Amino Acids (2013) 44:189–197
DOI 10.1007/s00726-012-1412-x

ORIGINAL ARTICLE

Divergent results induced by different types of septic shock in transglutaminase 2 knockout mice

Hyun Yoo · Eu-Ree Ahn · Se-Jin Kim · Seon-Hyeong Lee · Seung Hyun Oh · Soo-Youl Kim

Received: 25 June 2012 / Accepted: 28 September 2012 / Published online: 10 October 2012

Abstract Acute sepsis can be induced by cytokines such as TNF-α and biological products such as LPS. All of these agents cause systemic inflammation, which is characterized by hemodynamic shock and liver toxicity. However, the outcomes of different septic shock models were totally opposite in transglutaminase 2 knockout (TGase $2^{-/-}$) mice. The aim of our study was to clarify the role of TGase 2 in liver injury. Therefore, we explored the role of TGase 2 in liver damage using two different stress models: LPS-induced endotoxic shock and TNF-α/actinomycin D (ActD)-induced sepsis. TNF-α-dependent septic shock resulted in increased liver damage in TGase $2^{-/-}$ mice compared with wild-type (WT) mice, and was accompanied by increased levels of caspase 3 and cathepsin D (CTSD) in the damaged liver. Conversely, LPS-induced septic shock resulted in ablation of inflammatory endotoxic shock in TGase $2^{-/-}$ mice and decreased liver injury. We found that TGase 2 protected liver tissue from TNF-α-dependent septic shock by reducing the expression of caspase 3 and CTSD. However, TGase 2 differently participated in increased the hemodynamic shock in LPS-induced septic shock through macrophage activation rather than protecting direct liver damage. Therefore, these findings demonstrate that septic shock caused by different agents may induce different results in TGase $2^{-/-}$ mice depending on the primary target organs affected.

Keywords Transglutaminase · Acute liver failure · TNF-α · Cathepsin D · Caspase 3

Introduction

Transglutaminase 2 (TGase 2, E.C. 2.3.2.13, protein-glutamine γ-glutamyltransferase) is an enzyme that catalyses e-(γ-glutamyl)-lysine isopeptide cross-linking (Beninati and Piacentini 2004; Folk 1983). TGase 2 is associated with liver tissue remodeling, wound healing, cell adhesion, and apoptosis (Kim 2004; Mirza et al. 1997). Previously, we showed that TGase 2 induces NF-κB activation via I-κBα depletion in the absence of kinase activation (Lee et al. 2004; Park et al. 2006). In addition, TGase 2 over-expressing cells constantly maintained NF-κB activation upon TNF-α treatment compared with cells without TGase 2 expression (Park et al. 2011). From these findings, it appears that TGase 2 expression facilitates cell survival by enhancing NF-κB activity (Kim 2011).

The apoptotic role of TGase 2 has been debated, primarily because no spontaneous cell death was observed in TGase $2^{-/-}$ mice (De Laurenzi and Melino 2001, Nanda et al. 2001). TGase 2 was reported to promote cell survival against thapsigargin- or hypoxia-induced cell death (Jang et al. 2010; Yamaguchi and Wang 2006). The anti-apoptotic function of TGase 2 in vivo has been reported by Sarang et al., which

H. Yoo and E.-R. Ahn contributed equally to this study.

H. Yoo · E.-R. Ahn · S.-J. Kim · S.-H. Lee · S.-Y. Kim (✉)
Cancer Cell and Molecular Biology Branch, Division of Cancer Biology, Research Institute, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang 410-769, Republic of Korea
e-mail: kimsooyoul@gmail.com

S. H. Oh
Cancer Experimental Resources Branch, Division of Cancer Biology, Research Institute, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang 410-769, Republic of Korea

Present Address:
S. H. Oh
Gachon Institute of Pharmaceutical Sciences, Gachon University of Medicine and Science, 7-45 Songdo-dong, Yeonsu-gu, Incheon 406-840, Republic of Korea

showed that in TGase $2^{-/-}$ mice were sensitive to Fas-mediated cell death (Sarang et al. 2005). We also showed in two different liver injury models that injection of TNF-α with ActD or Concanavalin A results in increased hepatocyte apoptosis in TGase $2^{-/-}$ mice (Delhase et al. 2012). These data support a role for TGase 2 in inhibition of cell death mediated by members of the TNF/TNFR1 superfamily (Delhase et al. 2012). In the study, TGase 2 was identified as a downstream mediator of the anti-apoptotic response triggered upon NF-κB activation. Because these observations concurred with results from other acute liver injury models, such as lead nitrate (Falasca et al. 2005) and carbon tetrachloride treatment (Nardacci et al. 2003), we proposed a role for TGase 2 as a regulator of apoptotic proteases (Delhase et al. 2012). However, when LPS/galactosamine (Gal)-induced septic shock was elicited in TGase $2^{-/-}$ mice (Falasca et al. 2008), survival was dramatically increased compared with that in wild-type (WT) mice, an effect that was highlighted by a marked reduction in organ injury and limited infiltration by neutrophils. The increased survival of TGase $2^{-/-}$ mice in the LPS sepsis model indicates that TGase 2 plays an important role in endotoxemia, which is directly involved with NF-κB activation. This phenomenon was in agreement with other reports, including one showing induction of hepatic apoptosis upon treatment with anti-Fas antibody or alcohol (Tatsukawa et al. 2009).

The contradictory results obtained from these different experimental sets prompted investigation into how TGase 2 contributes to the septic process. Understanding the role of TGase 2 in sepsis is crucial due to the development of TGase 2 inhibitors for use in treating chronic inflammatory diseases or cancer.

