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Cell Injury, Repair, Aging and Apoptosis

Anti-Apoptotic Function of Gelsolin in Fas Antibody-Induced Liver Failure *in Vivo*

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Apoptosis is a key mechanism underlying fulminant hepatic failure. The role of gelsolin in such apoptotic pathways is not well understood because both pro-apoptotic and anti-apoptotic effects have been reported *in vitro*, depending on the cell type and *in vitro* expression model used. Therefore, we studied an *in vivo* model of hepatic failure by analyzing expression of gelsolin; intrahepatic activation of caspase-3, -8, and -9; and the extent of apoptosis in gelsolin knockout ($gsn^{-/-}$) versus wild-type mice ($gsn^{+/+}$) after exposure to stimulatory Fas antibody Jo-2. Gelsolin was expressed exclusively in sinusoidal lining cells, including sinusoidal endothelial cells and Kupffer cells, of $gsn^{+/+}$ mice. Compared with wild-type mice, Jo2-exposed $gsn^{-/-}$ mice showed significantly higher numbers of apoptotic cells in the liver (22 ± 9 versus 5 ± 4% terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive cells, $P = 0.002$); shorter survival ($P = 0.037$); and enhanced activation of caspase-3 ($P = 0.009$), -8 ($P = 0.004$), and -9 ($P = 0.004$). Furthermore, inhibition of caspase-3 with z-DEVD-fmk blocked Jo2-induced liver failure in all mice. Thus, our data on Jo2-induced hepatic failure suggest that gelsolin exerts an overall anti-apoptotic effect *in vivo*. Moreover, selective expression of gelsolin in sinusoidal endothelial cells indicates a pivotal role for interactions between sinusoidal endothelial cells and liver parenchymal cells in Fas ligand-mediated liver failure. *(Am J Pathol 2006, 168:778–785; DOI: 10.2353/ajpath.2006.050323)*

Apoptosis is mediated by a highly regulated sequence of proteolytic steps, centrally involving activation of caspases. Caspases are cytoplasmic proteases that are expressed as inactive zymogens that are activated post-translationally by proteolytic cleavage. On activation, caspase-3, the central effector caspase, cleaves several substrates that then lead to specific changes of apoptotic cell death. Several signaling pathways can activate caspase-3. First, caspase-3 is activated directly by the initiator caspase-8. Caspase-8 (also called FLICE) is the caspase most upstream in the apoptosis signaling cascade and can be directly activated by the death domain of the Fas receptor complex. Alternatively, caspase-3 is activated by the mitochondrial pathway involving caspase-9, in which caspase-8 cleaves BH3-interacting domain death agonist. This then triggers cytochrome *c* release from the mitochondria into the cytosol via voltage-dependent anion channels. Cytochrome *c* release into the cytosol activates caspase-9, which activates the effector caspase-3.

Cytosolic gelsolin is a major substrate of caspase-3. Physiologically, it severs and caps actin filaments in a Ca^{2+}- and pH-dependent manner. This function is highly relevant for dynamic changes of the actin cytoskeleton during cell motility.[1] In addition, divergent pro- and anti-apoptotic effects of gelsolin are recognized, depending on cell type and experimental conditions. In neutrophils, gelsolin is cleaved by caspase-3, resulting in an active fragment that rapidly degrades actin in a manner independent from regulation by Ca^{2+} and pH.[2] This degradation of actin contributes to apoptotic cell death. On the other hand, uncleaved full-length gelsolin can interact with mitochondrial voltage-dependent anion channels to inhibit cytochrome *c* release and subsequent apoptosis, as has been demonstrated in Jurkat cells overexpressing gelsolin.[3]

Apoptosis is a fundamental process in the pathogenesis of liver diseases. In particular, induction of apoptotic pathways is dramatically involved in the pathogenesis of fulminant hepatic failure (FHF). In FHF, liver integrity and life of affected humans is threatened in a few days to weeks. Several mouse models have been established to simulate and study inflammatory and apoptotic pathways leading to acute liver damage, including the concanava-

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lin A-induced liver failure model,[4] the galactosamine-lipopolysaccharide or -tumor necrosis factor model,[5] or the Fas antibody-induced liver failure model.[6] The model of Fas antibody-induced liver failure in mice represents an attractive tool to study the pathogenetic mechanisms that lead to apoptosis in fulminant hepatic failure *in vivo*. As established by Ogasawara et al,[6] intraperitoneal application of the agonistic Fas antibody Jo2 leads to FHF, and consequently death, in mice. Death occurs within 4 to 8 hours after application of Jo2. Histologically, the liver, but none of the other organs, shows abundant areas of focal hemorrhage and necrosis with high numbers of apoptotic cells.

