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Role of TNF-α Produced by Nonantigen-Specific Cells in a Fulminant Hepatitis Mouse Model

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In previous studies, the mechanisms of acute liver injury and virus exclusion have been examined using a model wherein HBsAg-specific CTL are injected into HBsAg transgenic (Tg) mice. The importance of the role of TNF-α in virus exclusion was shown, but its role in liver injury was unclear. We crossed the TNF-α knockout mouse and HBsAg-Tg mouse to establish the HBsAg-Tg/TNF-α KO mouse, and examined the influence of TNF-α on liver injury. The severity of liver damage, as determined by serum alanine aminotransferase activity, was ~100 times greater in HBsAg-Tg/TNF-α−/− mice than in HBsAg-Tg/TNF-α+/+ mice after i.v. administration of 5 × 10⁶ CTLs. This liver damage reached the peak of its severity within 24–48 h, and was restored 7 days later. Histopathological examination showed hepatocellular necrosis and inflammatory cell infiltrate 24 h after the CTL injection in HBsAg-Tg/TNF-α−/− mice but not in HBsAg-Tg/TNF-α+/+ mice. The liver damage was fatal for all HBsAg-Tg/TNF-α−/− mice that received 1.5 × 10⁷ CTLs. In contrast, 1.5 × 10⁷ CTLs could not kill the HBsAg-Tg/TNF-α−/− mice. The TNF-α production level was enhanced after the CTL injection in not only intrahepatic macrophages but also other types of mononuclear cells from non-HBsAg-Tg/TNF-α−/− mice. An adoptive transfer examination revealed that severe liver damage occurred in HBsAg-Tg/TNF-α−/− mice that had received mononuclear cells from TNF-α−/− mice. In conclusion, the present study provides evidence that TNF-α produced by intrahepatic non-Ag-specific inflammatory cells is critical in the development of lethal necroinflammatory liver disease. The Journal of Immunology, 2009, 182: 391–397.

Hepatitis B virus (HBV) is a nonlytic virus that does not directly infect cells and cause damage. Liver damage and viral clearance after an HBV infection are thought to be mediated by the host’s cellular immune response to viral Ags (1). CD8⁺ CTL play a critical role in the liver damage and viral clearance in HBV infections. These effector functions include the secretion of cytokines, such as IFN-γ and TNF-α, as well as cytolytic activity mediated by perforin and granzyme B (2–5). HBsAg transgenic (Tg) mice show no symptoms of liver disease until the adoptive transfer of HBsAg-specific CTLs, after which they develop a necroinflammatory liver disease that is histologically similar to acute viral hepatitis in man (6). The first step, which begins within 1 h of CTL administration, involves Ag recognition by the CTLs and delivery of a signal that results in the death of the hepatocyte. In the second step, which begins in 4–12 h, the CTLs recruit many host-derived inflammatory cells in their immediate vicinity, resulting in the formation of necroinflammatory foci. The third step is detectable 24–72 h after CTL administration: the livers display massive hepatocellular necrosis and an inflammatory cell infiltrate that consists principally of host-derived mononuclear cells and pronounced sinusoidal lining cell hyperplasia, which resembles the histopathological changes observed in patients dying from liver failure due to HBV-induced fulminant hepatitis. Using this murine fulminating hepatitis model, various studies have been conducted on viral clearance and liver disease in HBV infection.

TNF-α production is one of the earliest events in many types of liver injuries, and it triggers the production of other cytokines that together recruit inflammatory cells, kill hepatocytes, and initiate a hepatic healing response that includes fibrogenesis. The serum levels of TNF-α are significantly increased in patients with fulminant hepatitis (7). In viral hepatitis, elevated levels of plasma TNF-α and soluble TNFR are frequently observed (8). TNF-α is known to be released mainly by macrophages, but it also released by CD4⁺ and CD8⁺ T, B, NK (9), and dendritic cells (10). In particular, recent studies have shown that TNF-α is also released by CTLs (3) and contributes to CTL-mediated cytotoxicity, although its cytolytic activity is not as high as those of perforin and the Fas ligand (11–13).