To clarify the role of TGase 2 in liver injury, we explored the mechanism by which TNF-α treatment induces apoptosis. Acute liver failure commonly occurs due to increased production of chemokines and TNF-α by macrophages (Muto et al. 1988). TNF-α, a pro-inflammatory cytokine, induces systemic inflammation, which is characterized by hemodynamic shock and liver toxicity (Muto et al. 1988). In liver cells, TNF-α also induces expression of TGase 2, a cross-linking enzyme, through NF-κB activation; a role that is thought to be involved in liver fibrogenesis (Kuncio et al. 1998). Therefore, we explored the role of TGase 2 in liver damage using two different stress models: LPS-induced endotoxic shock and TNF-α/ActD-induced sepsis.

Materials and methods

Reagents

Caspase-3, PARP, and cathepsin D (CTSD) antibodies were purchased from Cell Signaling Technologies (Beverly, MA). The TGase 2 antibody was purchased from NeoMarkers (clone CUB 7402; Fremont, CA). Actinomycin D (ActD) and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St Louis, MO). Mouse recombinant TNF-α was obtained from R&D Systems (Minneapolis, MN).

Animals and experimental design

The 8- to 10-week-old male TGase $2^{-/-}$ mice used in this study were established previously and back-crossed with C57BL/6 mice over 20 generations (Kim et al. 2010). All experimental protocols were conducted in accordance with the Korean law on animal protection and approved by the Institutional Animal Care and Use Committee at the National Cancer Center. The animals were randomly divided into three groups: Group I, control animals that were injected intraperitoneally with PBS (pH 7.4); Group II, animals were injected intraperitoneally with 400 μg/head LPS; and Group III, animals were injected intraperitoneally with 10 μg/head ActD 5 min prior to intraperitoneal administration of 100 ng/head TNF-α. The mice were killed 16 h after the last treatment.

Preparation of immortalized MEFs

Mouse embryonic fibroblasts (MEFs) were prepared from individual embryos at embryonic day 13.5 following a standard protocol (Kim et al. 2010). After removal of the legs and liver, the remaining tissue was washed twice in Dulbecco's phosphate-buffered saline and then incubated with 100 μl trypsin/EDTA for 30 min. The tissue was dissociated to near-homogeneity in complete medium and transferred to a 150-mm dish. MEFs were maintained in Dulbecco's modified Eagle's medium supplemented with 10 % FBS and split every 2–3 days. To obtain natively immortalized MEFs, primary MEFs were maintained over 10 passages. All experiments were performed using immortalized MEF cells after 10 passages (Kim et al. 2010). The genotypes of the MEFs were identified and confirmed by PCR using genomic DNA from the yolk sac and from the MEFs.

Crystal violet staining

Cells were incubated with TNF-α/ActD in a CO_2 incubator for 4 h, washed twice with cold PBS, and fixed with cold methanol on ice for 10 min. Following fixation, crystal violet solution was added to each well and incubated for 15 min at RT. Residual crystal violet solution was removed by three washes in distilled water.

IN Cell analyzer 1000

Wild-type and TGase $2^{-/-}$ cells were seeded on 96 well plates and treated with TNF-α/ActD. After 24 h, the cells

were fixed in 3.7 % paraformaldehyde and stained with Hoechst and Annexin V Alexa-fluor 647 (Invitrogen, Carlsbad, CA). Cell number and Annexin V intensity were determined using the IN Cell analyzer 1000 (GE Healthcare, Cardiff) analysis software. Each experiment was repeated at least three times.

Apoptosis assay

Determination of apoptotic activity by Annexin V staining was performed using an Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, San Diego, CA) according to the manufacturer's instructions. TUNEL staining was performed on fixed tissues using the In Situ Cell Death Detection Kit (Roche, GER) following the manufacturer's instructions.

Cytokine secretion analyses

Mouse serum was evaluated using the Cytometric Bead Assays Mouse Inflammation Kit (CBA; BD Biosciences, San Diego, CA) following the standard procedure. Briefly, serum samples were incubated with antibody conjugated beads and a fluorochrome, and immediately analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). Results were plotted to determine the concentrations of cytokine proteins (IL-12, IL-6, MCP-1, TNF-α, and IFN-γ) using a standard curve.

Bone marrow derived macrophages (BMDM)

Bone marrow was flushed from WT and TGase $2^{-/-}$ mice and cultured for 1 week in complete macrophage medium [DMEM supplemented with 10 % FBS, 20 ng/ml macrophage-colony stimulating factor (R&D systems, Minneapolis, MN)] at 37 °C. After 7–10 days in culture, approximately 95 % of the adherent cells were macrophages (Vereyken et al. 2011) and were used for subsequent experiments.

Statistical analysis

All the data are representative of at least three independent experiments. Values are expressed as mean SD. Statistical analysis was performed using an unpaired Student's *t* test.

Results

Opposite effects in TGase $2^{-/-}$ mice using LPS/Gal and TNF-α/ActD septic models

To examine the response of TGase $2^{-/-}$ mice to septic shock, we employed two different septic shock models: LPS/Gal and TNF-α/ActD (Fig. 1). LPS/Gal treatment was strongly lethal in WT mice but had almost no lethal effect in TGase $2^{-/-}$ mice (Fig. 1a). Interestingly TNF-α/ActD treatment was lethal to TGase $2^{-/-}$ mice but caused almost no lethality in WT mice (Fig. 1b). These findings demonstrate that TGase $2^{-/-}$ mice are resistant to LPS/Gal-induced septic stress but vulnerable to TNF-α/ActD-induced septic stress.

