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Activation of Caspase 3 (CPP32)-Like Proteases Is Essential for TNF-α-Induced Hepatic Parenchymal Cell Apoptosis and Neutrophil-Mediated Necrosis in a Murine Endotoxin Shock Model

Hartmut Jaeschke,* Michael A. Fisher,* Judy A. Lawson,* Carol A. Simmons,* Anwar Farhood,† and David A. Jones3*  

Endotoxin (ET)-induced liver failure is characterized by parenchymal cell apoptosis and inflammation leading to liver cell necrosis. Members of the caspase family have been implicated in the signal transduction pathway of apoptosis. The aim of this study was to characterize ET-induced hepatic caspase activation and apoptosis and to investigate their effect on neutrophil-mediated liver injury. Treatment of C3Heb/FeJ mice with 700 mg/kg galactosamine (Gal) and 100 μg/kg Salmonella abortus equi ET increased caspase 3-like protease activity (Asp-Val-Glu-Asp-substrate) by 1730 ± 140% at 6 h. There was a parallel enhancement of apoptosis (assessed by DNA fragmentation ELISA and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay). In contrast, activity of caspase 1 (IL-1β-converting enzyme)-like proteases (Tyr-Val-Ala-Asp-substrate) did not change throughout the experiment. Caspase 3-like protease activity and apoptosis was not induced by Gal/ET in ET-resistant mice (C3H/HeJ). Furthermore, only murine TNF-α but not IL-1β increased caspase activity and apoptosis. Gal/ET caused neutrophil-dependent hepatocellular necrosis at 7 h (area of necrosis, 45 ± 3%). Delayed treatment with the caspase 3-like protease inhibitor Z-Val-Ala-Asp-CH₂F (Z-VAD) (10 mg/kg at 3 h) attenuated apoptosis by 81 to 88% and prevented liver cell necrosis (≤5%). Z-VAD had no effect on the initial inflammatory response, including the sequestration of neutrophils in sinusoids. However, Z-VAD prevented neutrophil transmigration and necrosis. Our data indicate that activation of the caspase 3 subfamily of cysteine proteases is critical for the development of parenchymal cell apoptosis. In addition, excessive hepatocellular apoptosis can be an important signal for transmigration of primed neutrophils sequestered in sinusoids. The Journal of Immunology, 1998, 160: 3480–3486.

Liver dysfunction and failure are common problems during endotoxemia and sepsis (1). It is generally accepted that proinflammatory cytokines, especially TNF-α, are critical for the pathophysiology (2–5). TNF-α has a wide variety of effects on leukocytes and liver cells that support inflammation; e.g., TNF-α is one of the mediators that induce up-regulation of the β2 integrin Mac-1 (CD11b/CD18) on neutrophils during endotoxia in vivo (6); TNF-α also primes Kupffer cells (7) and neutrophils (8) for release of cytoxic mediators. In addition, TNF-α activates the transcription factor NF-κB in endothelial cells and hepatocytes during endotoxia (9), which leads to the transcriptional activation of a number of proinflammatory genes, e.g., chemokines (10, 11), nitric oxide synthase (12, 13), and adhesion molecules such as ICAM-1 (14, 15), VCAM-1 (16), and selectins (9, 15). Therefore, TNF-α alone or in combination with IL-1 and complement is responsible for neutrophil sequestration in hepatic sinusoids during endotoxia and sepsis (14, 17). After transmigration, neutrophils attack parenchymal cells and cause severe liver cell necrosis (14, 16, 18). Thus, TNF-α is a critical early mediator for an acute inflammatory response in the liver during endotoxemia and sepsis.

TNF-α can act through binding to two different cell surface receptors, i.e., the 55-kDa TNF-R1 and the 75-kDa TNF-R2 (19, 20). Most of the proinflammatory effects of TNF-α are mediated through TNF-R1 (20). However, TNF-α can also induce apoptosis through the death domain of TNF-R1 (21). Recently, hepatocellular apoptosis has been characterized in various models of endotoxemia (22–25), and it was confirmed that this effect was mediated in vivo through TNF-R1 (25, 26). In all eukaryotic cells, the intracellular signal transduction pathway leading to apoptosis involves the activation of a cascade of cysteine proteases (caspases/ICE proteases) (27–29). Currently, there are 10 human caspases identified (30) with equivalent enzymes in the mouse (31). The activation of caspase 3 (CPP32)-like proteases in liver cells was observed during the development of apoptosis after various insults, i.e., anti-Fas Ab in vivo (32), TGF-β1 (33), staurosporine (33), and acetyl-Tyr-Val-Ala-Asp-CHO; CrmA, cowpox viral serpin cytokine response modifier A; Ac-YVAD-cmk, acetyl-Tyr-Val-Ala-Asp-chloromethylketone.

