Extensive apoptosis of leukocytes during sepsis and endotoxic shock constitutes an important mechanism linked to the excessive mortality associated with these disorders. Caspase inhibitors confer protection from endotoxin-induced lymphocyte apoptosis and improve survival, but it is not clear which caspases mediate lipopolysaccharide (LPS)–induced lymphocyte apoptosis and mortality. We report here that the apoptotic executioner caspase-7 was activated in the splenocytes of LPS-injected mice, suggesting a role for caspase-7 in lymphocyte apoptosis. Indeed, caspase-7–deficient mice were resistant to LPS-induced lymphocyte apoptosis and were markedly protected from LPS-induced lethality independently of the excessive production of serum cytokines. These results reveal for the first time a nonredundant role for caspase-7 in vivo and identify caspase-7 inhibition as a component of the mechanism by which caspase inhibitors protect from endotoxin-induced mortality. (Blood. 2009;113:2742-2745)

Introduction

Sepsis is the most common cause of mortality in patients treated in the intensive care setting, with more than 210 000 sepsis-related deaths occurring annually in the United States. Extensive apoptotic death of leukocytes is commonly observed in patients who died of sepsis and was suggested to contribute significantly to immune suppression and lethality. In this regard, synthetic caspase inhibitors and overexpression of the antiapoptotic protein Bcl-2 were shown to diminish lymphocyte apoptosis and improve survival in experimental sepsis models. However, it is currently incompletely understood which caspases promote lymphocyte apoptosis and contribute to lethality. Together with caspase-3, the executioner caspase-7 performs central roles in the execution phase of apoptosis by cleaving a large set of substrates, ultimately resulting in the morphologic and biochemical hallmarks of apoptosis such as DNA fragmentation. Caspase-3/-7 double-deficient mice were recently shown to exhibit embryonic lethality, whereas mice singly deficient in either caspase are born at normal Mendelian ratios and display no gross abnormalities when maintained on a C57BL/6 genetic background. At this stage, the precise roles of caspase-7 in the adult animals remain to be elucidated.

In this study, we show that caspase-7 was activated in splenocytes of lipopolysaccharide (LPS)–treated mice and that caspase-7–deficient mice were resistant to LPS-induced lymphocyte apoptosis. As a result, caspase-7 deficiency improved survival during endotoxemia without affecting cytokine levels.

Methods

Mice

Caspase-1−/−, caspase-3−/−, and caspase-7−/− mice were backcrossed to C57BL/6 background for 10 generations and have been described previously. Mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in a pathogen-free facility. The animal studies were conducted under protocols approved by St Jude Children’s Research Hospital Committee on Use and Care of Animals.

LPS-induced shock

Mice (6-8 weeks old) were injected intraperitoneally with either 20 or 30 mg kg−1 LPS from Escherichia coli (serotype 0111:B4; Sigma-Aldrich, St Louis, MO). The mice were monitored for signs of endotoxemia and lethality daily for 7 days. Differences in group survival were analyzed with the Kaplan-Meier test using Prism5 (GraphPad Software, La Jolla, CA). A value of P less than .05 was considered statistically significant.

Histology and apoptosis quantification

Caspase-7−/− and caspase-7−/− mice were sham-operated or injected with LPS in the peritoneum. Spleens were collected 24 hours later, fixed overnight at 4°C in 10% buffered formalin, and embedded in paraffin. Apoptotic lymphocytes were quantified both by light microscopy read of hematoxylin and eosin (H&E)–stained sections and by counting terminal dUTP nick end labeling (TUNEL) staining. Image acquisition was performed at room temperature on an Olympus BX41 microscope fitted with Olympus UPlan 20×/0.75 NA objective (Olympus America, Center Valley, PA) and equipped with a Spot Insight 3.2 digital camera and corresponding acquisition software (Diagnostic Instruments, Sterling Heights, MI). Data were analyzed with the Student t test. A value of P less than .05 was considered statistically significant.