Because both pro- and anti-apoptotic effects have been attributed to gelsolin in different *in vitro* experiments, depending on cell type and expression model, it remains difficult to understand the *in vivo* role of gelsolin. Therefore, we studied the *in vivo* role of gelsolin in Fas antibody-induced liver failure in gelsolin knockout mice ($gsn^{-/-}$) and wild-type mice ($gsn^{+/+}$).

Experimental Procedures

Fas Antibody-Induced Liver Failure

Experiments were performed in gelsolin null ($gsn^{-/-}$) mice kindly provided by D.J. Kwiatkowski.[7] $gsn^{-/-}$ and $gsn^{+/+}$ mice were housed under standard conditions. All procedures were performed according to approved protocols and recommendations for the proper use of laboratory animals and in agreement with the German legal requirements. Liver failure was induced by intraperitoneal application of 10 μg of Fas antibody Jo2 (IgG isotype, containing <0.01 lipopolysaccharide/μg antibody; BD Pharmingen, Franklin Lakes, NJ).

Survival

In a preliminary experiment, survival after Jo2 application was determined in eight $gsn^{-/-}$ mice versus eight $gsn^{+/+}$ mice that received no further intervention. Differences in survival between the groups were analyzed by the Kaplan-Meier method using the SPSS PC+ software package.

Apoptotic Changes 3 Hours after Jo2 Application

To analyze differences in the induction of apoptotic pathways at a defined point of time, 21 $gsn^{-/-}$ mice and 24 $gsn^{+/+}$ mice were sacrificed 3 hours after Jo2 application by cervical dislocation. Livers were shock-frozen in liquid nitrogen and stored at −80°C for further analysis including terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay; caspase-3, -8, and -9 activity assays; and Western blotting for gelsolin and active caspase-3, -8, and -9.

