HEPATOLOGY

HEPATOLOGY, VOL. 00, NO. 00, 2016

Opposing Role of Tumor Necrosis Factor Receptor 1 Signaling in T Cell–Mediated Hepatitis and Bacterial Infection in Mice

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Death receptor (DR) ligands such as tumor necrosis factor (TNF) have been identified as fundamental mediators of liver damage both in mouse models and in humans. While the essential site of function of DR signaling is conceivably the hepatocyte, a systematic analysis is missing. Using mice with conditional gene ablation, we analyzed the tissue-specific function of DR signaling in T cell–dependent (concanavalin A) and independent (lipopolysaccharide/galactosamine) hepatitis and in models of bacterial infection (*Listeria monocytogenes*, lipopolysaccharide). We report that lipopolysaccharide/galactosamine-induced liver injury depends on hepatocyte-intrinsic TNF receptor 1 (p55, TNFR1). In contrast, we show that T cell–induced hepatitis was independent of TNFR1 signaling in hepatocytes, T cells, or endothelial cells. Moreover, T cell–induced hepatitis was independent of hepatocyte-intrinsic Fas-associated protein with death domain, TNF-related apoptosis-inducing ligand receptor, or Fas signaling. Instead, concanavalin A–induced hepatitis was completely prevented in mice with myeloid-derived cell (MDC)–specific deletion of TNFR1. Significantly, however, mice lacking TNFR1 in MDCs succumbed to listeria infection, although they displayed similar sensitivity toward endotoxin-induced septic shock when compared to control mice. These results suggest that TNFR1 signaling in MDCs is a critical mediator of both the detrimental and the protective functions of TNF in T cell–induced hepatitis and bacterial infection, respectively. *Conclusion:* The critical site of action of DRs is completely dependent on the nature of hepatitis; the data specify MDCs as the essential cell type of TNFR1 function in T cell–mediated hepatitis and in the response to listeria, thereby identifying the opposing role of MDC TNFR1 in autoimmunity and bacterial infection. (HEPATOLOGY 2016; 00:000–000)

The orchestration of an efficient and regulated immune reaction against infection relies on the crosstalk of inflammatory cells. Proinflammatory and anti-inflammatory molecules such as cytokines and chemokines are the key messengers between cells of the innate and the adaptive immune system. The perception of these intercellular signals depends on cell surface receptors; however, the functional consequences of stimulation through a particular signaling molecule are context- and cell type–dependent, and incompletely understood.

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; CD, cluster of differentiation; ConA, concanavalin A; Cre, cyclization recombination; CXCL, chemokine (C-X-C motif) ligand; DR, death receptor; EC-KO, endothelial cell–specific knockout; FADD, Fas-associated protein with death domain; Gal, D-galactosamine; H&E, hematoxylin and eosin; IL, interleukin; LPC, liver parenchymal cell; LPS, lipopolysaccharide; MDC, myeloid-derived cell; PBS, phosphate-buffered saline; SEM, standard error of the mean; TC-KO, T cell–specific knockout; TNF, tumor necrosis factor; TNFR1, TNF receptor 1; TRAILR, TNF-related apoptosis-inducing ligand receptor.

Received September 8, 2015; accepted March 9, 2016.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.28551/suppinfo.

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Supported by the YAEL Stiftung, Hamburg, Germany (to H.E. and R.W.). M.A and G.K have been supported by the Innovative Medicines Initiative–funded project BeTheCure (115142-2, to G.K.) and European Research Council advanced grant MCs-inTEST (340217, to G.K.).

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DOI 10.1002/hep.28551

While the immune system plays a fundamental role in protecting the host against omnipresent infectious pathogens, abnormally functioning immune cells directed against self-antigens were identified as the cause of different autoimmune diseases. In humans, liver damage and hepatitis can be caused by many factors including infections, drugs or alcohol, as well as autoimmune disease. Autoimmune hepatitis (AIH) is a chronic liver disease of unknown etiology. While the pathogenesis of AIH remains incompletely understood, most evidence is consistent with a central role of autoreactive T cell function resulting in liver parenchymal death and chronic liver injury. Immunosuppressive therapy is the mainstay of treating AIH, but although it is highly effective, it has been associated with an increased risk for infectious complications.

Generally, death of hepatocytes and cholangiocytes (liver parenchymal cells [LPCs]) is the cardinal feature of liver damage and hepatitis. Death receptors (DRs) have been implicated as principal mediators of liver injury. LPCs are thought to be particularly susceptible to DR-induced killing given the ubiquitous expression of these receptors in the liver.[1] Tumor necrosis factor (TNF) as the prototypical DR ligand is a proinflammatory cytokine that exerts its functions through TNF receptors 1 (p55TNFR; TNFR1) and 2 (p75TNFR; TNFR2). Stimulation of TNFR1 leads to the assembly of a signaling complex that activates nuclear factor κB and mitogen-activated protein kinase pathways. The aggregation of a second complex includes binding of Fas-associated protein with death domain (FADD), which is an indispensable adaptor protein for the recruitment and activation of caspase-8 and the induction of apoptosis.[2] Moreover, in the liver, FADD was shown to promote both DR-dependent and independent cell death pathways.[3,4] Importantly, depending on the affected cell type and the balance of intracellular signaling pathways, TNF can induce death but also promote cell survival and immune activation in response to infection.[5-8] Drugs blocking TNF have become a central option in the treatment of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and AIH.[9] Importantly, however, interfering with TNF to treat autoinflammatory disease increases the risk of infectious side effects, reflecting again the dual role of this cytokine.

Mechanisms of liver injury are complex, and cell culture and *ex vivo* analysis were found insufficient to study the intricate interaction between LPCs and immune cells, cytokines, and the cell death–inducing machinery. Using mouse models of hepatitis and liver failure has become a standard to discern this complexity. The most widely studied mouse models of experimental hepatitis are concanavalin A (ConA)–induced and lipopolysaccharide + D-galactosamine (LPS/Gal)–induced liver injury. Treatment of mice with the plant lectin ConA induces a strong stimulation of the immune system, leading to liver damage that was reported to involve T cells and natural killer T cells, thereby recapitulating important aspects of AIH.[10] In contrast, the LPS/Gal model of liver failure is independent of T cells.[11] TNF has been implicated in the development of liver damage in both models.[8,12,13] After injection of LPS/Gal, TNF was released by Toll-like receptor 4–positive macrophages[14] and

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TNFR1-deficient mice were found to be protected against hepatitis.[8] T cell–mediated hepatitis induced by ConA was shown to be dependent on TNF–TNFR signaling as mice lacking TNF or TNFR1/2 were strongly protected.[12,15] Furthermore, it was shown for this model that TNF was released from both macrophages and T cells and that elimination of TNF from either source led to reduced levels of hepatitis.[16] TNF expression and induction of hepatitis required c-Jun NH2-terminal kinase in cells of the hematopoietic compartment as mice with selective loss of c-Jun NH2-terminal kinase 1/2 expression in hematopoietic cells exhibited a profound defect in the development of hepatitis.[17] From these data, it seems plausible that, in both models, TNF is produced by immune cells consequently directing liver injury by activation of DRs on LPCs.

The aim of this study was to examine the cell-specific function of the TNFR1 pathway in models of liver injury using mouse lines with tissue-specific deletion of TNFR1. We discovered that the site of the essential function of TNFR1 is entirely dependent on the model of hepatitis. Moreover, we show that myeloid-derived cell (MDC)–specific TNFR1 is critical for both the deleterious effect of TNF in T cell–mediated hepatitis and its protective role in bacterial infection.