The role of TNF-α is important not only in virus exclusion, but also in liver injury. However, in this model, it is considered that many host-derived inflammatory cells recruited by CTLs are critical in massive hepatocellular necrosis. In the present study, we crossed the TNF-α knockout (TNF-α−/−) mouse with the HBsAg-Tg mouse, to establish the HBsAg-Tg/TNF-α−/− mouse, and examined the influence of TNF-α produced by non-Ag-specific inflammatory cells on liver injury in a murine viral fulminant hepatitis model.

Materials and Methods

Mice

Male B10.D2 (H-2b) mice (age, 6–8 wk; weight, 25–30 g) were obtained from Japan SLC. The HBsAg-Tg mice lineage 107-5D (official designation

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2 Abbreviations used in this paper: HBV, hepatitis B virus; Tg, transgenic; sALT, serum alanine aminotransferase; MNC, mononuclear cell.

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Tg (Alb-1, HBV) Br66; inbred B10.D2, H-2b, in which the HBV envelope coding region is under the control of the mouse albumin promoter, was provided by Dr. F. V. Chisari (Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA). TNF-α−/− mice were produced by gene targeting as described previously (14) and backcrossed onto B10.D2 (H-2b). HBsAg-Tg/TNF-α−/− mice were produced by backcrossing TNF-α−/− mice with 107-5D.

Cell lines and reagents

P815 cells expressing HBV-preS1, 2, and S (P815-preS1), and HBsAg-specific, CD8+ CTL clones (6C2) were provided by Dr. F. V. Chisari (Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA). The clones are H-2d restricted, and can recognize an epitope (IPQSLDSWWTSL) located between residues 28 and 39 of HBsAg. Five days after the last stimulation with irradiated P815-preS1, the cells were washed and injected i.v. into HBsAg-Tg mice. (However, the phenotype of the 6C2, which we used in the present study, was changed in cytokines production. Therefore, we termed it 6C2-08.) Recombinant murine IFN-γ was obtained from R&D Systems.

Disease model

Five days after the last stimulation, the 6C2-08 cells were washed three times, suspended in PBS, and injected i.v. into HBsAg-Tg, non-Tg and HBsAg-Tg/TNF-α−/− recipients. Hepatocellular injury was monitored biochemically by measuring serum alanine aminotransferase (sALT) activity. At appropriate time points, mice were killed by cervical dislocation and necropsy was performed. Tissue samples were fixed in 10% formalin, embedded in paraffin, and sectioned; the sections were then stained with H&E. The intrahepatic distribution of HBV Ags was assessed by the indirect immunoperoxidase method using 3-aminio-9-ethyl carbazole.

Preparation of hepatic mononuclear cells and hepatocytes

Hepatic mononuclear cells (MNCs) (15) and hepatocytes (16) were isolated using the described simplified technique. The liver was perfused with liver perfusion and digestion medium (Life Technologies). Hepatocytes were separated from the MNCs by centrifugation at 50 × g for 2 min and were washed twice in complete RPMI 1640 (Nikken Biomedical Laboratory). MNC populations were purified using M-SMF (JINRO).

Real-time PCR

Total RNA was isolated and transcribed into cDNA using an RNaseasy Mini Kit and an Omniscript Reverse Transcriptase Kit (Qiagen GmbH). The resulting cDNA was used as a template for real-time PCR along with Kit and an Omniscript Reverse Transcriptase Kit (Qiagen GmbH). Total RNA was isolated and transcribed into cDNA using an RNeasy Mini Kit and an Omniscript Reverse Transcriptase Kit (Qiagen GmbH). The primer/probe sets for 18S were used as an internal control in each reaction (Applied Biosystems). Real-time PCR data were analyzed using the sequence detector software (Applied Biosystems).

TNF-α detection by ELISA

Macrophages, NK cells, CD4 T cells, and CD8 T cells were obtained from intrahepatic MNCs of HBsAg-Tg/TNF-α−/− mice treated with 6C2 cells with the help of an immunomagnetic separation system (MACS System), and cultured for 24 h. The concentrations of TNF-α in the culture supernatant were determined by an enzyme-linked absorbent assay kit for TNF-α (R&D Systems), according to the manufacturer’s instructions. The experimental results are expressed as the mean of triplicates ± SD of three independent experiments.