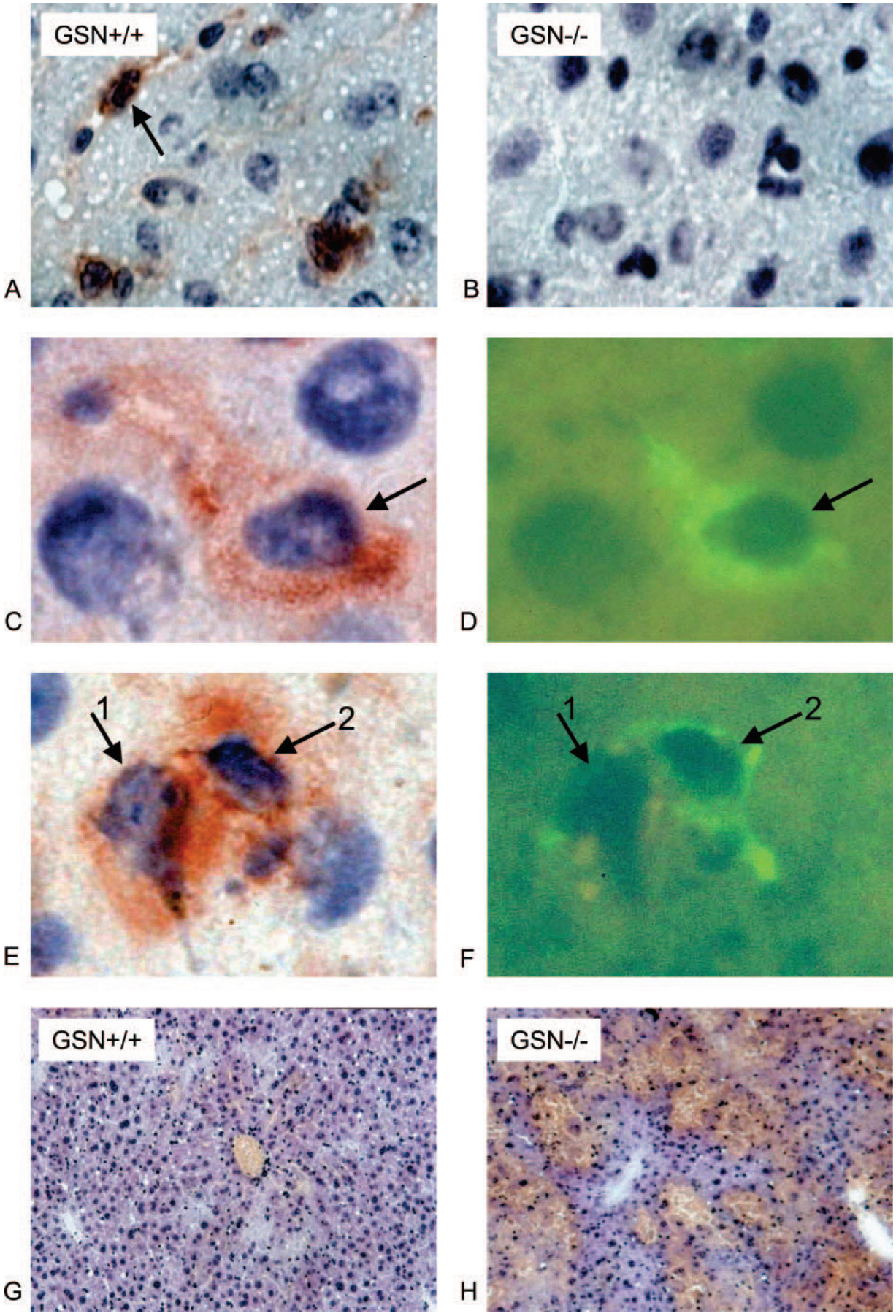
TUNEL Assay

The TUNEL test[8] was performed using the *In Situ* Cell Death Detection kit, POD (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Briefly, liver tissue was fixed with 4% paraformaldehyde (Sigma Chemicals, Munich, Germany) for 1 hour at 4°C. Endogenous peroxidase activity was blocked by incubating with 0.03% H_2O_2 for 5 minutes (Peroxidase Blocking Reagent; DAKO, Carpinteria, CA) for 30 minutes, and cells were permeabilized by 0.1% Triton X-100 in 0.1% sodium citrate. TUNEL reaction mixture was applied at 37°C for 60 minutes and visualized by horseradish peroxidase-conjugated sheep anti-fluorescein antibody (converter POD; Roche Diagnostics) and 3-amino-9-ethylcarbazole. Sections were then counterstained with hemalaun for 5 seconds. As negative controls, corresponding sections were treated in the same way without terminal deoxynucleotidyl transferase. TUNEL staining was quantified by counting TUNEL-positive liver cells in relation to TUNEL-negative liver cells per visual field at 400-fold magnification. TUNEL-positive cells were counted in at least 10 visual fields, and means of these counts were calculated for further statistical analysis.

Caspase-3, -8, and -9 Activity Assays

Caspase activities were measured by cleavage of specific fluorogenic substrates as previously published.[9] Substrates were Ac-DEVD-amino-4-trifluoromethyl coumarine (afc) (Ac-Asp-Glu-Val-asp-afc; Bachem, Heidelberg, Germany) for caspase-3, Ac-LETD-afc (Ac-Leu-Glu-Thr-Asp-afc; Alexis, Grünberg, Germany) for caspase-8, and Ac-LEHD-afc (Ac-Leu-Glu-His-Asp-afc; Bachem) for caspase-9.

Mouse liver was homogenized in 25 mmol/L *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (pH 7.5) buffer containing 0.1% Triton X-100, 5 mmol/L $MgCl_2$, 2 mmol/L dithiothreitol (DTT), and a protease-inhibitor cocktail (Complete; Roche Diagnostics), and centrifuged at 40,000 × *g*. Supernatant (10 μl) was added to 1500 μl of 100 mmol/L *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] buffer (pH 7.4) containing 2 mmol/L DTT. After addition of the fluorogenic substrate (12.5 μmol/L Ac-DEVD-afc, Ac-LETD-afc, or Ac-LEHD-afc), fluorescence was measured in 5-minute intervals (400 nm/505 nm; Shimadzu RF-5301PC fluorometer). The increase in fluorescence was linear between 5 and 35 minutes after adding the fluorogenic substrate. Caspase-3, -8, and -9 activities were calculated from the slope as fluorescence units per mg protein per minute of reaction time and converted to picomoles of substrate cleaved per milligram protein per minute based on a standard curve for afc. Protein concentration in the supernatant was determined by Bio-Rad *DC* Protein Assay (Bio-Rad, Munich, Germany) assay. Enzyme activity is expressed as means ± SD. In parallel control experiments, specificity of the fluorometric signal was confirmed by adding specific caspase inhibitors to the reaction mixture (caspase-3 inhibitor, z-DEVD-fmk


GSN+/+
A
GSN-/-
B
C
D
1
2
E
1
2
F
GSN+/+
G
GSN-/-
H

[Z-Asp(OMe)-Glu(OMe)-Val-DL-Asp(OMe)-fluoromethylketone; Bachem]; caspase-8 inhibitor, Ac-IETD-CHO [Ac-Ile-Glu-Thr-Asp-CHO; Alexis]; and caspase-9 inhibitor, Ac-LEHD-CHO [Ac-Leu-Glu-His-Asp-aldehyde; Bachem].