Materials and Methods

GENERATION AND HANDLING OF MICE

All mice were maintained in a C57BL/6 background. Animals were bred at the animal facilities of the University Medical Center Hamburg-Eppendorf (Hamburg, Germany), the University of Cologne (Cologne, Germany), and the Biomedical Sciences Research Center "Alexander Fleming" and received human care. All animal procedures were conducted in accordance with European, national, and institutional guidelines and protocols and approved by local government authorities (animal license number 86/11, Hamburg, Germany, and animal license number 2280/4-7-2011, Athens, Greece). TNFR1 (p55TNFR) floxed mice,[18] Fas floxed and TNF-related apoptosis-inducing ligand receptor (TRAILR) floxed mice crossed to AlfpCre have been described.[3] AlfpCre expressing mice[19] were crossed to $TNFR1^{FL}$ mice to create mice with liver parenchymal (i.e., hepatocyte and cholangiocyte)–specific deletion. LysMCre expressing mice[20] were crossed to $TNFR1^{FL}$ mice to obtain mice with myeloid-derived cell–specific deletion of TNFR1. Cluster of differentiation 4 (CD4)-Cre expressing mice (B6.Cg-Tg[Cd4-cre]1Cwi/BfluJ; The Jackson Laboratory) were crossed to $TNFR1^{FL}$ mice to receive T cell-specific deletion of TNFR1. Cdh-5 Cre[21] expressing mice have been crossed to $TNFR1^{FL}$ mice to obtain mice with deletion of TNFR1 in endothelial cells. The $3DR^{LPC\text{-}KO}$ mouse line was obtained by crossing male $NEMO^{FL/y}3DR^{FL/FL(3)}$ with $TNFR^{FL/FL}$;AlfpCre transgenic mice to obtain $NEMO^{WT}3DR^{FL}$ mice. $3DR^{FL}$ were intercrossed for homozygosity of the floxed DR alleles. For listeria infection experiments, heterozygous mice ($TNFR1^{fl/+}$) were mated to cytomegalovirus-Cre mice[22] to constitutively delete the sequences between the loxP sites. The newly generated knockout allele has been designated $TNFR1^{d}$.

GENOTYPING

The typing protocols of the mice used in this study have been described.[3,19,20,23]

CONA, LPS/GAL CHALLENGE AND LISTERIA INFECTION

ConA Challenge

Age-matched male mice between 8 and 12 weeks old were injected intravenously with 6-9 mg of ConA (Sigma) per kilogram of body weight. Animals were sacrificed 1-8 hours after ConA; blood, spleen, and liver were collected for analysis.

LPS/Gal, ConA/Gal, and LPS Challenge

Gal × HCl (0.7 mg/g body weight; Geneaxxon Bioscience, Germany) was administered intraperitoneally to age-matched and sex-matched animals 15 minutes before intraperitoneal injection of LPS (0.4 mg/g body weight, *Escherichia coli* O111:B4; Invivogen) or intravenous injection of ConA (6 mg/kg body weight). When challenged with LPS alone, age-matched and sex-matched mice received 20 mg/kg body weight intraperitoneally. Mice were monitored for survival up to 7 days. For detailed information on dosages see Supporting Information.

Listeria Infection

Mice were injected intraperitoneally with 10^4 colony-forming units of virulent *Listeria monocytogenes* strain L028 (kindly provided by Geneviere Milon, Pasteur Institute, France) and monitored for survival. Surviving animals were sacrificed 12 days after injection.

QUANTIFICATION OF CHEMOKINES AND CYTOKINES

Real-Time Polymerase Chain Reaction

Isolation of total RNA and complementary DNA synthesis were performed as described.[3] Gene-specific Taq Man assays were performed. Messenger RNA expression was normalized to the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase.

Enzyme-Linked Immunosorbent Assay

The following serum cytokines were measured using enzyme-linked immunosorbent assay kits following the manufacturers' instructions: TNF, interferon-γ, interleukin-4 (IL-4), IL-6, IL-10 (all BioLegend), IL-1β, and macrophage inflammatory protein 2 (R&D Systems). Serum levels of alanine aminotransferase (ALT) were measured using standard protocols in a Roche-Cobas Integra 400 plus analyzer.

IMMUNE CELL ISOLATION AND FLUORESCEIN ISOTHIOCYANATE ANALYSIS

To isolate the immune cells from the spleen and liver, the tissues were mechanically dissociated in 1 × phosphate-buffered saline (PBS) 1% fetal calf serum using a syringe plunger, passed through a 70-μm cell strainer, and centrifuged at 390*g* for 10 minutes. Liver immune cells were separated from hepatocytes by Percoll gradient centrifugation at 450*g* for 20 minutes. Erythrocytes were lysed in 0.15 M NH_4Cl, and the remaining cells were washed and resuspended in 1 × PBS 1% fetal calf serum. The cells were preincubated with rat antimouse CD16/CD32 (Mouse TruStain fcX) antibody for 10 minutes and then incubated with 4′,6-diamidino-2-phenylindole, CD45 (clone 30-F11), CD11c (clone M1/70), F4/80 (clone BM8), Ly6C (clone HK1.4), GR-1 (clone RB6-8C5), CD3 (clone 17A2), CD8 (clone 53-6.7), CD4 (clone RM4-5), and NK1.1 (clone PK136) antibodies (all BioLegend) for 20 minutes in the dark. After two washes, the cells were resuspended in 1 × PBS 1% fetal calf serum and analyzed using a BD LSR Fortessa (BD Biosciences, San Jose, CA); the data were analyzed using FlowJo_V10.

ANALYSIS OF LIVERS AND IMMUNOHISTOCHEMISTRY

Histology was determined using 3-μm-thick sections of formalin-fixed, paraffin-embedded liver tissues stained with hematoxylin and eosin (H&E). Immunohistochemical staining of sections was performed as described.[3] Antibodies used were antimouse Ly6G (clone 1A8; BioXCell) and antimouse F4/80 (clone A3-1; Abcam). Secondary antibodies used were goat antirat peroxidase–conjugated (Dianova) and rabbit antirat immunoglobulin horseradish peroxidase–conjugated (Dako). Protein block and a diaminobenzidine substrate chromogen system (Dako; K3466) were used in all stainings.

STATISTICS

The statistical significance of differences was determined by the Student *t* test using the program Prism (GraphPad Software, Inc.). Graphs show medians ± standard error of the mean (SEM) unless noted otherwise.

SUPPLEMENTAL MATERIAL

The Supporting Information comprises six figures including details on the generated mouse lines and supporting data on the role of inflammatory cells, cytokines, and oxidative stress.