Intracellular cytokine staining

For intracellular staining, the hepatic MNCs from the mice that were administered 6C2 cells were incubated for 4 h with brefeldin A (10 μg/ml). Then, these cells were fixed, permeabilized the Cytofix/Cytoperm buffer (BD Pharmingen), and stained with FITC-conjugated anti-mouse TNF-α (clone MP6-XT22; eBioscience). Samples were acquired on a FACStar flow cytometer and data analysis was conducted using the CellQuest software (BD Pharmingen).

Adaptive cell transfer

HBsAg-Tg/TNF-α−/− mice received CD4-positive splenocytes, CD8-positive splenocytes, DX5-positive splenocytes, and CD11b-positive splenocytes as well as non-CD4, non-CD8, non-DX5, and non-CD11b splenocytes (8 × 106/mouse), which were isolated by MACS from the non-HBsAg-Tg/TNF-α−/− mice 7 days before the CTL injection. To confirm the existence of the transferred cells, we labeled the isolated cells with CFSE and performed flow cytometric analysis. Approximately 4% of HLs were CFSE positive at 7 days after the injection. Hepatocellular injury was monitored biochemically by measuring the sALT activity.

Statistics

Values are expressed as means ± SEM. Differences between the experimental and control groups were analyzed by the Kruskal-Wallis test followed by Scheffe’s F test. Significance was established at p < 0.05.

Results

Induction of fulminant hepatitis by HBsAg-specific CTLs in HBsAg-Tg/TNF-α−/− mice, but not in HBsAg-Tg/TNF-α−/− mice

CTL clones 6C2-08 (1 × 107) were incubated with 1 × 106 irradiated stimulator cells (P815-preS1) in complete medium without EL-4 supernatant. 6C2-08 were collected 12 h after stimulation and analyzed for IFN-γ and TNF-α mRNA expression by 6C2-08 (Fig. 1A). The IFN-γ and TNF-α mRNA expression was enhanced in 6C2-08 after stimulation. HBsAg-Tg/TNF-α−/− and HBsAg-Tg/TNF-α−/− mice received a single i.v. injection of CTLs (5 × 106 cells/mouse). As shown in Fig. 1B, severe liver damage (as determined by the sALT levels) was observed in HBsAg-Tg/TNF-α−/− mice that were administered CTLs, but not in HBsAg-Tg/TNF-α−/− or non-HBsAg-Tg/TNF-α−/− mice. Histological changes in the livers of HBsAg-Tg/TNF-α−/−, HBsAg-Tg/TNF-α−/−, and non-HBsAg-Tg/TNF-α−/− mice were examined on days 2 and 5 after the CTL injection. A histological analysis revealed widely scattered necroinflammatory foci, containing mostly mononuclear cells and apoptotic hepatocytes in the livers of HBsAg-Tg/TNF-α−/− mice after the CTL injection (Fig. 1C). In contrast, in HBsAg-Tg/TNF-α−/− mice, invasion of a large amount of inflammatory cells and hepatocellular necrosis were not observed. Next, to confirm the HBsAg expression in hepatocytes after the CTL injection, we stained the liver tissues with anti-HBsAg mAb 5 days after the CTL injection. Immunohistopathological examination revealed that HBsAg expression in the hepatocytes of HBsAg-Tg/TNF-α−/− mice was down-regulated compared with that of HBsAg-Tg/TNF-α−/− mice.

CTL dose-dependent induction of liver disease and mortality in HBsAg-Tg/TNF-α−/− and HBsAg-Tg/TNF-α−/− mice

Disease severity was also strictly dependent on the CTL dose on the CTL dose in HBsAg-Tg/TNF-α−/− recipient mice (Fig. 2A). Liver damage was detectable biochemically at CTL doses above 1.5 × 107 CTLs per mouse in the HBsAg-Tg/TNF-α−/− mice. The resultant liver damage (as determined by the sALT levels) at a high dose (1.5 × 107 CTLs per mouse) was not severe enough to be fatal (1900 ± 187 IU/L). In contrast, in HBsAg-Tg/TNF-α−/− mice, the liver damage was severe enough to be fatal within 96 h in two of six recipients (33.3%) who were administered 0.8 × 107 HBsAg-specific CTLs, and it was fatal for all seven mice (100%) that received 1.5 × 107 CTLs (Fig. 2B).