Western Blotting for Gelsolin and Active Caspase-3

Extracts from mouse livers were prepared by lysing in radioimmunoprecipitation assay buffer (pH 7.55) containing phosphate-buffered saline, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and protease-inhibitor cocktail (Complete; Roche Diagnostics) followed by centrifugation at 40,000 × *g.* Total protein (10 μg) of each sample was loaded on a 10% SDS-polyacrylamide gel electrophoresis gel. After electrophoresis, protein was transferred to a polyvinylidene difluoride membrane (Bio-Rad). Blots were blocked overnight with 5% nonfat dry milk in TBST (50 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, and 0.05% Tween 20) at 4°C and probed with rabbit polyclonal antibodies against p17 subunit of caspase-3 (but not procaspase-3) (C 8487; Sigma Chemicals) and gelsolin[10] in 5% nonfat dry milk/TBST. Immunoblots were then processed with horseradish peroxidase-conjugated secondary antibody.

Bands of caspase-3 were detected using the ECL+Western Blotting Detection system (Amersham Biosciences, Buckinghamshire, UK) and high-performance chemiluminescence film (Hyperfilm ECL; Amersham Biosciences). Constitutively expressed and cleaved gelsolin was detected using enhanced chemiluminescence substrate (Lumi-Light Western blotting substrate; Roche Diagnostics) and a Roche Lumi-Imager.

Caspase-3 Inhibition in Vivo

Six $gsn^{-/-}$ and six $gsn^{+/+}$ mice were treated with the caspase-3 inhibitor z-DEVD-fmk before application of Jo2. Mice were sacrificed after 3 hours, and numbers of apoptotic cells were quantified with the TUNEL reaction.

Immunostaining Procedures

Sections from frozen liver tissue were stained by an indirect immunoperoxidase technique as described previously.[11] Briefly, endogenous peroxidase activity was blocked by 0.03% H_2O_2/$NaNO_3$ (Peroxidase Blocking Reagent; DAKO). The sections were incubated with polyclonal gelsolin antiserum[10] in antibody diluent with background reducing components (DAKO) at room temperature for 90 minutes. After washing in phosphate-buffered saline, peroxidase-coupled secondary antibody (Dianova, Hamburg, Germany) was applied for 30 minutes. Bound antibody was detected with 3-amino-9-ethylcarbazole (Sigma Chemicals). All sections were then counterstained with hemalaun. To determine gelsolin-expressing cell types, we performed double staining with gelsolin and fluorescein isothiocyanate-coupled antibodies specific for CD68 (Kupffer cells) (Clone FA-11; Serotec, Oxford, UK) and for mouse endothelial cells (ME-9F1).[11]

Statistical Analysis

All statistical calculations were performed using the SPSS PC+ software package. Data are given as means ± SD. Differences between the groups were calculated by the nonparametric Mann-Whitney *U*-test.

Results

Using immunohistochemistry, gelsolin expression was found in sinusoidal lining cells, vascular endothelial cells, and some scattered mononuclear cells in the $gsn^{+/+}$ wild-type mice. Double-staining experiments with markers for Kupffer cells and sinusoidal endothelial cells confirmed expression of gelsolin by both cell types. Hepatocytes did not express gelsolin (Figure 1, A, C, D, E, and F). In contrast, gelsolin could not be detected in the specimens obtained from $gsn^{-/-}$-deficient animals (Figure 1B).

To assess the effect of gelsolin expression, we determined survival after a single intraperitoneal injection of Fas antibody Jo2 in $gsn^{+/+}$ and $gsn^{-/-}$ mice. $gsn^{-/-}$ mice showed increased distress and more severe clinical illness (reduced mobility, social contact, and neglect of fur care) after Jo2 application compared with the wild-type animals. In particular, survival of $gsn^{-/-}$ mice was significantly shorter than that of $gsn^{+/+}$ mice ($P = 0.037$) (Figure 2). Six of eight $gsn^{-/-}$ mice died 322, 342, 342, 342, 348, and 366 minutes after Jo2 application, whereas only three of eight $gsn^{+/+}$ mice died at 592, 711, and 626 minutes, respectively.

To study pathways of apoptosis induction in more detail, we repeated intraperitoneal injection of Jo2 in both groups of animals and sacrificed the mice at 180 minutes. On macroscopic inspection, livers from $gsn^{-/-}$ mice were enlarged to a greater extent and were more hemorrhagic than those from $gsn^{+/+}$ mice. Liver damage was also much more pronounced in $gsn^{-/-}$ mice than in $gsn^{+/+}$ mice at the histomorphological level (Figure 1, G and H). We found significantly higher proportions of TUNEL-positive apoptotic cells in $gsn^{-/-}$ mice than in $gsn^{+/+}$ mice ($gsn^{-/-}$, 22 ± 9%; $gsn^{+/+}$, 5 ± 4%; P =

