</td>
<td>8.2 ± 1.3†</td>
<td>8.0 ± 0.2†</td>
<td>8.4 ± 0.9†</td>
</tr>
<tr>
<td>Ascorbate TMPD</td>
<td>1688 ± 102</td>
<td>1782 ± 351</td>
<td>1432 ± 251</td>
<td>2302 ± 205†</td>
<td>2689 ± 324†</td>
</tr>
<tr>
<td>Δψm</td>
<td>−220 ± 6</td>
<td>−190 ± 12*</td>
<td>−207 ± 2</td>
<td>−208 ± 2</td>
<td>−215 ± 2</td>
</tr>
</tbody>
</table>

Sample size is 12 in each group. Results were analyzed with one-way analysis of variance and Bonferroni’s multiple comparison post hoc adjustment. *p < 0.005 versus sham mice (Bonferroni adjusted; ten comparisons). †p < 0.005 versus CLP mice (Bonferroni adjusted; ten comparisons).

Ascorbate TMPD = N,N,N,N′-tetramethyl-p-phenylenediamine respiration rate (pmol oxygen/s/mg) with ascorbate (2 mmol/l) and TMPD (0.5 mmol/l) after rotenone complex I inhibition; Δψm = mitochondrial membrane potential (mV); RCR = respiratory control ratio of state 3 and state 4 respiration rates; State 3 = respiration rate (pmol oxygen/s/mg) with adenosine diphosphate (ADP) (1 mmol/l); State 4 = respiration rate (pmol oxygen/s/mg) with glutamate (5 mmol/l) malate (2 mmol/l) in absence of exogenous ADP; other abbreviations as in Table 1.
for pore opening significantly increased when compared with CLP mice (Fig. 4).

**Effects of cyclosporine and Bcl-2 overexpression on CLP-induced plasma nitrite/nitrate levels and effector caspase activation.** Plasma levels of nitrite/nitrate, an indicator of NO synthesis, were not increased in CLP mice at 18 h (nitrite/nitrate 15 ± 3 μmol/l in sham vs. 22 ± 8 μmol/l in CLP mice). Neither cyclosporine analogs (i.e., CsA and NIM811) nor Bcl-2 overexpression resulted in changes in plasma nitrite/nitrate levels in CLP mice (nitrite/nitrate 18 ± 4 μmol/l, 16 ± 7 μmol/l, and 24 ± 3 μmol/l, respectively). Heart caspase-9-like activities, which were largely prevented by cyclosporine analog treatment and Bcl-2 overexpression in septic mice (Fig. 5), increased in CLP mice at 18 h. Similar caspase-3-like activity increases were detected in CLP mice at 18 h; these were also prevented by cyclosporine analog treatment and Bcl-2 overexpression.

**DISCUSSION**

Although regional and microvascular perfusion abnormalities undoubtedly occur (1,2), recent investigations indicate that metabolism is also altered at the cellular level during sepsis, which might result in impaired cellular use of oxygen and organ dysfunction (3). Because mitochondria use >90% of total body oxygen consumption to generate adenosine triphosphate, function of vital organs such as the heart could be impaired as a consequence of abnormalities in mitochondrial bioenergetic processes. Here, we report the new, important information that improvement of mitochondrial function via permeability transition inhibition reduces heart and lung dysfunction and mortality in septic mice. To the best of our knowledge, the present report is the first to demonstrate that mitochondrial permeability transition might be directly responsible for vital organ dysfunction and mortality in chronic septic in mice.

The immunosuppressant drug CsA, which interacts with cyclophilin D, inhibits mitochondrial permeability transition (14,15). In isolated mitochondria, CsA prevents swelling and increases mitochondrial calcium retention capacity (11–13). In vivo, the inhibitory effect of CsA emphasized the critical importance of permeability transition pore in organ injury induced by ischemia reperfusion. At micromolar concentrations, CsA might significantly decrease infarct size in ischemia reperfusion of the heart (14,15) and the brain (24), whereas millimolar concentrations might cause organ toxicity though excessive free radical generation (25). Likewise, findings from our study and from other groups (26) suggest that treatment with high doses of CsA exacerbates mortality in CLP septic mice. Conversely however, we (27) and other groups (6,28) have reported that lower concentrations of CsA might attenuate tissue injury and abnormalities in mitochondrial function and protect against endotoxin-mediated myocardial dysfunction. Here, we provide the first evidence that cyclosporine derivatives and Bcl-2 overexpression fully afforded CLP septic mice protection against heart and lung derangements, which was associated with increased survival rate.

Among the deleterious changes induced by sepsis on vital organ function, impaired myocardial performance is a well-documented feature that greatly contributes to mortality (1,2). Although sepsis is generally viewed as a disease aggravated by an inappropriate inflammatory response to bacteria, implication of apoptosis has been recently demonstrated in the pathogenesis of sepsis (29). Moreover, we have demonstrated that activation of heart apoptosis pathways might directly impede ventricular contractile function via caspase-induced sarcomere disarray (23). Here, our results suggest that mitochondrial permeability transition, a major event in apoptotic mitochondrial pathway (10), is implicated in septic ventricular contractile dysfunction via abnormalities in heart mitochondrial bioenergetics. Mitochondrial permeability transition and dissipation of membrane potential induced by CLP sepsis were associated with impaired respiratory capacity under phosphorylating conditions (state 3) and respiration increases under non-phosphorylating conditions (state 4), which lead to reduced RCR (state 3/state 4 respiratory rates). In contrast, reductions in RCR were prevented by cyclosporine analogs and overexpression of Bcl-2; this indicates global improvement of mitochondrial functional integrity. Our results are thus consistent with recent studies suggesting that sepsis-mediated mitochondrial injury correlates with impaired respiration efficacy of
mitochondria, which was prevented by cyclosporine derivatives (6,28).

Limitations of the study are mainly that treatment with cyclosporine analogs and Bcl-2 overexpression might improve organ dysfunction and survival in sepsis through mechanisms independent of mitochondrial permeability transition. For example, overexpression of Bcl-2 and CsA treatment might be beneficial in different models of sepsis via reduction in proinflammatory mediator synthesis and lymphocyte apoptosis. Here, we observed that neither cyclosporine analogs nor overexpression of Bcl-2 in CLP mice altered plasma nitrite/nitrate and TNF-alpha (S. Lancel, unpublished data, January 2005) levels. Beneficial effects of treatment with cyclosporine analogs could also be attributed to their known effects on calcineurin pathways. However, the fact that CsA and NIM811 (a non-immunosuppressive drug that inhibits mitochondrial permeability transition) but not tacrolimus (a potent immunosuppressive drug that has no effects on mitochondrial permeability transition) were able to offer protection suggests that reduction in sepsis-induced myocardial dysfunction and mortality rate is related to the unique effects of cyclosporine derivatives on the mitochondrial function. We have shown that, during endotoxemia, increased heart caspase-3-like activity could participate directly in myocardial dysfunction. Here, increases in caspase-9- and -3-like activities were detected in the hearts of CLP mice. Reduction in caspase-3-like activities in CLP mice treated with cyclosporine analogs and overexpression of Bcl-2 could account at least in part for observed beneficial effects on heart function.

In conclusion, our study provides strong evidence that septic vital organ dysfunction can be prevented by inhibiting mitochondrial permeability transition. Regarding the critical role of heart failure in the pathophysiology of septic shock, our study also indicates a potentially new therapeutic approach for treatment of sepsis syndrome.
REFERENCES