TNF-α/ActD, but not LPS/Gal, induces significant amounts of apoptosis in the livers of TGase $2^{-/-}$ mice

The acute sepsis model induced by TNF-α/ActD causes systemic inflammation, which is associated with liver toxicity. To examine liver toxicity induced by LPS/Gal and TNF-α/ActD, liver tissue was evaluated by TUNEL staining and immunoblotting (Fig. 2). The TUNEL assay showed a

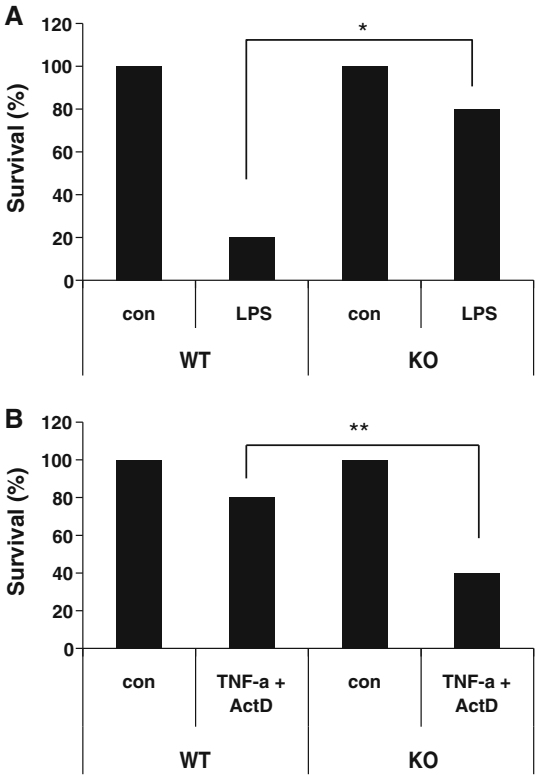

Fig. 1 Mortality rates differed between WT and TGase $2^{-/-}$ mice depending on the sepsis models used. **a** Age-matched WT and TGase $2^{-/-}$ mice sensitized with D-galactosamine (0.5 mg/g) to enhance the toxicity of LPS were injected with LPS (0.4 mg/head). **b** ActD (10 μg/head) was injected intraperitoneally before the intraperitoneal administration of TNF-α (0.1 μg/head). Each mouse was monitored for 24 h. $n = 5$ mice/group. Experiments were repeated twice, and the averages are presented with the SD. *, ** $P < 0.05$ was considered statistically significant. *WT* wild type, *KO* TGase 2 knock-out

significant increase in the number of apoptotic cells in the livers of TGase $2^{-/-}$ mice exposed to TNF-α/ActD compared with those of WT mice (Fig. 2a, b). However, there was no difference in apoptotic induction between WT and TGase $2^{-/-}$ mice subjected to LPS/Gal treatment (Fig. 2a, b). Interestingly, liver tissue in TGase $2^{-/-}$ mice showed about 160 % increase in TNF-α/Act-induced apoptosis to compare to LPS-induced apoptosis (Fig. 2a, b). Immunoblot analysis of cleaved caspase 3 and PARP showed increased cleavage only in TGase2$^{-/-}$ mice exposed to TNF-α/ActD, while TGase2$^{-/-}$ mice exposed to LPS did not show any cleavage of caspase 3 or PARP (Fig. 2c). These data agree with the results of the TUNEL assay.

TGase2$^{-/-}$ MEFs are susceptible to TNF-α/ActD-induced apoptosis

To determine whether the susceptibility of liver tissue to TNF-α/ActD-induced apoptosis is a general phenomenon that also occurs in other tissues, MEF cells were examined after TNF-α/ActD treatment (Fig. 3). After treatment with LPS or TNF-α/ActD for 4 h, colonies were visualized by crystal violet staining, and fluorescence image acquisition was performed with an IN Cell Analyzer 1000 automated confocal laser scanning microscope (Fig. 3a). LPS-induced apoptosis was less than 2.5-fold lower both in MEFs from WT and TGase $2^{-/-}$ mice. MEFs from TGase $2^{-/-}$ mice