Results

LIVER PARENCHYMAL TNFR1 SIGNALING IS DISPENSABLE FOR T CELL–MEDIATED HEPATITIS

For ConA-induced hepatitis, it has been suggested that DR ligands mediate damage by acting directly upon the liver parenchyma.[12,24,25] Because FADD is the main adaptor transmitting apoptotic signals by all known DRs, we were intrigued to find that mice lacking this molecule selectively in the liver parenchyma ($FADD^{LPC\text{-}KO}$) were sensitive toward ConA: after challenge with ConA, $FADD^{LPC\text{-}KO}$ mice showed

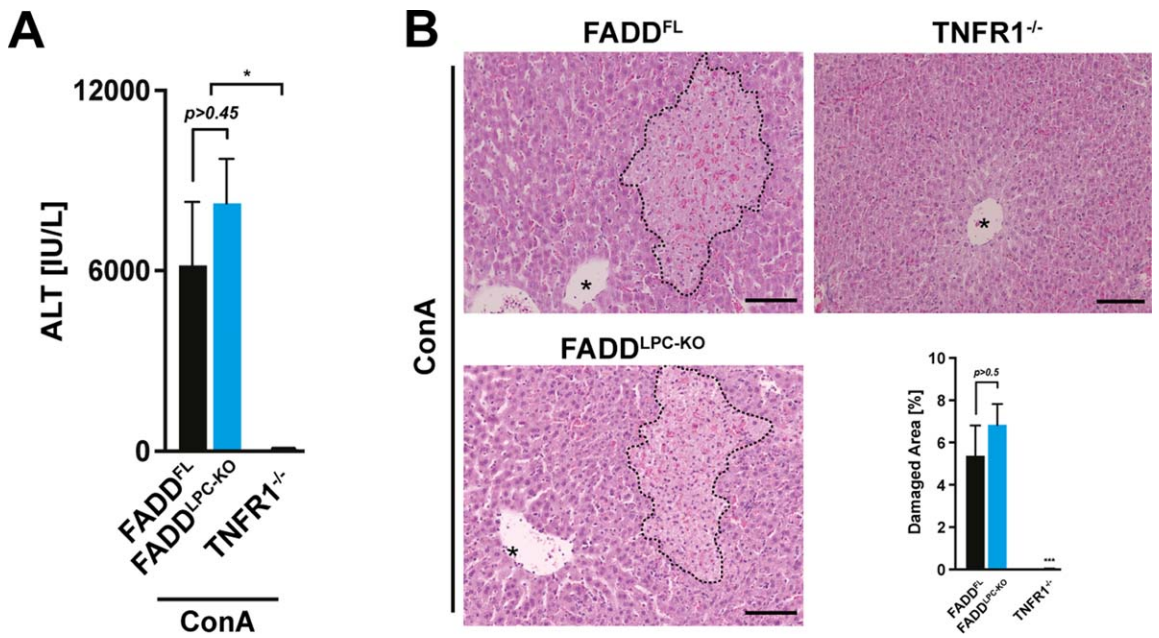

FIG. 1. Liver parenchymal FADD is dispensable in ConA-induced hepatitis. (A) Serum ALT levels 8 hours after ConA injection of mice with the indicated genotypes (n = 12, 9, and 5, respectively). (B) H&E liver stainings of representative damaged area 8 hours after ConA injection. Graph shows quantification of damaged liver area (mean and SEM). In photographs, * indicates central vein, bars = 50 μm, and dashed line surrounds damaged area. $^{*}P < 0.05$; $^{***}P < 0.001$.

similar levels of serum ALT and damaged liver area upon histological analysis as their littermate controls (Fig. 1). In contrast and consistent with the findings of others,[12] we confirmed that mice with a complete deletion of TNFR1 ($TNFR1^{-/-}$) were fully protected against ConA-induced liver injury (Fig. 1).

Our finding that the presence of FADD in LPCs was dispensable for ConA-induced hepatitis but that $TNFR1^{-/-}$ mice were protected prompted us to consider two possibilities for the role of TNF in T cell–mediated liver injury: (1) there is an LPC-dependent but FADD-independent role for TNFR1 or (2) a different cell type than the liver parenchyma is the site of the essential function of TNFR1. To test the hypothesis that TNF directly mediates liver injury by acting upon hepatocyte-intrinsic or cholangiocyte-intrinsic TNFR1, we generated mice lacking the receptor specifically in the liver parenchyma ($TNFR1^{LPC\text{-}KO}$; Supporting Fig. S1). $TNFR1^{LPC\text{-}KO}$ mice challenged with ConA were as sensitive toward liver injury as their littermate controls as shown by similar serum ALT levels and similar extent of damaged liver area (Fig. 2A). We could also not detect statistically significant differences in the increase of the spleen size between $TNFR1^{LPC\text{-}KO}$ and control mice (Fig. 2B). Conversely, in the LPS/Gal model of acute liver failure, we found $TNFR1^{LPC\text{-}KO}$ mice completely protected. Whereas all control mice died within 10 hours after challenge with LPS/Gal, $TNFR1^{LPC\text{-}KO}$ animals survived (Fig. 2C; Supporting Fig. S1). While we found a disrupted liver architecture and very high serum ALT levels already 5 hours after LPS/Gal injection in control mice, the $TNFR1^{LPC\text{-}KO}$ mice presented with normal liver architecture and low serum ALT levels even at 10 hours after LPS/Gal injection (Fig. 2D). After LPS/Gal challenge, $TNFR1^{LPC\text{-}KO}$ mice failed to display cleaved caspase-3–positive hepatocytes, while control animals showed abundant areas of positive cells (Supporting Fig. S4). Interestingly, ConA-injected animals failed to show cleaved caspase-3–positive cells (Supporting Fig. S4).

Apart from TNF, also FasL and TRAIL have been implicated in ConA-induced liver injury.[26-28] Because ablation of FADD or TNFR1 selectively in LPCs failed to protect mice from ConA-induced hepatitis, we assumed that also mice with disruption of the other DRs should not be protected against ConA-induced liver damage. To test this hypothesis, we took