Figure 1. A–F: Intrahepatic gelsolin expression: *In situ* staining of gelsolin in a $gsn^{+/+}$ mouse (**A**) and in a $gsn^{-/-}$ mouse (**B**) visualized by immunoperoxidase reaction. Strong gelsolin staining was seen in sinusoidal lining cells (←) in $gsn^{+/+}$ mice, whereas no gelsolin staining was detected in $gsn^{-/-}$ mice. Double-staining experiments with gelsolin (**C**, ←) and CD68 (**D**, ←) confirmed gelsolin expression by Kupffer cells. Furthermore, also sinusoidal endothelial cells express gelsolin, as demonstrated by double staining with gelsolin antibody (**E**, ← 1 + 2) and ME-9F1, an antibody specific for endothelial cells (**F**, ← 2). **Arrow 1** marks a Kupffer cell expressing gelsolin and is negative for the endothelial cells marker. **G** and **H:** Liver histology from a $gsn^{+/+}$ (**G**) and a $gsn^{-/-}$ mice (**H**) 180 minutes after Jo2 injection. Note, only small areas of liver cell damage could be detected in $gsn^{+/+}$ mice, whereas large areas of hemorrhaged and destroyed liver parenchyma were found in $gsn^{-/-}$ mice 3 hours after Jo2 application.

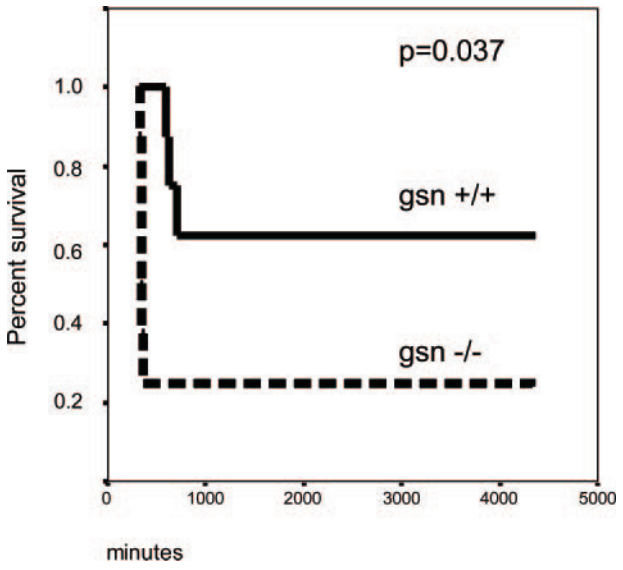


Figure 2. Survival after Jo-2 application. Survival after injection of the Fas antibody Jo2 in 8 $gsn^{+/+}$ versus 8 $gsn^{-/-}$ mice. More $gsn^{-/-}$ (6 of 8) than $gsn^{+/+}$ mice (3 of 8) died after Jo2 application, and survival time was significantly shorter in $gsn^{-/-}$ mice than in $gsn^{+/+}$ mice ($P = 0.037$).

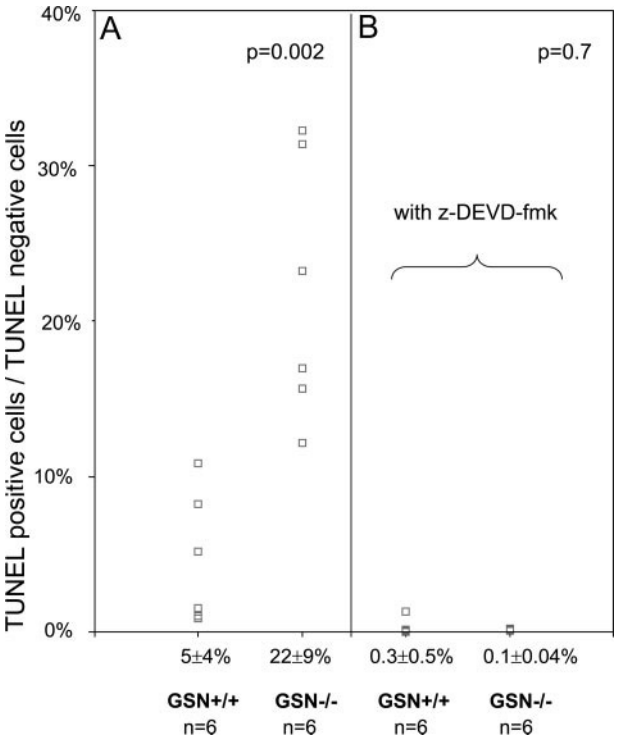


Figure 3. Apoptotic cells after Jo-2 application. Number of TUNEL-positive apoptotic cells 3 hours after Fas antibody injection. In $gsn^{+/+}$ mice (**A**, first line), the number of apoptotic cells was significantly lower compared with $gsn^{-/-}$ mice (**A**, second line). In mice that were pretreated with the caspase-3 inhibitor z-DEVD-fmk (**B**), numbers of TUNEL-positive cells were markedly decreased, independently from gelsolin expression.