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* Abbreviations used in this paper: NF-κB, nuclear factor κB; ET, endotoxin; Gal, galactosamine; ALT, alanine aminotransferase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; ICE, IL-1β-converting enzyme; Z-VAD, Z-Val-Ala-Asp-CH₂F; Ac-DEVD-MCA, acetyl-Asp-Glu-Val-Asp-(4-methylcoumaryl)-7-amide; Ac-DEVD-Ald, acetyl-Asp-Glu-Val-Ala-CHO; Ac-YVAD-Ald, acetyl-Tyr-Val-Ala-Asp-CHO; CrmA, cowpox viral serpin cytokine response modifier A; Ac-YVAD-cmk, acetyl-Tyr-Val-Ala-Asp-chloromethylketone.

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The kinetics of the proteolytic cleavage of the substrates was monitored in a fluorescence microplate reader (F-max; Molecular Devices, Corp., Sunnyvale, CA) using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The fluorescence intensity was calibrated with standard concentrations of MCA, and the caspase activity was calculated from the slope of the recorder trace and expressed in picomols per minute per mg of protein. The protein concentrations in the supernatant were determined with the bichinchoninic acid kit (Sigma). For the inhibitor studies in vitro, Ac-DEVD-Asporchisensated TUNEL assay (Oncor, Gaithersburg, MD) and a MicroProbe staining station (Fisher Scientific, Pittsburgh, PA). Frozen liver samples were cryosectioned (6 μm; two sections/sample, 150 μm apart) using a Leitz 1720 cryostat (W. Nuhsbaum, McHenry, IL) and assayed for direct immunoperoxidase detection of digoxigenin-labeled genomic DNA. Staining was conducted according to the manufacturer’s kit protocol for fresh frozen tissue samples. DNase 1 treatment of additional sections, to nick all DNA, served as positive controls at a concentration of 1 μg/ml determined after a preliminary dilution experiment (Applegate DNase 1, Oncor). This was prepared and conducted according to manufacturer’s instructions. TUNEL-stained mouse liver tissue sections were quantitated for apoptotic cells using computer-assisted image analysis and Optimas software (Bioscan, Edmonds, WA). Visual images from TUNEL-stained slides were captured with a digital camera attached to a microscope with modifications of previously published methods (38). A color threshold was applied (black brown for diaminobenzidine) to match visualization of apoptotic cells. Ten random microscopic regions of interest (each covering a surface area of 0.55 mm²) were evaluated per tissue slice (two slides/sample) and for the number of apoptotic cells captured into computer memory and downloaded into Excel 4.0 (Microsoft Co, Redmond, WA) spreadsheets for statistical analysis.

For the cell death detection ELISA (Boehringer Mannheim, Indianapolis), a 20% homogenate (50 mM sodium phosphate buffer (120 mM NaCl, 10 mM EDTA) was prepared and centrifuged at 14,000 × g. Diluted supernatant was used for the ELISA. In this test, the kinetics of product generation (Vmax) is a measure of DNA fragmentation. The Vmax values obtained for untreated controls (100%) are compared with those in treated groups. The assay allows the specific quantitation of histone-associated DNA fragments (mono- and oligonucleosomes) in the cytoplasmic fraction of cell lysates and was used extensively to demonstrate apoptosis in the liver in vivo (22, 23, 26).