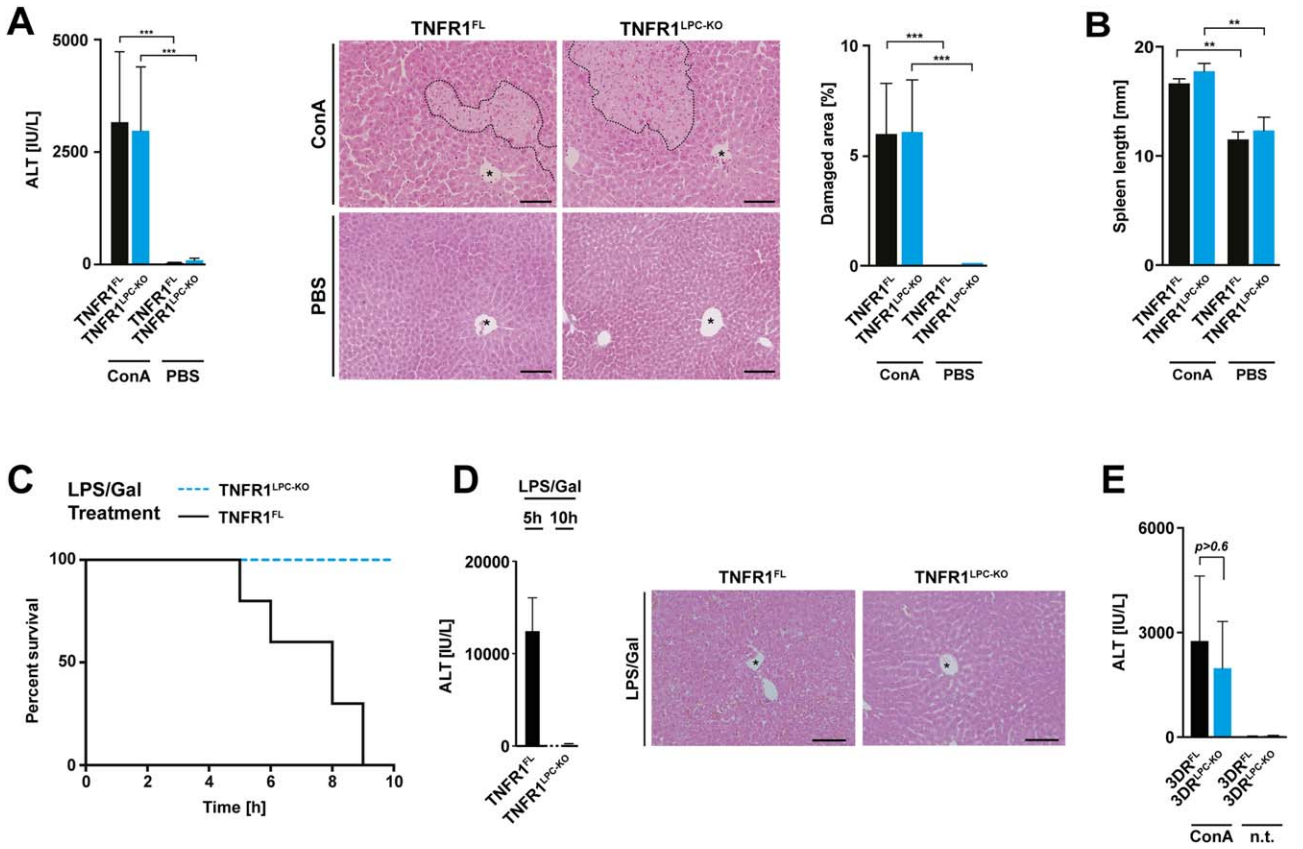

FIG. 2. Liver parenchymal death receptor signaling is dispensable in ConA-induced hepatitis. (A) Serum ALT levels and quantification of damaged liver area 8 hours after ConA or PBS injection of $TNFR1^{LPC\text{-}KO}$ and $TNFR1^{FL}$ mice (n = 14, 12, 2, and 3, respectively). H&E liver stainings show representative damaged area. (B) Spleen length 8 hours after injection of ConA or PBS. (C) Survival of $TNFR1^{LPC\text{-}KO}$ and $TNFR1^{FL}$ mice after LPS/Gal treatment (n = 5 and 4, respectively). (D) Serum ALT levels for $TNFR1^{LPC\text{-}KO}$ and $TNFR^{FL}$ 10 and 5 hours after LPS/Gal injection, respectively. Representative H&E staining of liver from $TNFR1^{LPC\text{-}KO}$ and control mice after LPS/Gal. (E) Serum ALT levels 8 hours after ConA or PBS injection of $3DR^{LPC\text{-}KO}$ and $3DR^{FL}$ mice (n = 6, 12, 3, and 2, respectively). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. In photographs, * indicates central vein, bars = 50 μm, and dashed line surrounds damaged area. Column graphs show mean and SEM. Abbreviation: n.t., not treated.

advantage of mice with a compound knockout of the DRs TNFR1, Fas, and TRAILR specifically in the liver parenchyma ($3DR^{LPC\text{-}KO}$; Supporting Fig. S1). As expected, $3DR^{LPC\text{-}KO}$ mice exhibited similar levels of liver damage as their floxed littermates after challenge with ConA (Fig. 2E). Taken together, our results are consistent with the indispensable need of FADD for the activation of procaspases downstream of DR signaling. However, after ConA challenge, as opposed to LPS/Gal-induced hepatitis, liver injury is not mediated through DRs of the liver parenchyma. Instead, with these results we predicted that a different cell population must be the site of the essential function of TNFR1 in T cell–mediated hepatitis.

T CELL–SPECIFIC AND ENDOTHELIAL CELL–SPECIFIC TNFR1 IS DISPENSABLE IN EXPERIMENTAL HEPATITIS

T cells and liver resident natural killer T cells were shown to be of critical importance in ConA-induced hepatitis.[10,27] Moreover, it has been demonstrated in detailed genetic analyses that TNF was released from both macrophages and T cells after ConA challenge.[16] Hence, we were interested to test if T cells could also be the critical target for TNF through TNFR1 in ConA-induced hepatitis. We therefore generated mice with a T cell–specific knockout of TNFR1 ($TNFR1^{TC\text{-}KO}$; Supporting Fig. S1).

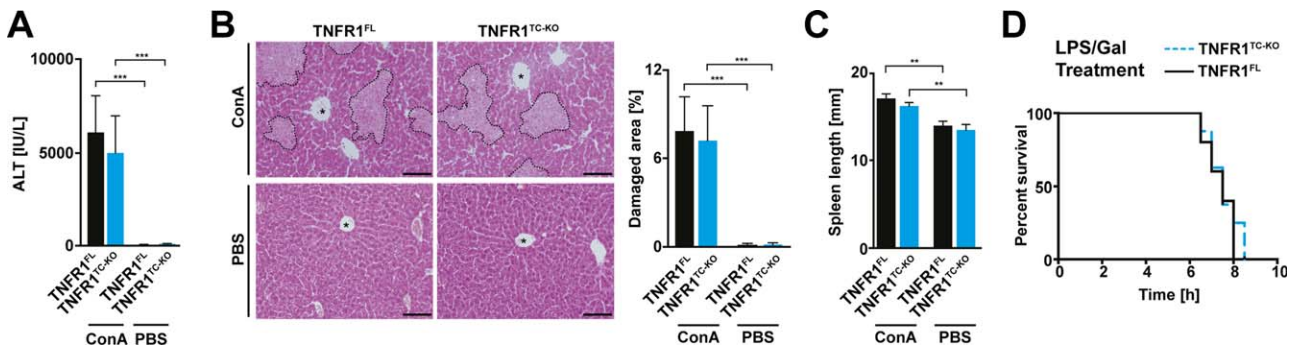

FIG. 3. TNFR1 in T cells is dispensable in experimental hepatitis. (A) Serum ALT levels and (B) quantification of damaged liver area of TNFR1$^{TC\text{-}KO}$ and TNFR1^{FL} mice 8 hours after ConA or PBS injection (n = 15, 15, 2, and 3, respectively). H&E liver stainings show representative damaged area. (C) Spleen length 8 hours after injection of ConA. (D) Survival of TNFR1$^{TC\text{-}KO}$ and TNFR1^{FL} mice after LPS/Gal injection (n = 8 and 5, respectively). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Column graphs show mean and SEM. In photographs, * indicates central vein, bars = 50 μm, and dashed line surrounds damaged area.

TNFR1$^{TC\text{-}KO}$ mice showed similar sensitivity to ConA-induced liver damage as control mice as indicated by statistically insignificant differences in serum ALT levels and the amount of degenerated liver area (Fig. 3A,B). Furthermore, we did not observe relevant differences in the increase of spleen size between the knockout and the control mice after challenge with ConA (Fig. 3C). When challenged with LPS/Gal, no difference could be detected between TNFR1$^{TC\text{-}KO}$ and control mice as measured by overall survival (Fig. 3D).

Endothelial cells function as important gatekeepers and regulators in the orchestration of inflammatory responses. Moreover, within the context of ConA-induced hepatitis it has been suggested that liver sinusoidal endothelial cells are critically involved in liver injury by mediating a hypercoagulative state.[29] We therefore asked if endothelial cells were the essential cell type for TNFR1 function in T cell–mediated hepatitis. To this end we generated mice with a selective deletion of TNFR1 in endothelial cells (Supporting Fig. S1). TNFR1 endothelial cell–specific knockout mice (TNFR1$^{EC\text{-}KO}$) and their littermate controls displayed similar levels of liver damage, as measured by serum ALT levels and damaged area quantification on H&E sections (Fig. 4A,B). The increase in spleen size was also similar between TNFR1$^{EC\text{-}KO}$ and control mice (Fig. 4C). Interestingly, when challenged with LPS/Gal, no difference could be detected between TNFR1$^{EC\text{-}KO}$ and control mice based on overall survival (Fig. 4D). Collectively, these data demonstrate that LPS/Gal-mediated and T cell–mediated hepatitis is caused independently of TNFR1 signaling in T cells or endothelial cells.