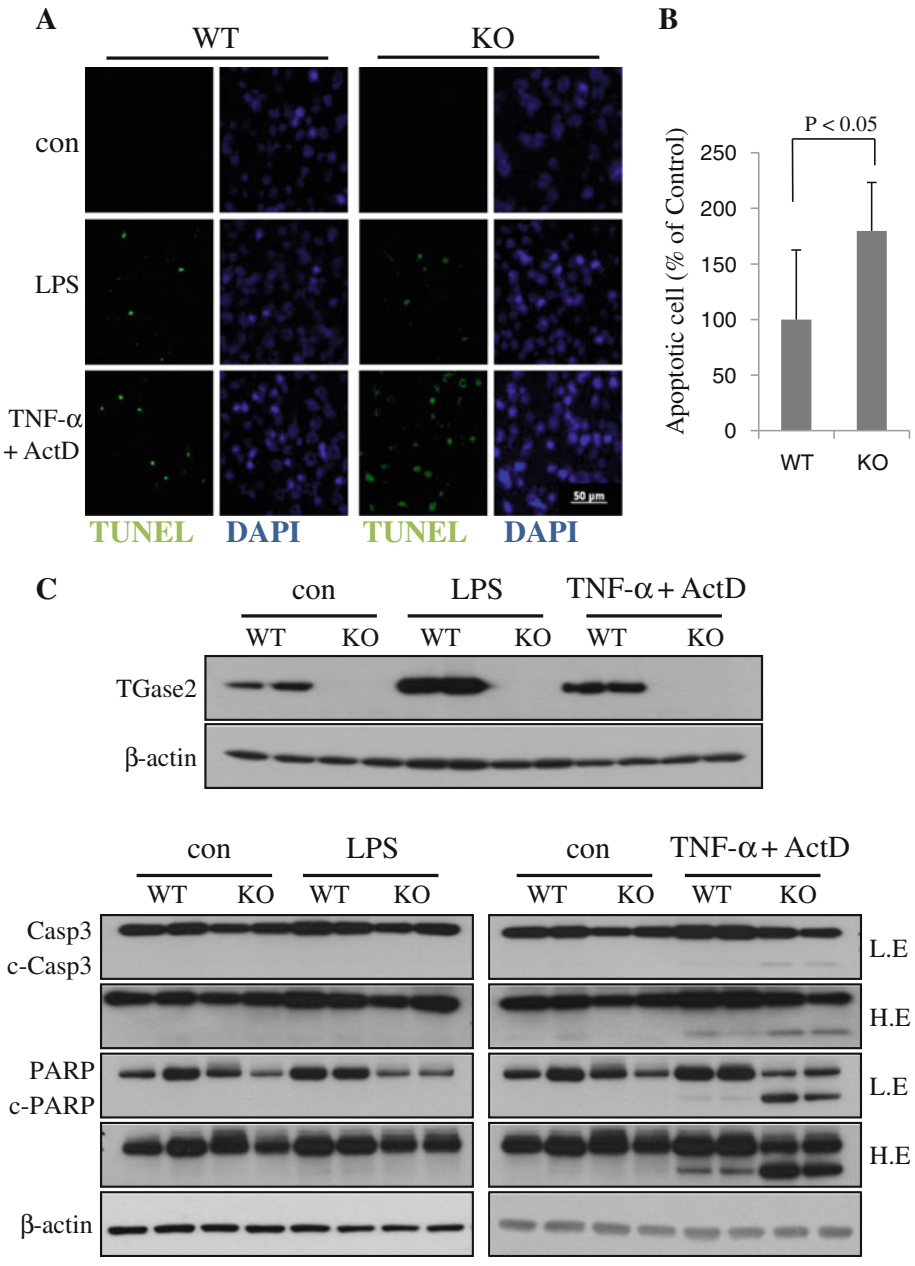

Fig. 2 TGase 2 gene ablation led to increased apoptosis. **a**, **b** Liver samples from WT or TGase $2^{-/-}$ mice treated with TNF-α/ActD or LPS/Gal for 16 h were subjected to TUNEL assay to detect apoptotic hepatocytes. Y axis represents % of control as a number of dead cells in wild type treated with TNF-α + Act D set 100 %. **c** Liver tissues from WT and TGase $2^{-/-}$ mice were homogenized, and TGase 2, caspase-3, and PARP were analyzed by immunoblotting. *HE* high expose, *LE* low expose, *con* control)

showed over 11-fold increased susceptibility to TNF-α/ActD-induced apoptosis, whereas MEFs from WT mice were 6-fold increased (Fig. 3a, b). Therefore, TGase $2^{-/-}$ MEFs are over 3-fold susceptible to TNF-α/ActD-induced apoptosis to compare to LPS-induced apoptosis. Immunoblot analysis of caspase 3 and PARP activation revealed increased activation of caspase 3 and PARP only in whole cell extracts of TNF-α/ActD-treated MEFs from TGase $2^{-/-}$ mice, while almost no change has been detected in whole extracts of LPS-treated MEFs both from WT and TGase $2^{-/-}$ mice (Fig. 3c).

Increased TNF-α/ActD-mediated apoptosis in TGase $2^{-/-}$ mice is associated with restoration of cathepsin D (CTSD)

Binding of TGase 2 to CTSD results in the depletion of CTSD both in vitro and in MEFs, leading to decreased levels of apoptosis (Kim et al. 2011). To determine whether CTSD contributes to apoptosis induced by TNF-α/ActD treatment, immunohistochemical analysis was performed on livers from WT and TGase2$^{-/-}$ mice (Fig. 4a). A significant increase in CTSD expression was revealed by