Isolation of mouse liver cells

Animals (n = 4 per group) were anesthetized with a ketamine mixture (225 mg/kg ketamine; 11.4 mg/kg xylazine; 2.3 mg/kg acepromazine) i.m. The liver was perfused free of blood in an open system for 5 to 10 min using an oxygenated Ca²⁺-free Hanks’ buffer. A collagenase-supplemented (25 mg/100 ml buffer) Hanks’ buffer was used to digest the liver. When digestion was obtained (~10 min), the liver was removed, minced, and strained through a tissue sieve. Cells were then centrifuged at 50 × g for 3 min. The supernatant (nonparenchymal cells) was removed and saved. The pellet (parenchymal cells) was resuspended in Hanks’ buffer and spun at 50 × g for 3 min. The supernatant was combined with the supernatant from the first spin, and the pellet was resuspended. Cell fractions were then spun at 600 × g for 10 min. The supernatants were discarded, and the nonparenchymal pellet was resuspended in pronase buffer (200 mg/50 ml buffer) and stirred for 10 min to remove any hepatocytes in the suspension.
cells in controls (about 0.2% of hepatocytes) and no significant change up to 4 h after Gal/ET treatment (Fig. 3). However, at 5 and 6 h, the number of apoptotic cells increased dramatically reaching 13% at 6 h (65-fold increase). These data indicate that the increase of proapoptotic caspase activity correlated with the increase of apoptotic cell death in this model. Because the intact tissue did not allow us to distinguish which liver cell types had been affected, parenchymal cells (hepatocytes) and nonparenchymal cells (mixture of Kupffer cells and endothelial cells) were isolated 5.5 h after Gal-ET administration. Measurement of DEVD-MCA caspase activity and DNA fragmentation showed significant increases only in hepatocytes but not in the nonparenchymal cell fraction (Fig. 4).

To further characterize the type of caspase involved, inhibitor studies were performed using liver homogenate of Gal/ET-treated animals (6 h). The inhibitor of caspase 3-like proteases, Ac-DEVD-Ald, dose dependently inhibited the enzyme activity in the liver (Fig. 5). An IC_{50} of 16.75 nM was calculated from these data. In contrast, the caspase 1 inhibitor Ac-YVAD-Ald or CrmA, an effective inhibitor of caspase 1 and the proapoptotic caspase 8 had no or very limited effects on Gal/ET-induced protease activity in the liver. The extrapolated IC_{50} for YVAD-Ald was 19.6 μM.

To identify the critical mediators involved in the activation of caspases in vivo, ET-resistant animals, which do not generate cytokines in response to ET, were treated with Gal/ET. In contrast to the sensitive animals, ET-resistant animals did not show enhanced caspase 3-like protease activity or evidence for increased DNA fragmentation (Table I). Injection of TNF-α, IL-1α, or IL-1β in combination with Gal indicated that only Gal/TNF-α was able to induce an increase in caspase 3-like protease activity and apoptosis in ET-resistant (Table I) and in ET-sensitive animals (data not shown). These findings suggest that ET-induced proapoptotic caspase activation and apoptotic cell death in hepatocytes is mediated by TNF-α.

Because we previously provided evidence that neutrophils are critical for ET-induced hepatocellular necrosis in this model, we tested the hypothesis that caspase activation and apoptosis are relevant for the inflammatory response. A large group of animals was treated with Gal/ET. After 3 h, i.e., at a time when TNF-α has been generated and TNF-α-mediated inflammatory responses were initiated (NF-κB activation, transcription of adhesion molecules) (2, 6, 9, 14, 16, 18), animals either were left untreated (disease control) or were injected with the caspase inhibitor Z-VAD in DMSO (10 mg/kg) or with DMSO alone (1 ml/kg). Z-VAD or vehicle treatment was repeated twice at 90-min intervals. Gal/ET administration caused severe liver injury at 7 h as indicated by a significant increase in plasma ALT activities and widespread hepatocellular necrosis (45% of hepatocytes) (Fig. 6a). Z-VAD, in contrast to the vehicle DMSO, effectively inhibited liver injury. Necrotic cell injury was almost eliminated; i.e., only few individual hepatocytes could be identified as necrotic. In contrast, Z-VAD did not reduce the overall number of neutrophils in the liver (Fig. 6b). However, virtually all neutrophils (>95%) remained localized within sinusoids. In the vehicle group, 30 to 35% of neutrophils were extravasated. To demonstrate the effect of Z-VAD treatment on caspase activity and apoptosis, the experiment was repeated, and the animals were killed at 6 h (before the onset of massive hemorrhage and necrosis). Z-VAD completely inhibited caspase 3-like protease activity in the liver but had no effect on the baseline caspase 1 activity (Fig. 7). Z-VAD treatment also significantly reduced the increase in apoptotic cell death as determined by two independent criteria, DNA fragmentation (81% inhibition) and TUNEL assay (88% reduction of apoptotic cells) (Table II).
Discussion

The principal objective of this investigation was to study the role of caspase activation in hepatocellular apoptosis and neutrophil-induced necrosis during ET-mediated liver injury in vivo. Our data demonstrated a substantial increase of caspase activity with DEVD-MCA, a substrate of caspase 3-like proteases. However, no increase of activity was seen with YVAD-MCA, a substrate more specific for the caspase 1 subfamily. Furthermore, the enhanced caspase activity could be inhibited by DEVD-Ald but not by YVAD-Ald or CrmA. These data suggest that in the Gal/ET model in vivo, there is a predominant activation of the caspase 3-like subfamily of proteases. Caspase activation correlated with development of hepatocellular apoptosis in vivo. These results are consistent with critical involvement of caspase 3 subfamily members in apoptosis in a variety of cell types (27–29). The effect of