MDCS ARE THE SITE OF ESSENTIAL TNFR1 FUNCTION IN T CELL–MEDIATED HEPATITIS

Macrophages and neutrophils have been demonstrated to fulfill fundamental and nonredundant functions in T cell–mediated liver injury.[10,30] After injection of ConA, we found a more than 20-fold increase in neutrophils in the livers of wild-type mice and that circulating neutrophils in the blood doubled in number (data not shown). To address the role of MDC-specific TNFR1, we crossed TNFR1 floxed mice with LysM-Cre transgenic mice to generate TNFR1$^{MDC\text{-}KO}$ mice (Supporting Fig. S1). Strikingly, upon ConA challenge, TNFR1$^{MDC\text{-}KO}$ mice were completely protected against liver damage as measured by serum ALT levels and quantification of damaged liver area upon histological evaluation (Fig. 5A,B). Interestingly, however, when we measured the increase in spleen size upon challenge with ConA we could not find statistically significant differences between the protected TNFR1$^{MDC\text{-}KO}$ mice and the control mice (Fig. 5C).

ConA-mediated liver damage has been linked to the function of different cytokines and chemokines. We therefore analyzed their dynamics in TNFR1$^{MDC\text{-}KO}$ compared to control mice (Fig. 5D-F). Remarkably, we did not determine statistically significant differences in serum concentrations of TNF, interferon-γ, or other inflammatory cytokines between TNFR1$^{MDC\text{-}KO}$ and control mice (Fig. 5D). For serum IL-10 concentrations we found higher levels in TNFR1$^{MDC\text{-}KO}$ mice, although this trend failed to reach statistical significance

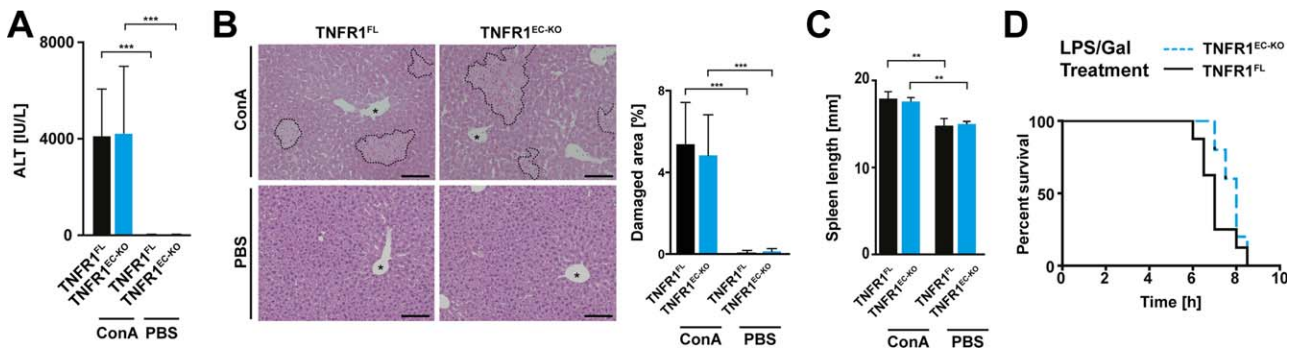

FIG. 4. TNFR1 in endothelial cells is dispensable in experimental hepatitis. (A) Serum ALT levels and (B) quantification of damaged liver area of TNFR1^{EC-KO} and TNFR1^{FL} mice 8 hours after ConA or PBS injection (n = 12, 13, 5, and 5, respectively). H&E liver stainings show representative damaged area. (C) Spleen length 8 hours after injection of ConA. (D) Survival of TNFR1^{EC-KO} and TNFR1^{FL} mice after LPS/Gal treatment (n = 5 and 8, respectively). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Column graphs show mean and SEM. In photographs, * indicates central vein, bars = 50 μm, and dashed line surrounds damaged area.

(Fig. 5D). TNFR1^{MDC-KO} mice had significantly lower expression for chemokine (C-C motif) ligand 2, as measured by quantitative polymerase chain reaction; and we observed a trend toward lower expression of chemokine (C-X-C motif) ligand 1 (CXCL1) and CXCL9 in TNFR1^{MDC-KO} mice (Fig. 5F). Hence, we asked if chemoattraction of inflammatory cells could be affected by ablation of TNFR1 in MDCs. However, when we quantified the extent of neutrophil, macrophage, and T-cell invasion into the liver we could not detect marked changes in TNFR1^{MDC-KO} mice (Supporting Fig. S2).

THE SITE-SPECIFIC FUNCTION OF TNFR1 IN EXPERIMENTAL HEPATITIS IS CONTEXT-DEPENDENT

Because we found that TNFR1^{MDC-KO} mice were protected from ConA-induced hepatitis, we were interested in whether the MDC-specific function of TNFR1 was also critical in LPS/Gal-induced liver injury. TNFR1^{MDC-KO} mice were as sensitive toward LPS/Gal as their littermate controls (Fig. 6A). Challenge with ConA alone has been shown to cause liver failure by inducing necrotic cell death, while in the presence of Gal it leads to hepatocyte death both by apoptosis and by necrosis.[31] Considering that pretreatment with Gal sensitized mice against LPC-specific, TNFR1-driven liver failure when challenged with LPS (Fig. 2C,D), we expected that challenge with ConA in the presence of Gal[31] would render TNFR1^{MDC-KO} mice more susceptible to liver failure. Indeed, upon pretreatment with Gal, TNFR1^{MDC-KO} mice died shortly after injection of ConA; and we found increased serum ALT levels compared to Gal treatment alone (Fig. 6B,C). Interestingly, in sharp contrast to TNFR1^{MDC-KO} and floxed control mice, we found that mice with an LPC-selective ablation of TNFR1 survived the ConA/Gal challenge (Fig. 6D). In conjunction with the results presented above, these data demonstrate that the mechanism of damage in TNFR1-mediated liver injury majorly depends on the competence of LPCs to induce the prosurvival pathway.