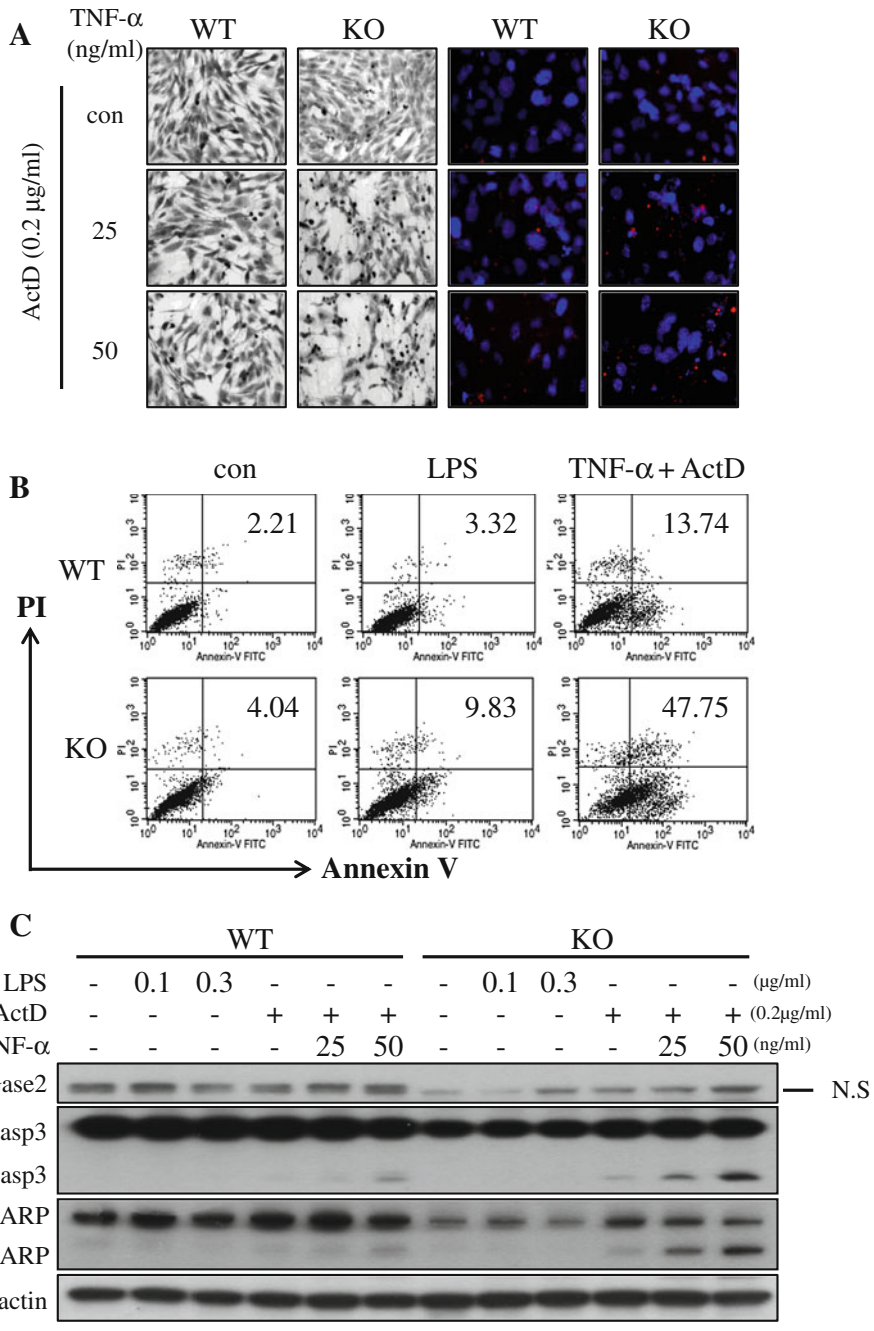

Fig. 3 MEFs from TGase $2^{-/-}$ mice were more susceptible to TNF-α/ActD-induced cell death than wild-type mice. MEF cells were stimulated with LPS or TNF-α/ActD for 4 h. **a** After treatment, colonies were visualized by crystal violet staining. Fluorescence image acquisition was performed with IN Cell Analyzer 1000 automated confocal laser scanning microscope. **b** Expression of Annexin V was analyzed by flow cytometry. **c** Whole-cell lysates were subjected to immunoblot analysis of the indicated proteins. *ActD* actinomycin D, *NS* non-specific band

immunohistochemical staining of livers from TGase2$^{-/-}$ mice (Fig. 4a) and by immunoblotting of whole cell extracts from TGase2$^{-/-}$ mice (Fig. 4b). The mature form of CTSD was markedly increased in TGase2$^{-/-}$ mice, which is indicative of an increase in apoptosis (Fig. 4c).

The endotoxic effect of LPS/Gal-induced septic shock is dramatically reduced in TGase2$^{-/-}$ mice

Because LPS/Gal-induced apoptosis was not significant in the liver tissues of WT and TGase2$^{-/-}$ mice (Fig. 2), the susceptibility of TGase2$^{-/-}$ macrophages to LPS/Gal septic shock was examined in BMDMs. LPS/Gal treatment for 6 h did not increase apoptosis in BMDMs, as shown by FACS analysis of Annexin V staining in BMDMs from WT and TGase 2$^{-/-}$ mice (Fig. 5a). Immunoblotting also showed no change in the expression of caspase 3 or PARP following LPS/Gal or TNF-α/ActD treatment. However, COX-2 expression was increased in BMDMs from WT mice exposed to LPS/Gal treatment (Fig. 5b). When the levels of inflammatory cytokines in the serum from mice, including IFN-γ, MCP-1, IL-6, and TNF-α, were measured following treatment with LPS/Gal or TNF-α/ActD, the results showed that LPS/Gal treatment induced increased levels of all cytokines in sera from WT mice (Fig. 5c), which may be associated with hemodynamic shock. Sera from TGase 2$^{-/-}$ mice treated with LPS/Gal showed clearly reduced levels of IFN-γ and IL-6 (Fig. 5c). However, TNF-α/ActD treatment from TGase 2$^{-/-}$ mice resulted in markedly reduced levels of IL-6 and TNF-α compared with WT mice (Fig. 5c).

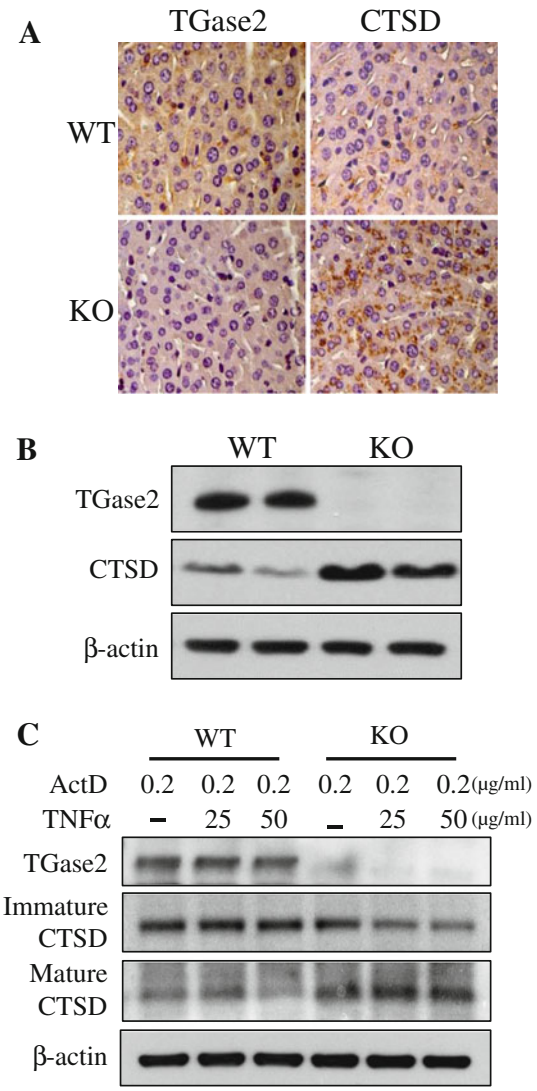

Fig. 4 TNF-α/ActD-mediated apoptosis in TGase 2$^{-/-}$ mice was associated with CTSD repletion. **a** Immunohistochemical analysis of CTSD expression showed an inverse relationship between TGase 2 expression and CTSD levels in the livers of WT and TGase 2$^{-/-}$ mice treated with TNF-α/ActD. **b** Whole liver lysates were subjected to immunoblot analysis to detect CTSD. **c** MEFs from WT and TGase 2$^{-/-}$ cells were treated with TNF-α/ActD for 4 h, and the lysates were subjected to immunoblot analysis to detect CTSD. *CTSD* cathepsin D