FIGURE 2. Detection of hepatocellular DNA fragmentation using the TUNEL method as described in Materials and Methods. Arrows indicate apoptotic cells. A, Untreated control; B, liver section of an ET-sensitive mouse 6 h after treatment with Gal/ET; C, liver section of an ET-resistant mouse 6 h after Gal/ET treatment; D, a liver section that was pretreated with DNase 1 to nick all DNA, served as positive control. All samples: ×312.5.

FIGURE 3. Quantitative analysis of apoptotic cells. TUNEL-stained mouse liver tissue sections were quantitated for apoptotic cells using computer-assisted image analysis as described in Materials and Methods. Cells were counted in 20 random fields (×125) of 2 slides from each liver and expressed as apoptotic cells per field. Data represent means ± SE of six to eight animals. *, p < 0.05 (compared with t = 0).

FIGURE 4. Caspase activity and DNA fragmentation in parenchymal cells (hepatocytes) and nonparenchymal cells in untreated controls and 6 h after i.p. administration of 700 mg/kg Gal and 100 μg/kg S. abortus equi endotoxin (Gal/ET). Caspase activity was determined by measuring the proteolytic cleavage of the caspase 3 substrate Ac-DEVD-MCA. 100% value: 24.6 ± 4.5 pmol of MCA/min/mg protein (DEVD). DNA fragmentation was measured by ELISA; values are reported as percent changes of v_max obtained from controls (=100%). Data represent means ± SE of four animals per group. *, p < 0.05 (compared with control).
Gal/ET treatment on hepatic caspase activity was not observed in ET-resistant animals that do not generate cytokines in response to ET. Furthermore, caspase activation could be induced with TNF-α but not with IL-1 in ET-resistant and -sensitive mice. These findings strongly indicate that Gal/ET-induced activation of caspase 3-like proteases in vivo and hepatocellular apoptosis is mediated by TNF-α. Other stimuli that induced caspase activation in hepatocytes, e.g., Fas Ab (32), TGF-β1 (33), and hypoxia-reoxygenation (34), showed increased protease activity with DEVD-MCA and YVAD-MCA as substrates. Inhibitors of caspase 1 (YVAD-Ald, YVAD-cmk) and caspase 3 (DEVD-Ald, Z-VAD) inhibited hepatocyte apoptosis in vitro (34, 39) and after Fas Ab administration in vivo (32, 39). On the basis of these data, Rouquet et al. (40) suggested that at least two distinct pathways of Fas signaling exist in hepatocytes. Activation of caspase 1- and caspase 3-like proteases are required, but these pathways involve different sub-classes of serine proteases and can be selectively modulated by protein tyrosine kinase inhibitors (40). Interestingly, actinomycin/TNF-α-induced apoptosis in isolated hepatocytes and in vivo could be induced by YVAD-cmk (39). In contrast, our data indicated that Gal/TNF-α-mediated apoptosis correlated only with increased caspase 3-like activity and could only be inhibited with predominantly caspase 3 inhibitors, e.g., DEVD-Ald. This would suggest that there might also be multiple pathways for TNF-α signaling of apoptosis.

Hepatocellular apoptosis has been described in several models of ET shock (22–26); however, the importance of apoptosis for the overall injury and organ failure in these models remained unclear. Quantitative analysis of apoptotic and necrotic parenchymal cells

![Figure 5](image-url)  
**FIGURE 5.** Dose-dependent inhibition of caspase activity (Ac-DEVD-MCA) in liver homogenate derived from animals treated with 700 mg/kg Gal/100 μg/kg ET for 6 h. Inhibitors (CrmA, Ac-DEVDAld, or Ac-YVAD-Ald) were added to the supernatant 15 min before the substrate.

![Figure 6](image-url)  
**FIGURE 6.** Liver injury, as assessed by plasma ALT activities and histologically by the area of necrosis (A), and hepatic neutrophil sequestration (B) were evaluated in C3Heb/FeJ mice before (controls, C) and 7 h after the combined administration of 700 mg/kg Gal and 100 μg/kg *S. abortus equi* endotoxin (G/E). Some animals received 3 × 10 mg/kg of the caspase inhibitor Z-VAD; the drug was injected 3, 4.5, and 5.5 h after G/E administration. Vehicle control animals received DMSO (1 ml/kg) at the same time. Data represent means ± SE of eight animals per group. *, p < 0.05 (compared with control); #, p < 0.05 (ZVAD vs DMSO).