MDC TNFR1 HAS A PROTECTIVE FUNCTION IN BACTERIAL INFECTION

Our results demonstrate that mice with LPC-specific and MDC-specific deletion of TNFR1 were protected against LPS/Gal-induced and ConA-induced hepatitis, respectively. Interestingly, in contrast to this detrimental function of TNFR1, it was recently demonstrated that TNFR in macrophages played a fundamental role in orchestrating the defense against *E. coli* in a model of urinary tract infection.[6] Upon infection with *L. monocytogenes*, hepatocytes and macrophages have been identified as critical sites for bacterial inoculation and proliferation. As mice with complete ablation of TNFR1 were found to succumb to listeria infection,[8] we suspected that the sites of the essential function of TNFR1 could be hepatocytes or

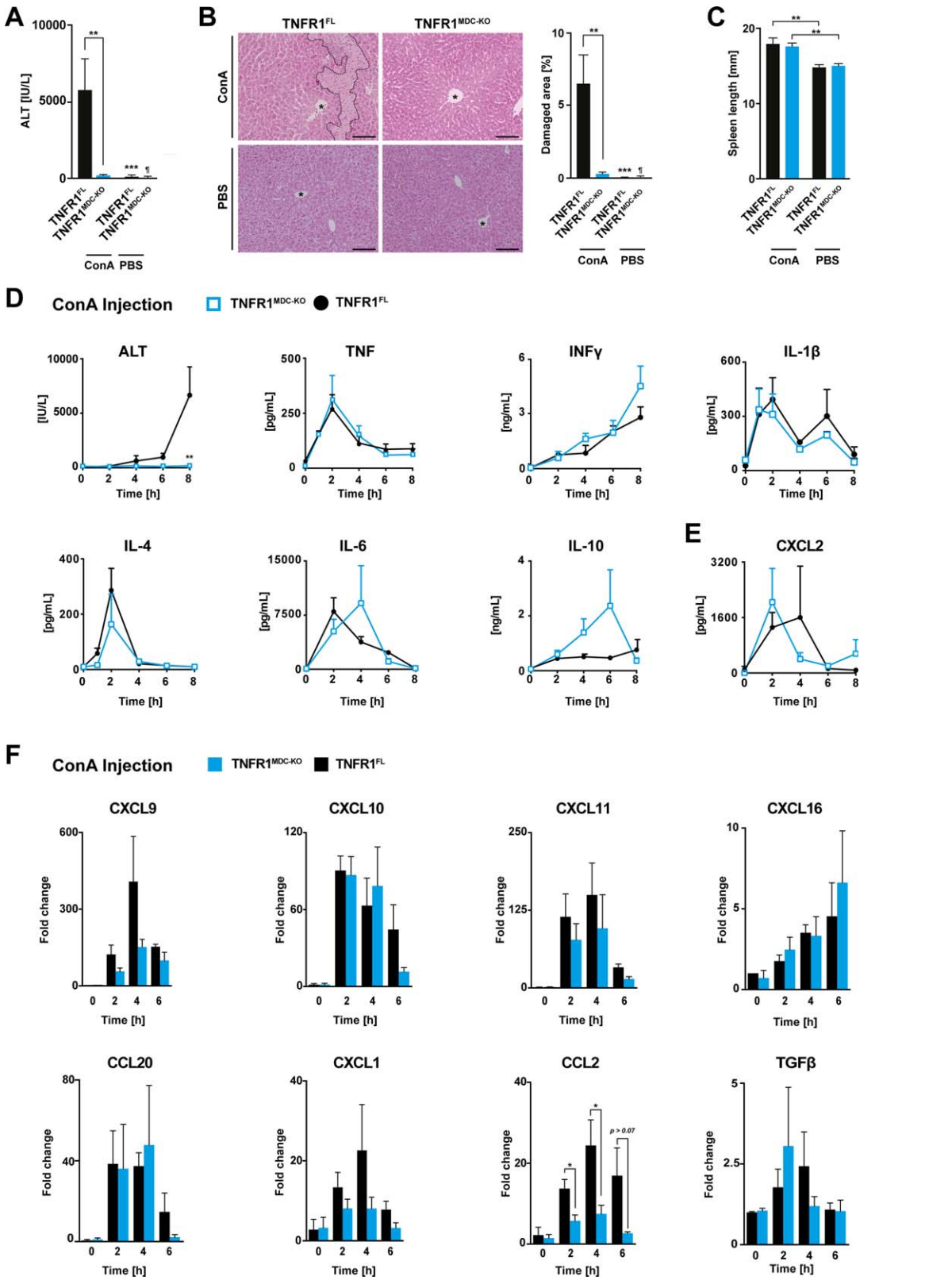

FIG. 5. MDCs are the site of essential TNFR1 function in T cell–mediated hepatitis. (A) Serum ALT levels and (B) H&E liver stainings with quantification of damaged liver area of mice with indicated genotypes 8 hours after challenge (n = 15, 17, 5, and 5, respectively). (D) Serum ALT levels and designated serum cytokine concentrations of $TNFR1^{MDC\text{-}KO}$ and $TNFR1^{FL}$ mice 0-8 hours after ConA injection (n = 2-8 per time point). (E,F) Serum concentrations of chemokines after injection of ConA as measured by enzyme-linked immunosorbent assay and quantitative polymerase chain reaction. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{¶}P > 0.18$. Graphs show mean and SEM. In photographs, * indicates central vein, bars = 50 μm, and dashed line surrounds damaged area. Abbreviation: INF, interferon

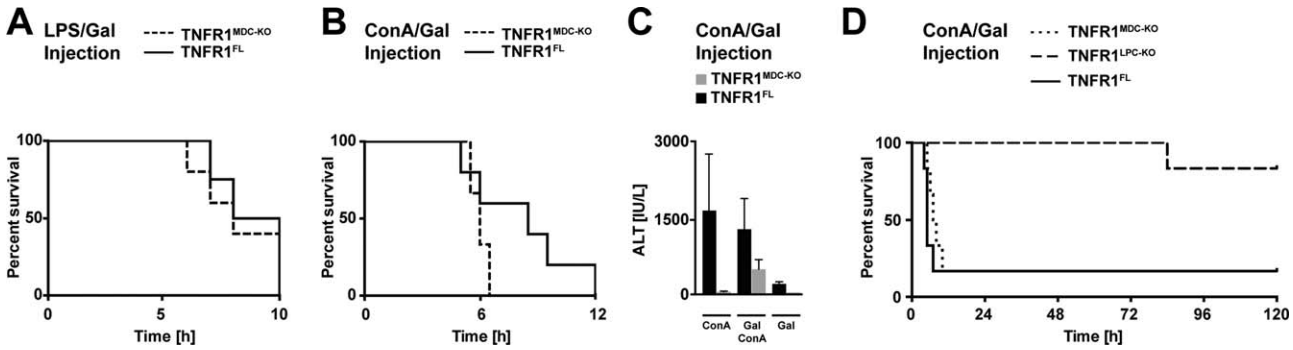

FIG. 6. Context dependency of cell-specific TNFR1 in experimental hepatitis. (A) Survival of $TNFR1^{MDC\text{-}KO}$ and $TNFR1^{FL}$ mice after LPS/Gal (n = 4 and 4, respectively) and (B) ConA/Gal injection (n = 3 and 5, respectively). (C) Serum ALT levels of $TNFR1^{MDC\text{-}KO}$ and $TNFR1^{FL}$ mice 5 hours after ConA/Gal injection (n = 2-4, mean and SEM). (D) Survival of $TNFR1^{LPC\text{-}KO}$ and control mice after ConA/Gal injection.

MDCs. When we challenged the respective tissue-specific knockout mice with listeria, we found that mice with an LPC-selective ablation of TNFR1 were protected, while mice with MDC-specific deletion of TNFR1 succumbed to listeria similarly to the $TNFR1^{-/-}$ mice (Fig. 7A). Given this important role of MDCs as the target for TNF in combatting listeria infection we asked whether TNFR1 in MDCs would also be critical in endotoxic shock induced by LPS injection. Interestingly, $TNFR1^{MDC\text{-}KO}$ mice were as sensitive as control mice upon injection of a median lethal dose of LPS (Fig. 7B). Moreover, upon injection of LPS (median lethal dose) neither LPC, T-cell, nor endothelial cell TNFR1 played a critical role as indicated by the statistically insignificant differences in the survival of the respective mice and their controls (Fig. 7C-E).