0.002; Figure 3A), including hepatocytes and sinusoidal lining cells. Unlike numbers of apoptotic cells, numbers of inflammatory cells were low. In the liver specimens from $gsn^{+/+}$ mice treated with Fas antibody, cleavage of gelsolin into a 58-kd fragment was demonstrated, whereas the amount of uncleaved gelsolin was reduced (Figure 4). In liver homogenates from $gsn^{-/-}$ mice that were blotted for control, neither constitutively expressed gelsolin nor a cleavage product was detectable (Figure 4).

Nevertheless, induction of apoptosis by Jo2 was dependent on caspase-3 activation in both groups of mice, because induction of TUNEL-positive cells was almost completely prevented by the caspase-3 inhibitor z-DEVD-fmk (Figure 3B). Moreover, increased intrahepatic apoptosis in $gsn^{-/-}$ mice corresponded to an increased functional activity of the effector caspase-3, as assessed quantitatively by fluorometric assays (Figure 5A) and semiquantitatively by Western blot (Figure 6). Furthermore, activities of activator caspases 8 and 9 were also significantly higher in the $gsn^{-/-}$ mice than in the wild-type animals as measured fluorimetrically (Figure 5, B and C).

Discussion

Divergent effects of gelsolin have been reported for the control of apoptosis in different *in vitro* models, due to its interactions with apoptosis regulatory pathways at different sites. First results reported by Kothakota et al[2] suggested pro-apoptotic functions of gelsolin. In these experiments, gelsolin was identified as a substrate of caspase-3 and was shown to degrade actin filaments in a rapid, calcium-independent fashion after activation. Consequently, gelsolin-deficient neutrophils showed delayed onset of apoptosis.[2] These pro-apoptotic results were confirmed by transfecting COS-7 cells with truncated gelsolin[12] and by adenoviral transfer of the N-terminal gelsolin fragment to vascular smooth muscle cells.[13]

Based on these experiments, we first expected $gsn^{-/-}$ mice to be protected against Fas-induced apoptosis; however, survival of $gsn^{-/-}$ mice was significantly reduced compared with $gsn^{+/+}$ mice. This increased mortality of $gsn^{-/-}$ in response to Jo2 was associated with markedly increased numbers of TUNEL-positive apoptotic cells and increased activation of caspase-3 in the $gsn^{-/-}$ mice. Thus, our findings suggest that the *in vivo* net effect of gelsolin is anti-apoptotic. Furthermore, our data suggest that the anti-apoptotic effect of gelsolin is localized upstream of caspase-3 and also involves caspase-8 and -9 activation.

Anti-apoptotic effects of gelsolin have also been reported by other groups.[3,14–19] These anti-apoptotic effects were attributed either to the formation of a complex of gelsolin with phosphatidylinositol-4,5-biphosphate reducing activation of caspase-3 and -9[16] or to inhibition of cytochrome *c* release from the mitochondria[3,14,17] due to stabilization of the mitochondrial voltage-dependent anion channel.[3] Importantly, Jurkat cells become resistant against several apoptosis-inducing stimuli such as Fas antibodies, ceramide, and dexamethasone on transfection with gelsolin, whereas morphology of F-actin or levels of Fas and Bcl-2 family members were not altered.[18] Nevertheless, no influence of gelsolin on apoptosis was

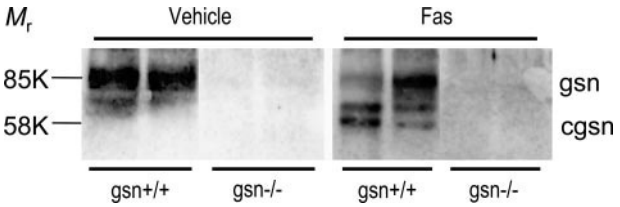


Figure 4. Gelsolin cleavage after Jo-2 application. Constitutively expressed gelsolin (gsn) and gelsolin cleavage products (cgsn) in Fas- versus vehicle-treated wild-type mice ($gsn^{+/+}$). Liver homogenates were subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis using gelsolin antiserum. The specific gelsolin band at 85 kd is decreased in Fas-treated mice, whereas the band of the 58-kd cleavage product is increased. The 67-kd band occurs irrespective of the Jo-2 application. Liver homogenates of $gsn^{-/-}$ mice did not show specific bands. Each lane represents proteins prepared from the liver of an individual animal.

reported by Posey et al,[20] who analyzed gelsolin-overexpressing Jurkat cells, CTLL-20 cells, and Ba/F3 cells.