![Figure 7](image-url)  
**FIGURE 7.** Increase in hepatic caspase activity before (controls, C) and 6 h after i.p. administration of 700 mg/kg Gal and 100 μg/kg *S. abortus equi* endotoxin (G/E). Caspase activity was determined by measuring the proteolytic cleavage of the caspase 1 substrate Ac-YYAD-MCA and the caspase 3 substrate Ac-DEVD-MCA. Data represent means ± SE of five animals per group. *, p < 0.05 (compared with controls).

### Table I. Caspase activation and apoptosis in ET-resistant animals

<table>
<thead>
<tr>
<th></th>
<th>DEVD (pmol/min/mg protein)</th>
<th>YVAD (pmol/min/mg protein)</th>
<th>DNA Fragmentation Vmax (%)</th>
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<tr>
<td>Controls</td>
<td>28.7 ± 3.0</td>
<td>86.2 ± 4.3</td>
<td>100 ± 5</td>
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<td>Gal/ET</td>
<td>25.7 ± 4.7</td>
<td>100.6 ± 5.5</td>
<td>78 ± 13</td>
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<td>Gal/TNF-α</td>
<td>190.3 ± 44.5*</td>
<td>92.1 ± 2.7</td>
<td>410 ± 5*</td>
</tr>
<tr>
<td>Gal/IL-1α</td>
<td>34.1 ± 4.5</td>
<td>99.6 ± 6.6</td>
<td>88 ± 13</td>
</tr>
<tr>
<td>Gal/IL-1β</td>
<td>34.6 ± 6.0</td>
<td>101.0 ± 4.1</td>
<td>83 ± 19</td>
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</tbody>
</table>

* Hepatic caspase activity and DNA fragmentation (% of control) as indicator for apoptosis were determined in C3Heb/FeJ (ET-resistant) mice 5 h after administration of 700 mg/kg Gal in combination with 100 μg/kg *S. abortus equi* ET (Gal/ET), 15 μg/kg murine recombinant TNF-α (Gal/TNF-α), 13 μg/kg murine rIL-1α (Gal/IL-1α), or 23 μg/kg murine rIL-1β (Gal/IL-1β). DNA fragmentation was measured by ELISA; values are reported as % changes of Vmax obtained from controls (100%). Caspase activity was determined by measuring the proteolytic cleavage of the caspase 1 substrate Ac-YYAD-MCA and the caspase 3 substrate Ac-DEVD-MCA. Data represent means ± SE of five animals per group. *, p < 0.05 (compared with controls).
necrotic. These data indicate that quantitatively, apoptotic cell death could not explain the three- to fourfold higher number of necrotic cells 1 h later. Thus, apoptosis appears to be a trigger mechanism for the more severe attack of neutrophils. This hypothesis was investigated by the use of caspase inhibitors, which allowed the selective blockage of apoptosis. Administration of Z-VAD in vivo inhibited caspase 3-like activity (DEVD-MCA) but not caspase 1 activity (YVAD-MCA) and protected effectively against Fas-mediated apoptosis (32). Furthermore, Z-VAD blocked apoptosis (TGF-β1, staurosporine) but not necrosis (staurosporine) in isolated hepatocytes (41). Kinetic analysis in hepatocyte lysate showed that Z-VAD is substantially less potent than DEVD-Ald in inhibiting DEVD-AFC cleavage (33). However, similar inhibition curves indicated that the mechanism of action was the same; i.e., both inhibitors acted as suicide substrates for caspase 3 (33). YVAD-Ald acted as a suicide substrate for caspase 1 but was a competitive inhibitor for caspase 3 with a very high Kᵢ of 12.6 μM (33). Our data agree with these findings. Whereas DEVD-Ald was a highly potent inhibitor (IC₅₀ of 16.75 nM) for increased caspase activity in the liver homogenate, YVAD-Ald inhibited this activity with an IC₅₀ of ~19.6 μM. Because Z-VAD completely prevented the increase in hepatic caspase activity and apoptosis, it further supports the conclusion that caspase 3-like proteases are involved in hepatic parenchymal cell apoptosis in Gal/ET-treated animals.