Taken together, these data show that, apart from its detrimental function in T cell–mediated hepatitis, MDC TNFR1 fulfilled a protective function in bacterial infection, although TNFR1 in neither of the examined cell types was critical to altering overall survival in endotoxic shock induced by LPS.

Discussion

It has been reported that TNF signaling is required for hepatocyte cell death in mouse models of hepatitis. Our results confirm the essential function of TNFR1. When using mouse lines with complete gene knockout, the complex crosstalk of immune cells and the liver parenchyma is difficult to interpret; however, by using mice with conditional gene knockout, immune cells have been identified as the source of TNF in ConA-induced and LPS/Gal-induced hepatitis.[16,17] Yet, the target cell type for binding of TNF remained unknown. That mice challenged with LPS after being pretreated with Gal develop hepatocyte death and liver failure, which is mediated by TNFR1 on LPCs, was certainly the favored concept. However, an unexpected finding of this study was that TNFR1 plays no major role in LPCs but instead does play a role in MDCs in T cell–mediated, ConA-induced hepatitis. These data do not conflict with findings that hematopoietic cells release TNF in a c-Jun NH2-terminal kinase 1/2–dependent manner in ConA-induced hepatitis.[17] Instead, the fact that we failed to detect changes in serum levels of TNF rather suggests that after ConA injection, TNF is initially released from MDCs and other immune cells and subsequently binds to TNFR1 in the same or a different subset of MDCs. This interpretation would be consistent with the recently suggested concept of crosstalk between sentinel and helper macrophages as the source and target of TNF in bacterial infection, respectively.[6] Schiwon et al. demonstrated a macrophage TNFR-dependent release of CXCL2 that allowed entry of neutrophils into the uroepithelium. However, in ConA-induced liver injury, we could not detect differences either in the serum concentration of CXCL2 or in the recruitment of neutrophils into the liver, suggesting that a different effector pathway mediates the deleterious function of TNFR1 in this model of hepatitis.

Our findings further exclude a role of Fas and TRAILR in LPCs in ConA-induced hepatitis. It is

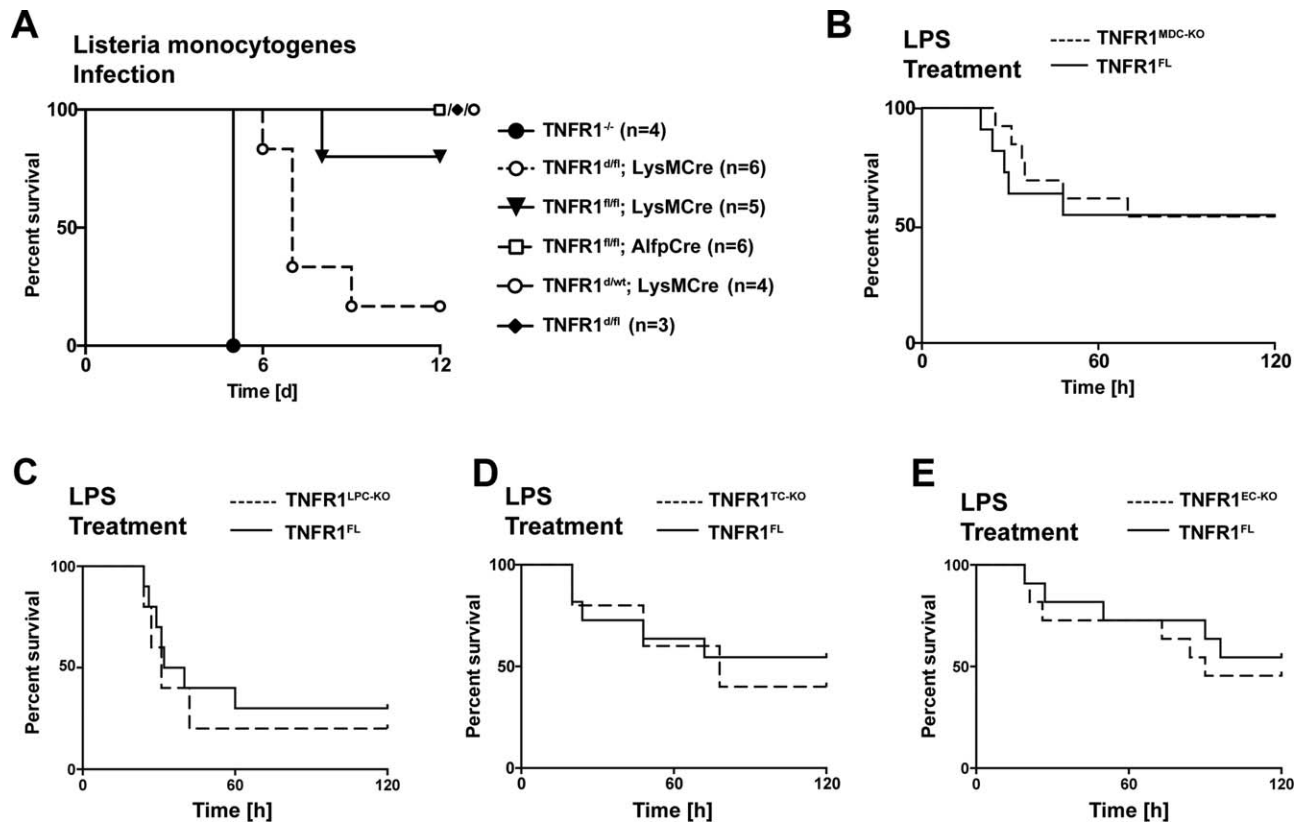

FIG. 7. Protective function of myeloid cell–derived TNFR1 in infection. (A) Survival of mice with MDC-specific deletion of TNFR1 and control mice after infection with *Listeria monocytogenes*. $TNFR1^{d}$ knockout allele for TNFR1 (see also Materials and Methods). Survival of (B) $TNFR1^{MDC\text{-}KO}$ (n = 13 and 11, respectively), (C) $TNFR1^{LPC\text{-}KO}$ (n = 5 and 10, respectively), (D) $TNFR1^{TC\text{-}KO}$ (n = 5 and 11, respectively), and (E) $TNFR1^{EC\text{-}KO}$ (n = 11 and 11, respectively) and $TNFR^{FL}$ control mice after LPS injection.

certain that under specific conditions such as pretreatment with Gal (this study) or in the absence of nuclear factor κB,[3,4] liver parenchymal DR signaling determines liver injury. Nonetheless, liver parenchymal DRs are not required in ConA-induced hepatitis with a functional hepatocyte survival pathway. These findings are of importance because they underline the significance of a cell death effector system that functions independently of DR ligands such as oxidative stress (Supporting Fig. S6A), perforin-induced cell death,[32] or liver parenchymal necrosis triggered by disturbances of the microcirculation.[29] Importantly, however, our results on the DR TNFR1 also demonstrate that within the context of an impaired survival pathway the site of the essential TNF function is the liver parenchyma but not MDCs. Impairment of the survival pathway (Gal pretreatment) sensitized mice toward LPC-intrinsic TNFR1-mediated liver injury, in the context of both ConA and LPS injection. In contrast, ConA-induced, T cell–dependent hepatitis requires MDC-intrinsic, but not LPC-intrinsic, TNFR1. Principally, the site-specific role of TNFR1 in experimental hepatitis is manifold, and the competence of hepatocytes to induce the prosurvival pathway is a critical determinant. In the absence of nuclear factor κB or pretreatment with Gal, TNF causes hepatocyte apoptosis (positive for cleaved caspase-3; Supporting Fig. S4; Fig. 8A). Importantly, the inability to activate the survival pathway renders hepatocytes sensitive to TNF also in the context of ConA challenge as demonstrated by our results showing that $TNFR1^{LPC\text{-}KO}$, but not $TNFR1^{MDC\text{-}KO}$, mice were protected after injection of ConA/Gal. In contrast, in the context of a functional prosurvival response, ConA-induced hepatitis completely depends on TNFR1 in MDCs (Fig. 8B). Interestingly, we found that this cell death is