Discussion

In this study, we found that TGase 2$^{-/-}$ mice respond differently to different types of septic shock. LPS/Gal-induced sepsis is more damaging to immune surveillance cells than to tissue cells, while TNF-α/ActD-induced sepsis affects liver tissue cells rather than immune cells. LPS/Gal-induced sepsis stimulates the production of multiple endotoxic cytokines through macrophage activation, leading to tissue damage, while TNF-α/ActD-induced sepsis triggers severe liver damage through activation of apoptosis. Both LPS/Gal and TNF-α/ActD treatment appear to induce TGase 2 expression through NF-κB activation (Kuncio et al. 1998). To mount an effective defence against agents such as LPS, TGase 2 must be induced in macrophages. To protect against chemically induced tissue damage, TGase 2 must be induced in the damaged tissue. Thus, TGase 2$^{-/-}$ mice are affected in different ways depending on the source of septic shock.

Opposite effects in TGase 2$^{-/-}$ mice were observed from septic models using LPS/Gal and TNF-α/ActD (Fig. 1). LPS/Gal treatment was strongly lethal in WT mice but had almost no lethal effect in TGase 2$^{-/-}$ mice (Fig. 1a). Interestingly, TNF-α/ActD treatment was lethal to TGase 2$^{-/-}$ mice but caused almost no lethality in WT mice (Fig. 1b). These findings demonstrate that TGase 2$^{-/-}$ mice are resistant to LPS/Gal-induced septic stress but vulnerable to TNF-α/ActD-induced septic stress. TNF-α/ActD, but not LPS/Gal, induces significant amounts of apoptosis in the livers of TGase 2$^{-/-}$ mice (Fig. 2). Immunoblot analysis of cleaved caspase 3 and PARP