This observation of both pro- and anti-apoptotic effects has been explained by the fact that the function of gelsolin depends on its state of cleavage, with anti-apoptotic effects associated with uncleaved full-length gelsolin[14,17,18] and pro-apoptotic effects occurring when gelsolin is cleaved by caspase-3.[2] Our experiments cannot be reconciled with the hypotheses that the function of gelsolin depends on its cleavage by caspase-3 and that the anti-apoptotic effect of uncleaved gelsolin is switched into a pro-apoptotic function by cleavage in all situations because we found an overall anti-apoptotic effect of gelsolin despite conspicuous cleavage of gelsolin.

Of note, our experiments differ from previous data in that we studied apoptosis induction in vivo using an animal model instead of in homogenous cell population under *in vitro* cell culture conditions. Thus, our results may reflect the complex interactions between sinusoidal lining cells and hepatocytes in the hepatic microenvironment during induction of apoptosis. Importantly, cellular expression of gelsolin in the liver is limited to sinusoidal lining cells including sinusoidal endothelial cells, Kupffer cells (Figure 1; Ref. 21), and hepatic stellate cells[21] but not hepatocytes. In contrast, Fas is expressed in both hepatocytes and sinusoidal lining cells. Thus, increased mortality of $gsn^{-/-}$ mice in our model of FHF may reflect an important role of sinusoidal lining cells concerning hepatic damage and death of hepatocytes in the model of Jo2-induced liver failure.[22–24] Although the exact mechanism underlying these interactions remains to be elucidated, several factors should be discussed. It might be possible that sinusoidal lining cells are the primary target of Jo2-induced liver failure because Jo2 first has to pass along sinusoidal lining cells when reaching the liver via the portal tract. Secondary to damage of sinusoidal lining cells, intraparenchymal hemorrhage and death of hepatocytes may occur.[22–24] This concept is confirmed by extensive studies of Jodo et al,[23] who investigated the early stages of hepatic apoptosis induction after Fas antibody injection. In these experiments, sinusoidal lining cells had become apoptotic already 1 hour after antibody injection. At this early time point, hepatocytes remained TUNEL negative without any signs of apoptosis. Two hours after injection of the Fas antibody, apoptosis of