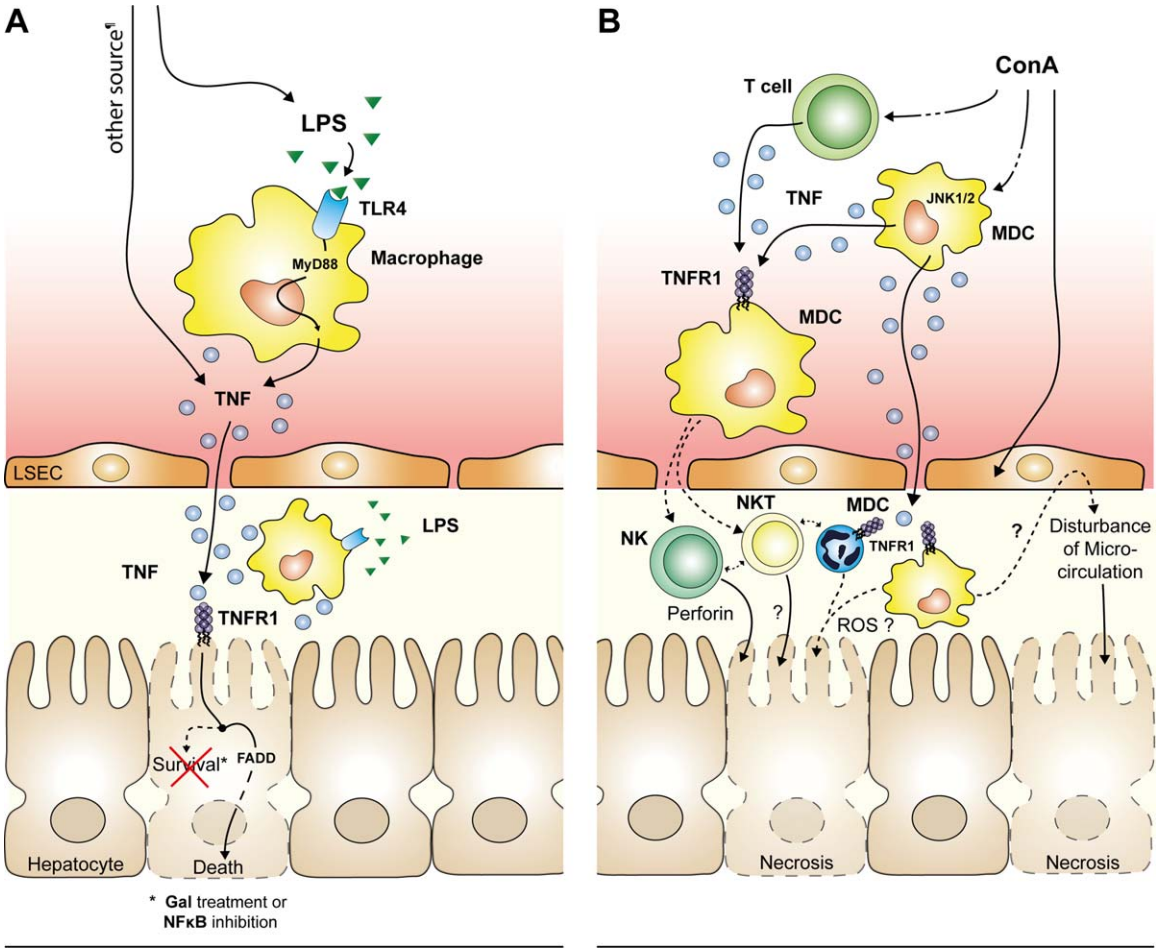

FIG. 8. Proposed concept for the cell–specific function of TNFR1 in different models of experimental hepatitis. (A) Essential function of hepatocyte-intrinsic TNFR1 in the context of an inhibited survival pathway. ¶Note that TNF could be induced through LPS but also through other sources such as ConA (see Fig. 6). (B) Essential role of MDC-intrinsic, but not hepatocyte-intrinsic, TNFR1 in the context of a functional survival pathway in the T cell–dependent model of ConA-induced liver injury. Note that, as opposed to (A), the effector phase of cell death induction in (B) is independent of liver parenchymal DRs and FADD. While cell-type specific TNFR1 fulfills deleterious functions in hepatitis TNFR1, in MDCs it has an additional protective function in the defense against bacteria such as in listeria infection (Fig. 5). Abbreviations: LSEC, liver sinusoidal endothelial cell; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor κB; NKT, natural killer T; ROS, reactive oxygen species; TLR4, Toll-like receptor 4.

independent of caspase-3 activation and that it could be prevented by inhibition of oxidative stress and necrostatin-1[33] (Supporting Fig. S4 and S6).

Our findings identify the critical role of MDC-intrinsic TNFR1 in T cell–mediated hepatitis. Remarkably, apart from this detrimental role, MDC-specific TNFR1 also fulfills a protective function in bacterial infection, as indicated by our results showing that $TNFR1^{MDC\text{-}KO}$ mice succumbed to listeria infection. These data suggest that the same cell type from the compartment of MDCs could fulfill an ambivalent function in autoimmune disease and infection. Alternatively, different populations of MDCs such as neutrophil granulocytes and different subsets of macrophages could fulfill a distinct function. Further experiments with ablation of genes from the TNF pathway selectively within the subcompartments of the hematopoietic fraction are required to determine the exact sites of release and binding of TNF in order to unravel its opposing functions in disease.

Anti-TNF agents have become an enormous success in the clinical management of chronic inflammatory conditions such as rheumatoid arthritis or inflammatory bowel disease, albeit anti-TNF treatment can provoke serious infectious complications. Interestingly, the anti-TNF antibody infliximab has been established

as a rescue therapy for patients with difficult-to-treat AIH when standard treatment is not tolerated or inefficient.[9] While anti-TNF is thought to neutralize soluble and membrane-bound TNF and might also trigger apoptosis of T cells in AIH,[9] the mechanism of action is unrecognized and the role of MDCs has not been addressed. Our findings in murine T cell–induced hepatitis suggest that TNF could similarly act upon MDCs resulting in T-cell activation in human AIH. Of note, our data imply that the observed susceptibility to infections upon treatment with anti-TNF agents could at least in part be explained by the function of TNFR1 on MDCs.

In conclusion, this study reveals that the essential site of the detrimental function of TNFR1 is dependent on the nature of hepatitis. Within the context of an impaired survival pathway, liver injury critically depends on hepatocyte-intrinsic TNFR1. MDC-intrinsic, but not hepatocyte-intrinsic, TNFR1, in contrast, is essential for T cell–mediated hepatitis. Importantly, however, TNF also fulfills an antibacterial function through TNFR1 on MDCs. The results describe a divergent role of MDC TNFR1 in autoimmunity and infection.

Acknowledgment: M.A. and G.K. thank Panagiotis Athanasakis and Spyros Lalos for excellent technical assistance and the InfrafrontierGR infrastructure (ERDF/NSRF 2007-2013) for mouse hosting and phenotyping facilities.

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Supporting Information

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