TNF-α production by hepatic macrophages, NK cells, CD4 T cells, and CD8 T cells in HBsAg-Tg/TNF-α−/− mice after the CTL injection

As shown in from several publications (7, 17), patients with fulminant hepatitis have increased serum TNF-α levels. Therefore, we next isolated macrophages, NK cells, CD4 T cells, and CD8 T cells from the intrahepatic MNCs of HBsAg-Tg/TNF-α−/− mice that had received HBsAg-specific CTLs by immunomagnetic separation and examined which cell fractions produced TNF-α. Twenty-four hours after the CTL injection, the TNF-α mRNA expression was enhanced in all the cell fractions, and it was decreased
48 h after the CTL injection (Fig. 3A). Next, we measured the TNF-α protein production levels in hepatic MNCs after the administration of HBsAg-specific CTLs by ELISA and intracellular cytokine staining. As shown in Fig. 3B, the level of TNF-α secreted by CD11b-positive cells and DX5-positive cells from HBsAg-Tg/TNF-α−/− mice treated with CTLs was increased
icient mice were administered 1.5 × 10⁷ HBsAg-specific CTLs. A, TNF-α mRNA expression by intrahepatic CD11b⁺, NK⁺, CD8⁺, and CD4⁺ cells from HBsAg-Tg/TNF-α⁻/⁻ mice that were administered HBsAg-specific CTLs. B, Hepatic CD11b⁺, NK⁺, CD8⁺, and CD4⁺ cells were purified with the MACS system (purity of each cell fraction, >95%). The mRNA levels for TNF-α were normalized to that of 18S mRNA. Representative charts derived from the analyses of three mice per group. C, Flow cytometric analysis for intracellular TNF-α production by intrahepatic CD11b⁺, NK⁺, CD8⁺, and CD4⁺ cells from HBsAg-Tg/TNF-α⁻/⁻ mice that were administered HBsAg-specific CTLs were purified by the MACS system. These cells were cultured for 24 h. TNF-α concentrations in the culture supernatants were measured by ELISA. C. Flow cytometric analysis for intracellular TNF-α production by hepatic CD11b⁺, NK⁺, CD8⁺, and CD4⁺ cells obtained from mice 24 h after the injection of CTLs and cultured for 4 h in brefeldin A.

Role of TNF-α-producing non-Ag-specific cells

The requirement of TNF-α-producing cells for the development of hepatitis was further evaluated with the help of experiments involving the adoptive transfer of macrophages, NK cells, CD4 T cells, and CD8 T cells from non-HBsAg-Tg/TNF-α⁻/⁻ mice into HBsAg-Tg/TNF-α⁻/⁻ mice. HBsAg-Tg/TNF-α⁻/⁻ recipient mice were administered 1.5 × 10⁷ HBsAg-specific CTLs 5 days after the i.v. injection of freshly isolated macrophages, NK cells, CD4 T cells, and CD8 T cells from HBsAg-Tg/TNF-α⁻/⁻ mice. We confirmed the existence of the CFSE-labeled transferred cells by flow cytometric analysis (data not shown). As shown in Fig. 4, the sALT levels several days after the CTL injection were significantly increased in HBsAg-Tg/TNF-α⁻/⁻ mice pretreated with isolated macrophages, NK cells, CD4 T cells, and CD8 T cells from TNF-α⁻/⁻ mice compared with the sALT levels in non-pretreated HBsAg-Tg/TNF-α⁻/⁻ mice.

Effect of IFN-γ on HBsAg-Tg/TNF-α⁺/⁺ and HBsAg-Tg/TNF-α⁻/⁻ mice

IFN-γ and TNF-α were thought to be critical components in this murine fulminant hepatitis model (6). We examined the direct effect of IFN-γ on HBsAg-Tg/TNF-α⁺/⁺ and HBsAg-Tg/TNF-α⁻/⁻ mice. As shown in Fig. 5A, recombinant murine IFN-γ administration caused marked elevations in sALT activity in the HBsAg-Tg/TNF-α⁻/⁻ compared with the HBsAg-Tg/TNF-α⁺/⁺ mice.
mice. In contrast, recombinant murine TNF-α administration caused a similar elevation in sALT activity in the HBsAg-Tg/TNF-α+/+ and HBsAg-Tg/TNF-α−/− mice, but it did not enhance the sALT levels in non-HBsAg-Tg mice (data not shown). Histopathological examination revealed the presence of small necroinflammatory foci and apoptotic hepatocytes in the liver of HBsAg-Tg/TNF-α+/+ mice after the administration of recombinant IFN-γ; however, these changes were not observed in the liver of HBsAg-Tg/TNF-α−/− mice (Fig. 5B).