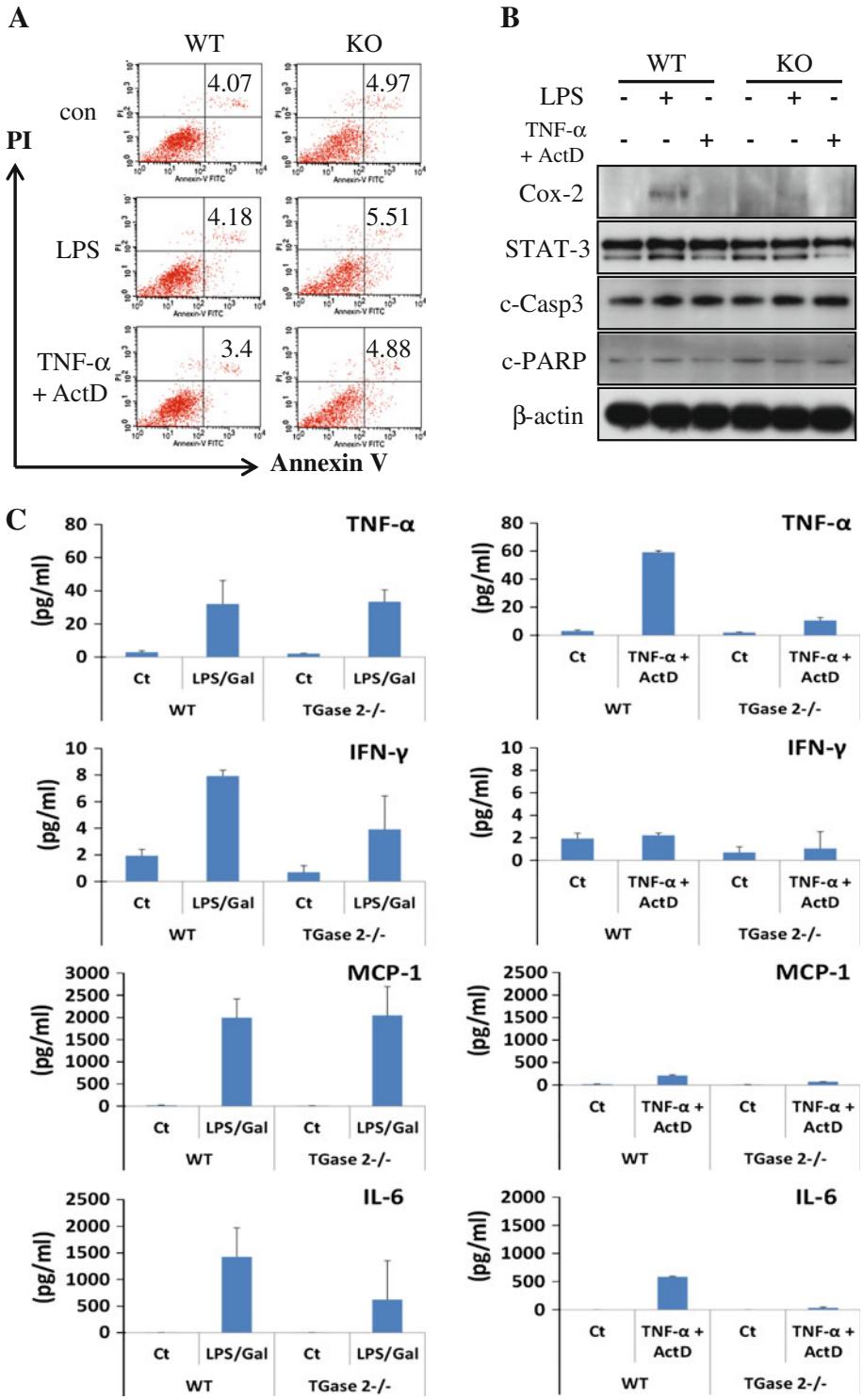

Fig. 5 Inflammatory protein and cytokine expression were decreased in TGase $2^{-/-}$ mice. BMDM cells were treated for 6 h with LPS (200 ng/ml) or TNF-α (50 ng/ml)/ActD (20 ng/ml). **a** Cells were labeled with Annexin V and analyzed using a FACScan flow cytometer to quantify fluorescence. Annexin V-positive cells were defined as apoptosis. **b** BMDM cells were treated for 6 h with LPS or TNF-α/ActD, and whole-cell lysates were subjected to immunoblot analysis to detect the indicated proteins. **c** Cytokine production in serum from WT and TGase $2^{-/-}$ mice treated intraperitoneally with TNF-α/ActD or LPS/Gal. IFN-γ, IL-6, MCP-1, and TNF-α expression were quantitatively measured with the BD™ CBA Mouse Inflammation Kit. *Con* control

showed increased cleavage only in TGase2$^{-/-}$ mice exposed to TNF-α/ActD, while TGase2$^{-/-}$ mice exposed to LPS did not show any cleavage of caspase 3 or PARP (Fig. 2c). With regard to liver injury, there are conflicting results regarding the role of TGase 2 in different injury models. Carbon tetrachloride (CCl_4) treatment of TGase $2^{-/-}$ mice increases the incidence of post-injury death relative to that in WT mice (Nardacci et al. 2003). The authors postulated that this is because TGase 2 plays a protective role by supporting tissue stability and repair. In addition, lead nitrite ($PbNO_3$) treatment for 5 days in TGase $2^{-/-}$ mice results in increased inflammation and apoptosis in the liver (Falasca et al. 2005), which is believed to be due to decreased apoptosis-dependent

phagocytosis by macrophages (Falasca et al. 2005) or impaired maturation of autophagosomes, which induces autophagy in MEFs (D'Eletto et al. 2009). Conversely, TGase $2^{-/-}$ mice showed decreased hepatic apoptosis following treatment with anti-Fas antibody or alcohol (Tatsukawa et al. 2009). Mallory body (MB) formation assays (3,5-diethoxycarbonyl-1,4-dihydrocollidine, DDC-fed) in TGase $2^{-/-}$ mice revealed decreased MB formation and liver hypertrophy compared with WT mice (Tatsukawa et al. 2009). This was reported to be because TGase 2 induces hepatocyte apoptosis via Sp1 cross-linking and inactivation, with resultant inhibition of c-Met expression required for hepatic cell viability (Tatsukawa et al. 2009). Sp1 depletion by TGase 2 might be one explanation for the increased survival of TGase $2^{-/-}$ mice treated with DDC-fed. However, activation of immune cells such as Kupffer cells may be more responsible for MB formation and liver hypertrophy induced in DDC-fed or alcoholic liver hypertrophy, because both of these stressors significantly induce activation of immune cells. TGase 2 may contribute to activation of Kupffer cells, which may in turn lead to increased IL-6-mediated recruitment of immune cells to the damaged liver tissue (Tacke et al. 2009). Recently, Oh et al. reported that TGase 2 is responsible for IL-17 production by T-cells following to the induction of IL-6 in lung epithelia cells in a lung fibrosis model (Oh et al. 2011). Therefore, the effect of TGase 2 activation in Kupffer cells might be of interest for future investigation.

In this study, we found that TGase$2^{-/-}$ MEFs are over 3-fold susceptible to TNF-α/ActD-induced apoptosis to compare to LPS-induced apoptosis (Fig. 3). Immunoblot analysis of caspase 3 and PARP activation revealed increased activation of caspase 3 and PARP only in whole cell extracts of TNF-α/ActD-treated MEFs from TGase $2^{-/-}$ mice (Fig. 3c). It is quite interesting because cell growth and normal survival without stress are almost same between MEFs from WT and TGase $2^{-/-}$ mice (Fig. 3a). There is a non-specific band which was increased by LPS- or TNF-α treatment, however, the size of the molecule is relatively smaller than WT TGase 2. By analysis of TGase activity using extracts from TGase$2^{-/-}$ MEFs, we could not detect TGase activity at all in vitro (unpublished). However, there is a possibility that certain environmental stress may induce other type(s) of TGase. It may be an intriguing question to be answered in future. Conversely, we also found that the endotoxic effect of LPS/Gal-induced septic shock is dramatically reduced in TGase$2^{-/-}$ mice (Fig. 5). Although LPS/Gal-induced apoptosis was not significant in the liver tissues of WT and TGase$2^{-/-}$ mice (Fig. 2), inflammatory marker such as COX-2 was increased in BMDMs from WT mice exposed to LPS/Gal treatment (Fig. 5b). LPS/Gal treatment induced increased levels of all cytokines including IFN-γ, MCP-1, IL-6, and TNF-α in sera from WT mice (Fig. 5c), which may be associated with hemodynamic shock. Because LPS/Gal treatment was strongly lethal in WT mice but had almost no lethal effect in TGase $2^{-/-}$ mice (Fig. 1a). This demonstrated that TGase $2^{-/-}$ mice are resistant to LPS/Gal-induced septic stress.

In summary, the effects of septic shock observed in TGase $2^{-/-}$ mice are dependent on the source of the septic shock and the cells and tissues are affected, such as liver cells or macrophages. Therefore, great care must be taken when selecting therapeutic agents that inhibit TGase 2, particularly with regard to their potential to cause liver damage.

Acknowledgments This study was supported by a research grant (NCC1110011-2) from the National Cancer Center of Korea and by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 20110027248).