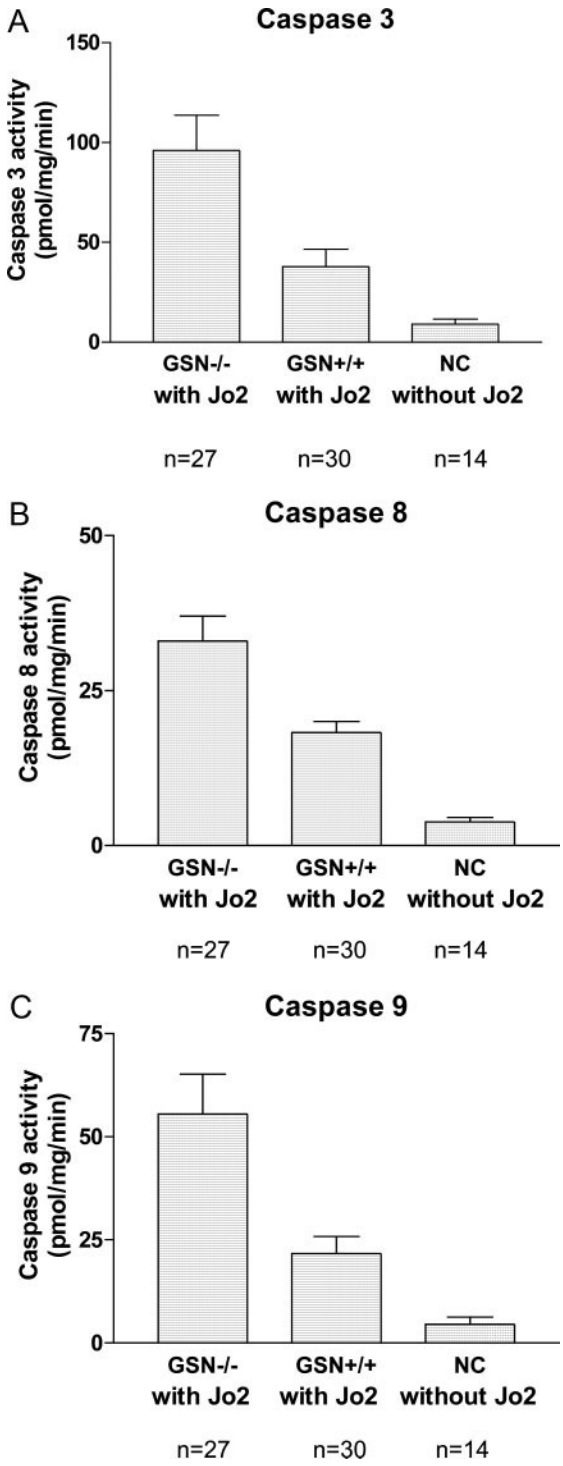


Figure 5. Caspase-3, -8, and -9 activity after Jo-2 application. Fluorometric analysis of the activities of caspase-3 (**A**), -8 (**B**), and -9 (**C**) in livers of 21 $gsn^{-/-}$ and 24 $gsn^{+/+}$ mice 3 hours after application of the Fas antibody Jo2. Activities of caspase-3, -8, and -9 were significantly higher in $gsn^{-/-}$ mice than in $gsn^{+/+}$ mice.

sinusoidal cells lead to intrahepatic hemorrhage and secondary apoptosis of hepatocytes. These data clearly demonstrate that sinusoidal apoptosis precedes the cell death of hepatocytes. The hypothesis of primary damage of sinusoidal endothelial cells by the Fas antibody Jo2 is

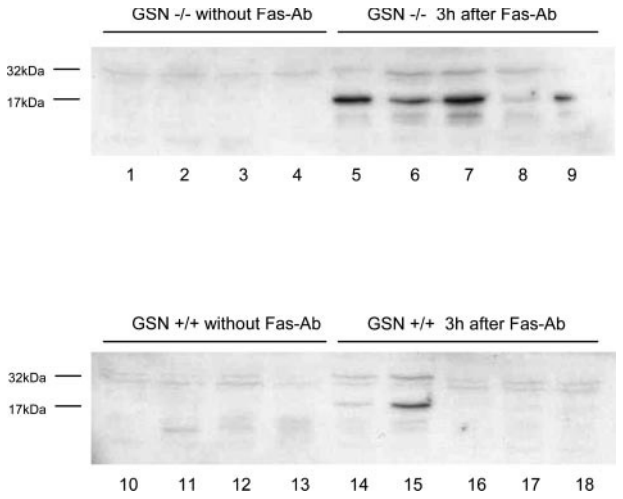


Figure 6. Western blot of caspase-3. Western blot analysis of caspase-3 in livers of nine $gsn^{-/-}$ (1 to 9) and nine $gsn^{+/+}$ (10 to 18) mice. Five $gsn^{-/-}$ (5 to 9) and five $gsn^{+/+}$ mice (14 to 18) were analyzed 3 hours after application of the Fas antibody Jo-2, whereas four mice each (1 to 4 and 10 to 14) were analyzed as controls without Jo-2 application. Detection of the 17-kd cleavage product confirms activation of caspase-3. In only two $gsn^{+/+}$ mice could we detect some caspase-3 cleavage products at 17 kd (14 and 15), whereas high amounts of gsn cleavage were found in all $gsn^{-/-}$ mice that received the Fas antibody (5 to 9). In contrast, no caspase-3 cleavage could be observed in the control mice without Fas antibody.

further supported by data published by Zinn et al[22] and by Janin et al.[24] In biodistribution studies Zinn et al demonstrated that the Fas antibody Jo2 binds primarily to the sinusoidal endothelium, whereas Janin et al identified extensive, disseminated endothelial cell apoptosis 2 hours after Fas antibody injection as a major pathomechanism of Fas antibody-induced liver failure.

This concept is also strongly supported by the pattern of gelsolin expression in Jo2-induced liver failure, because gelsolin is not expressed by hepatocytes but by sinusoidal lining cells. In addition, it has been proposed that release of large quantities of actin during fulminant hepatic failure may result in spontaneous polymerization of actin filaments in the microcirculation. An actin scavenger system involving gelsolin and group-specific component protein (Gc-protein) has been proposed to prevent actin filament deposition in the microcirculation.[25] In line with this concept, gelsolin serum levels are decreased in fulminant hepatic failure and inversely correlated to disease severity.[26] Thus, $gsn^{-/-}$ mice may have reduced capacity to dispose of actin in the circulation, possibly making them more susceptible to actin toxicity associated with severe organ damage. Alternatively, gelsolin knockout may affect motility and cellular remodeling of sinusoidal endothelial cells contributing to hepatic perfusion damage.

Taken together, our data demonstrate an overall antiapoptotic effect of gelsolin *in vivo*, substantiating the important regulatory role of gelsolin in the hepatic environment. Furthermore, our data confirm the crucial function of sinusoidal endothelial cells in hepatic survival during apoptotic stimuli by Fas induction.

Acknowledgments

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