Z-VAD treatment not only prevented caspase activation and apoptosis but also suppressed liver cell necrosis. Previous studies showed that parenchymal cell necrosis could be attenuated by antibodies against β₂ integrins on neutrophils (35), ICAM-1 on sinusoidal endothelial cells and hepatocytes (14) as well as Vcam-1 on sinusoidal lining cells (16). Neutrophil transmigration in hepatic sinusoids has been identified as a critical step for neutrophil-mediated injury in this model (18). Antibodies to ICAM-1 or Vcam-1 blocked neutrophil extravasation and therefore prevented liver cell necrosis (14, 16). These data suggest that neutrophils are essential for the development of hepatocellular necrosis in this model. Thus, TNF-α initiates two separate responses in the liver, an apoptotic response and an inflammatory response. Treatment with Z-VAD was started at 3 h after Gal/ET administration.

Since formation of TNF-α (2, 14), neutrophil sequestration in the hepatic vasculature (18, 35), activation of NF-kB (9, 37), mRNA formation of ICAM-1 (14, 15), Vcam-1 (16), and selectins (15), and even in part adhesion molecule protein synthesis (16, 42) already occurs before the 3-h time point, Z-VAD could have not affected the proinflammatory response in the liver. The only major events of the inflammatory response that occur after 3 h are neutrophil transmigration and the adherence-dependent cytotoxicity against hepatocytes. The fact that preventing apoptosis in hepatocytes suppresses neutrophil transmigration suggests that hepatocytes undergoing apoptosis represent a signal for neutrophil migration and attack on parenchymal cells. Generally, apoptosis is considered a physiologic way to remove unwanted cells without generating an inflammatory response (43, 44). In contrast, our data suggest that apoptosis may be able to aggravate an inflammatory response. How can these opposite viewpoints be reconciled? First of all, our data do not argue against the fact that single-cell apoptosis will not trigger an inflammatory response. Our data suggest that in the presence of activated and primed neutrophils in hepatic sinusoids, a large number of parenchymal cells undergoing apoptosis at the same time can represent a stimulus for these leukocytes to transmigrate and attack. Recently (45), it was reported that infection of hepatocytes with Listeria monocytogenes causes apoptosis and the generation of a neutrophil chemotactic factor. However, it was unclear whether the increased neutrophil chemotaxis was actually dependent on apoptosis or was more related to Listeria infection. Nevertheless, this observation is consistent with the presence of neutrophils around infected hepatocytes in vivo (45). Neutropenia experiments in this model indicate that a major function of neutrophils is the removal of infected hepatocytes undergoing apoptosis (45). In the Gal/ET model, triggering the transmigration of primed neutrophils may have a similar purpose, and this may be a general mechanism for removal of a large number of apoptotic cells. However, as demonstrated after Gal/ET treatment, the further activation of neutrophils in the liver vasculature bears the risk of additional damage to healthy tissue. Further studies are necessary to identify the nature of the chemotactic stimulus generated by apoptotic parenchymal cells.

In summary, a selective activation of caspase 3-like proteases was observed in the liver that correlated with the development of apoptosis in parenchymal cells after Gal/ET treatment. Caspase activation and apoptosis during endotoxemia in vivo was mediated by TNF-α. Injection of Z-VAD, an effective inhibitor of the caspase 3 subfamily, prevented caspase activation, apoptosis, and liver cell necrosis. Z-VAD did not affect neutrophil sequestration in sinusoids but inhibited transmigration. These data indicate that activation of the caspase 3 subfamily is critical for the development of parenchymal cell apoptosis. In addition, excessive hepatocellular apoptosis can be an important signal for transmigration of primed neutrophils sequestered in sinusoids. Thus, proapoptotic caspases may be a promising therapeutic target for ET- and sepsis-related liver failure.

References

Table II. Effect of the caspase inhibitor Z-VAD on Gal/ET-induced apoptosis

<table>
<thead>
<tr>
<th>Data Points</th>
<th>DNA Fragmentation (V₅₀ₕ_max (%))</th>
<th>TUNEL Assay (Apoptotic Cells/Field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>100 ± 13</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Gal/ET</td>
<td>489 ± 7*</td>
<td>165.5 ± 35.2*</td>
</tr>
<tr>
<td>Gal/ET + DMSO</td>
<td>515 ± 11*</td>
<td>197.8 ± 44.5*</td>
</tr>
<tr>
<td>Gal/ET + Z-VAD</td>
<td>177 ± 16†</td>
<td>24.0 ± 12.1†</td>
</tr>
</tbody>
</table>
CASPASE ACTIVATION AND ET-INDUCED LIVER INJURY