Western blotting

Mice (6-8 weeks old) were injected intraperitoneally with 20 mg kg−1 LPS from E coli (serotype 0111:B4; Sigma-Aldrich). Spleens were collected and splenocyte extracts were transferred to nitrocellulose membranes, immunoblotted with primary antibodies, and proteins were detected by enhanced chemiluminescence. Antibodies against active caspase-3, active caspase-7, and Grb2 were purchased from Cell Signaling Technology (Danvers, MA).
The antibody against caspase-1 was kindly provided by Dr Peter Vandena- 
bbee (Ghent University, Zwijnaarde, Gent, Belgium).

Measurement of cytokines

Serum cytokines and chemokines were measured with Multiplex assay 
(Bio-Rad, Hercules, CA). Data were analyzed with the Student t test. 
A value of \( P \) less than .05 was considered statistically significant.

Results and discussion

Extensive lymphocyte apoptosis is evident in the spleen, intestinal 
lamina propria, and in lymphoid organs of patients who died of 
sepsis\(^2,19\) and studies that used Bcl-2-overexpressing mice\(^7,8,11\) and 
synthetic caspase inhibitors\(^8,12\) demonstrated its importance for 
shock-induced lethality. Lethal endotoxia is a widely used 
experimental model that mimics many features of septic shock, 
including elevated cytokine production and extensive leukocyte 
apoptosis.\(^8,20\) However, it is currently not well understood which 
caspases contribute to endotoxia-associated lymphocyte apoptosis. 
To study whether caspase-7 was implicated in endotoxin-
induced lymphocyte apoptosis, spleens of caspase-7\(^{-/-}\) and caspase-
7\(^{-/-}\) mice were collected 24 hours after intraperitoneal LPS 
jection (20 mg kg\(^{-1}\)). Microscopic analysis of H&E-stained 
sections showed that the morphology of a significant number of 
lymphocytes in the splenic white pulp of caspase-7\(^{-/-}\) mice was 
consistent with apoptosis (Figure 1A top panel). Notably, this 
apoptotic phenotype was markedly reduced in the splenic white 
pulp of caspase-7\(^{-/-}\) mice (Figure 1A bottom panel). To quantify 
these differences, apoptotic lymphocytes in spleens of wild-type 
and caspase-7\(^{-/-}\) mice were stained with TUNEL. As expected, a 
significant number of lymphocytes stained positive for TUNEL in 
spleen of LPS-treated caspase-7\(^{-/-}\) mice (Figure 1B top panel). In 
contrast, splenic lymphocytes of caspase-7\(^{-/-}\) were markedly 
protected from endotoxin-induced apoptosis (Figure 1B bottom 
panel). The number of TUNEL-positive lymphocytes in each 
genotype was quantified by counting the number of apoptotic cells 
in 5 randomly chosen high-power fields (\( \times 400 \)) in the splenic 
white pulp of each mouse (\( n = 4 \)). The number of apoptotic 
lymphocytes was significantly (\( P = .005 \)) lower in caspase-7\(^{-/-}\) 
mice compared with LPS-treated caspase-7\(^{-/-}\) mice (Figure 1C). 
These results demonstrate that caspase-7 is essential for endotoxin-
induced lymphocyte apoptosis in vivo.

Binding of LPS to receptors on leukocytes triggers the production 
of potent proinflammatory cytokines such as interleukin-\( \beta \) 
(IL-\( \beta \)), IL-18, tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)), and IL-6 as well as 
chemokines such as CXCL1/KC and CXCL2/MIP-\( \alpha \).\(^{21,22}\) 
These cytokines and chemokines are believed to be important 
mediators of organ injury in endotoxic shock.\(^{20,21,23}\) Therefore, we 
addressed whether caspase-7 mediates lymphocyte apoptosis 
directly by regulating the secretion of proinflammatory cytokines 
and chemokines. After endotoxin challenge, we observed that amounts 
of all cytokines and chemokines measured in the serum of 
caspase-7\(^{-/-}\) mice were not significantly different from those 
found in the serum of wild-type mice (Table 1). These results 
indicate that caspase-7 is not required for the secretion of proinflam-
matory cytokines and chemokines during endotoxia and suggest 
that caspase-7 contributes to splenocyte apoptosis independent of 
 systemic cytokine release. Indeed, an antibody directed against 
active caspase-7 confirmed the potent activation of caspase-7 in 
splenocytes of LPS-injected mice (Figure 1D). Caspase-7 activa-
tion was not observed in splenic extracts of phosphate-buffered 
saline (PBS)-injected mice, indicating that caspase-7 activation in 
splenocytes was associated with endotoxia. The absence of 
immunoreactive bands in splenic extracts of LPS-treated caspase-
7\(^{-/-}\) mice confirmed that the antibody was specific for caspase-7 
and did not cross-react with other caspases. In addition to 
caspase-7, caspase-3 and the inflammatory caspase-1 also were 
averted in splenic extracts of LPS-challenged mice (Figure 1E). 
Caspase-1 and caspase-3 activation were associated with endoto-
xemia because processing was not observed in splenic extracts of 
sham-operated mice.