**Kinetics of hepatocyte TNFR mRNA expression in HBsAg-Tg/TNF-α+/+ mice after the CTL injection**

Next, we tested the TNFR 1 and 2 mRNA expressions in hepatocytes after the CTL injection. The expression of TNFR 1 mRNA in hepatocytes in the HBsAg-Tg/TNF-α+/+ mice was obviously enhanced (more than 40-fold) after the CTL injection and peaked 24 h thereafter (Fig. 6). In contrast, the expression of TNFR 2 mRNA in hepatocytes in the HBsAg-Tg/TNF-α+/+ mice was unchanged after the CTL injection.

**Discussion**

Fulminant hepatitis is a clinical syndrome consisting of sudden and severe liver injury that results in hepatic encephalopathy and acute liver failure (17, 18). The rate of mortality from fulminant hepatitis remains very high, although intensive medical care and implementation of the latest therapies, including liver transplantation, are progressing. The HBsAg-Tg mouse model contributed to the study on the mechanisms of fulminant hepatitis (4, 6). Previously, various reports provided HBV transgenic mice. The HBsAg transgenic mouse lineages 107-5D, pFC80-219, or HBV transgenic mouse lineages 1.3.32 were suitable for examining CTL-induced hepatitis (19). Lethal fulminant hepatitis was only observed when the HBsAg transgenic mouse lineages 107-5D received HBsAg-specific CTL clones. In lineages pFC80–219 and lineages 1.3.32 HBV Tg mice, lethal hepatitis was not caused by the high-dose CTL injection. Recently, many reports have discussed the influence of the genotypes on outcome of HBV infections. It was reported that the intracellular accumulation of HBV DNA and Ags may play a role in inducing liver damage (20). Moreover, HBV genotype B was an independent risk factor for the development of fulminant hepatitis, and the expression of intracellular Ags in HBV genotype B was the highest (20, 21). Therefore, the HBsAg transgenic mouse lineages 107-5D may be suitable for the analysis of human fulminant hepatitis because the HBsAg particles were retained within the endoplasmic reticulum of the hepatocyte in this lineage. However, it is thought that the HBsAg transgenic mouse lineages 107-5D should be used only for the analysis of the fulminant hepatitis and not acute hepatitis or chronic hepatitis. Previous studies have described the relative hierarchy of the perforin-granzyme, FasL-Fas, IFN-γ, and TNF-α death pathways in the pathogenesis of the necroinflammatory liver disease induced by HBsAg-specific CTL clones in HBsAg transgenic mice (3, 6). Kondo et al. (22) demonstrated that liver injury was attenuated by blocking the FasL-Fas and TNF-α-TNFR pathway in the fulminant hepatitis model. In contrast, it was reported that IFN-γ-dependent signals are primarily responsible for killing HBsAg-positive hepatocytes irrespective of the presence or absence of FasL and Fas.
hepatocytes are much less sensitive to destruction by TNF-α than by the other death pathways in the fulminant hepatitis model (3). This discrepancy remains unsolved. However, the difference might be due to the difference in the methods used to block the FasL and Fas signaling. Nakamoto et al. (3) used Fas and FasL knockout mice for blocking the signaling. In contrast, the use of the soluble form of Fas has also been reported. We previously reported that anti-TNF-α Abs were only modestly protective against CTL-induced liver disease. In contrast, Kondo et al. (22) appropriated soluble TNFRβ-Fc for the TNF-TNFR blocking experiment. TNFRβ-Fc greatly inhibited the increase in the ALT level in the fulminant hepatitis model. Thus, in the present study, we examined the role of TNF-α using TNF-α knockout mice. The proinflammatory cytokine TNF-α is thought to play a critical role in acute viral hepatitis (7, 8, 23). We established the HBsAg-Tg (lineage 107-5D)/TNF-α−/− mouse strain and have shown that TNF-α secreted by intrahepatic non-Ag-specific inflammatory cells plays a critical role in the development of acute and lethal necroinflammatory liver disease.