References

Beninati S, Piacentini M (2004) The transglutaminase family: an overview (minireview article). Amino Acids 26:367–372

D'Eletto M, Farrace MG, Falasca L, Reali V, Oliverio S, Melino G et al (2009) Transglutaminase 2 is involved in autophagosome maturation. Autophagy 5:1145–1154

De Laurenzi V, Melino G (2001) Gene disruption of tissue transglutaminase. Mol Cell Biol 21:148–155

Delhase M, Kim SY, Lee H, Naiki-Ito A, Chen Y, Ahn ER et al (2012) TANK-binding kinase 1 (TBK1) controls cell survival through PAI-2/serpinB2 and transglutaminase 2. Proc Natl Acad Sci USA 109:E177–E186

Falasca L, Iadevaia V, Ciccosanti F, Melino G, Serafino A, Piacentini M (2005) Transglutaminase type II is a key element in the regulation of the anti-inflammatory response elicited by apoptotic cell engulfment. J Immunol 174:7330–7340

Falasca L, Farrace MG, Rinaldi A, Tuosto L, Melino G, Piacentini M (2008) Transglutaminase type II is involved in the pathogenesis of endotoxic shock. J Immunol 180:2616–2624

Folk JE (1983) Mechanism and basis for specificity of transglutaminase-catalyzed epsilon-(gamma-glutamyl) lysine bond formation. Adv Enzymol Relat Areas Mol Biol 54:1–56

Jang GY, Jeon JH, Cho SY, Shin DM, Kim CW, Jeong EM et al (2010) Transglutaminase 2 suppresses apoptosis by modulating caspase 3 and NF-kappaB activity in hypoxic tumor cells. Oncogene 29:356–367

Kim SY (2004) New target against inflammatory diseases: transglutaminase 2. Arch Immunol Ther Exp (Warsz) 52:332–337

Kim SY (2011) Transglutaminase 2: a new paradigm for NF-kappaB involvement in disease. Adv Enzymol Relat Areas Mol Biol 78:161–195

Kim DS, Kim B, Tahk H, Kim DH, Ahn ER, Choi C et al (2010) Transglutaminase 2 gene ablation protects against renal ischemic injury by blocking constant NF-kappaB activation. Biochem Biophys Res Commun 403:479–484

Kim SJ, Kim KH, Ahn ER, Yoo BC, Kim SY (2011) Depletion of cathepsin D by transglutaminase 2 through protein cross-linking promotes cell survival. Amino Acids [Epub ahead of print]

Kuncio GS, Tsyganskaya M, Zhu J, Liu SL, Nagy L, Thomazy V et al (1998) TNF-alpha modulates expression of the tissue transglutaminase gene in liver cells. Am J Physiol 274:G240–G245

Lee J, Kim YS, Choi DH, Bang MS, Han TR, Joh TH et al (2004) Transglutaminase 2 induces nuclear factor-kappaB activation via a novel pathway in BV-2 microglia. J Biol Chem 279:53725–53735

Mirza A, Liu SL, Frizell E, Zhu J, Maddukuri S, Martinez J et al (1997) A role for tissue transglutaminase in hepatic injury and fibrogenesis, and its regulation by NF-kappaB. Am J Physiol 272:G281–G288

Muto Y, Nouri-Aria KT, Meager A, Alexander GJ, Eddleston AL, Williams R (1988) Enhanced tumour necrosis factor and interleukin-1 in fulminant hepatic failure. Lancet 2:72–74

Nanda N, Iismaa SE, Owens WA, Husain A, Mackay F, Graham RM (2001) Targeted inactivation of Gh/tissue transglutaminase II. J Biol Chem 276:20673–20678

Nardacci R, Lo Iacono O, Ciccosanti F, Falasca L, Addesso M, Amendola A et al (2003) Transglutaminase type II plays a protective role in hepatic injury. Am J Pathol 162:1293–1303

Oh K, Park HB, Byoun OJ, Shin DM, Jeong EM, Kim YW et al (2011) Epithelial transglutaminase 2 is needed for T cell interleukin-17 production and subsequent pulmonary inflammation and fibrosis in bleomycin-treated mice. J Exp Med 208:1707–1719

Park SS, Kim JM, Kim DS, Kim IH, Kim SY (2006) Transglutaminase 2 mediates polymer formation of I-kappaBalpha through C-terminal glutamine cluster. J Biol Chem 281:34965–34972

Park KS, Kim DS, Ko C, Lee SJ, Oh SH, Kim SY (2011) TNF-alpha mediated NF-kappaB activation is constantly extended by transglutaminase 2. Front Biosci (Elite Ed) 3:341–354

Sarang Z, Molnar P, Nemeth T, Gomba S, Kardon T, Melino G et al (2005) Tissue transglutaminase (TG2) acting as G protein protects hepatocytes against Fas-mediated cell death in mice. Hepatology 42:578–587

Tacke F, Luedde T, Trautwein C (2009) Inflammatory pathways in liver homeostasis and liver injury. Clin Rev Allergy Immunol 36:4–12

Tatsukawa H, Fukaya Y, Frampton G, Martinez-Fuentes A, Suzuki K, Kuo TF et al (2009) Role of transglutaminase 2 in liver injury via cross-linking and silencing of transcription factor Sp1. Gastroenterology 136(1783–1795):e1710

Vereyken EJ, Heijnen PD, Baron W, de Vries EH, Dijkstra CD, Teunissen CE (2011) Classically and alternatively activated bone marrow derived macrophages differ in cytoskeletal functions and migration towards specific CNS cell types. J Neuroinflammation 8:58

Yamaguchi H, Wang HG (2006) Tissue transglutaminase serves as an inhibitor of apoptosis by cross-linking caspase 3 in thapsigargin-treated cells. Mol Cell Biol 26:569–579