To examine the survival of caspase-7\(^{-/-}\) mice when challenged 
with a lethal dose of LPS, wild-type and caspase-7\(^{-/-}\) mice were 
jected with 20 mg kg\(^{-1}\) of LPS intraperitoneally. By 72 hours, all 
caspase-7\(^{-/-}\) mice survived, whereas approximately 60% of the
wild-type mice succumbed to LPS administration (Figure 1F). Caspase-7 activation in LPS-activated macrophages was recently demonstrated to require the caspase-1 inflammasome.24 To compare the resistance of caspase-1−/− and caspase-7−/− mice, a cohort of age-matched caspase-1−/− mice was challenged with 20 mg kg−1 of LPS. In line with the reported resistance of caspase-1−/− mice,18 all caspase-1−/− mice survived the insult (data not shown). We repeated the experiment with an increased dose of 30 mg kg−1 of LPS and included a cohort of caspase-3−/− mice to allow comparison across the different genotypes. The complete caspase-1−/− group survived the increased LPS dose, whereas the survival rate was approximately 50% for the cohort of caspase-7−/− mice (Figure 1G). In contrast to caspase-7−/− mice, all caspase-3−/− deficient mice succumbed at a rate comparable with that observed with wild-type mice (Figure 1G). These results demonstrate that the absence of caspase-7 confers significant protection against LPS-induced mortality and support the notion that caspase-3 and caspase-7 are functionally distinct in the adult animal.16,24 In addition, caspase-1−/− mice were significantly (P = 0.01) more resistant to endotoxemia compared with caspase-7−/− mice. One explanation is that in addition to the reduced lymphocyte apoptosis shared with caspase-7−/− mice,10 proinflammatory cytokine levels are significantly attenuated in caspase-1−/− mice18,25 but unaffected in caspase-7−/− mice (Table 1). Caspase-1 may therefore function upstream of caspase-7 during endotoxemia, as recently observed in LPS-activated macrophages.24 Alternatively, a caspase-1–independent mechanism may account for the activation of caspase-7 in LPS-challenged mice.

Regardless, we showed here that caspase-7−/− mice display a marked protection against LPS-induced lymphocyte apoptosis and endotoxemia-associated mortality despite elevated cytokine levels. These results demonstrate for the first time a nonredundant in vivo role for caspase-7 and identify caspase-7 inhibition as a potential mechanism by which caspase inhibitors protect from endotoxin-induced lymphocyte apoptosis and lethality. Indeed, studies that use Bcl-2–overexpressing mice and synthetic caspase inhibitors identified leukocyte apoptosis as a major contributor of sepsis- and endotoxemia-induced lethality.7-12 Thus, therapeutics targeting apoptosis of lymphoid tissues constitute promising new approaches for the treatment of sepsis and endotoxemia. The results presented here suggest caspase-7 inhibition as a reasonable approach that warrants further study.

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Authorship

Contribution: M.L. and T.-D.K. designed the research, performed experiments, analyzed data and wrote the paper; L.M., P.M., and D.C.J.S. performed experiments; histologic evaluation and immunohistochemistry were performed by J.A.D.; pathologic evaluation and immunohistochemistry were performed by J.A.D.; J.Y.C. and L.O.M. performed statistical analysis; F.C. and D.C.J.S. edited successive drafts of the manuscript.

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References


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Caspase-7 deficiency protects from endotoxin-induced lymphocyte apoptosis and improves survival