TNF-α is a pleiotropic cytokine that induces cellular responses such as proliferation, production of inflammatory mediators, and cell death, and plays a major role in the pathogenesis of septic shock and wasting syndrome. In the liver, TNF-α is involved in the pathophysiology of viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease, and ischemia-reperfusion injury. TNF-α plays a dichotomous role in the liver, where it not only acts as a mediator of cell death but also induces hepatocyte proliferation and liver regeneration. In particular, in HBV-related acute hepatitis, TNF-α is thought to contribute to the viral clearance and development of the necroinflammatory liver disease. It was reported that mice that express HBV envelope proteins in their hepatocytes develop acute viral hepatitis after adoptive transfer of CD8-positive, HBsAg-specific CTL lines and clones (6, 24). Various established HBsAg-specific CTL clones produce TNF-α following HBsAg stimulation (2). Moreover, it was reported that anti-TNF-α Abs (the soluble form of TNFR) markedly blocked the development of hepatitis induced by HBsAg-specific CTLs in HBsAg-Tg mice (22). These results indicated that TNF-α is deeply involved in the progress of liver injury in acute viral hepatitis. Although activated HBsAg-specific CTLs produce TNF-α in fulminant hepatitis, it is considered that TNF-α secreted from only HBsAg-specific CTLs is not enough to cause necroinflammatory liver disease because only 22 CTLs infiltrate in the liver tissue per mm² (25). Therefore, TNF-α secreted by non-Ag-specific cells seems to play a critical role in the fulminant hepatitis, but this issue remains to be elucidated.

In this study, we first found that necroinflammatory liver disease did not occur in HBsAg-Tg/TNF-α−/− mice that had received even a large number of HBsAg-specific CTLs. Furthermore, there were no deaths among HBsAg-Tg/TNF-α−/− mice that had received 1.5 × 10⁷ HBsAg-specific CTLs, which caused lethal liver disease in all HBsAg-Tg/TNF-α−/− mice (Fig. 2B). Although the peak of severe liver injury was observed within 24–48 h after the CTL injection in this murine hepatitis model, similar injury was not observed in HBsAg-Tg/TNF-α−/− mice at the same time (Fig. 1B). When HBsAg-specific CTLs were administered at an extremely high dose into HBsAg-Tg/TNF-α−/− mice, the sALT level slightly increased at 96 h after the CTL injection (Fig. 2A). These data suggest that TNF-α secreted by host cells other than Ag-specific CTLs plays a critical role in the acute progression of necroinflammatory liver disease, and it may cause acute hepatitis to develop into lethal liver disease.

In previous studies, it was thought that intrahepatic macrophages mainly produced TNF-α and had the potential to damage hepatocytes in the murine hepatitis model (26). In this model, it was clear that cells of the monocyte/macrophage lineage dominated the inflammatory infiltrate (6). Because the irradiation slightly attenuated the liver injury in this model but did not prevent the massive destruction of most hepatocytes, it can be said that the macrophage lineage plays the major role in this murine fulminant hepatitis (6). However, NK-positive cells (including NKT cells) are also resistant to irradiation (27) and are the second largest mononuclear cell population in the liver after macrophages (Kupffer cells). Therefore, it is possible that the TNF-α secreted by NK-positive cells plays an important role in this model. In contrast, T cells are sensitive to irradiation, and non-Ag-specific T cells in the liver were killed by the irradiation. Thus, the mild decrease in the sALT levels with irradiation as described above can be accounted for by the depletion of the non-Ag-specific T cells in the liver. Taken together, TNF-α secreted by NK-positive cells and non-Ag-specific T cells including CD8-positive cells may play a partially important role in this model.

In vivo, IFN-γ is released by activated HBsAg-specific CTLs when they recognize Ags. Pretreatment with anti-IFN-γ Abs in this murine fulminant hepatitis model completely inhibited the CTL-induced liver injury (6). IFN-γ administration caused marked elevations in the sALT levels in HBsAg-positive Tg mice, whereas it was entirely nontoxic to HBsAg-negative Tg controls (28). In contrast, the Tg and control mice were similarly sensitive to the hepatotoxic effects of TNF-α (28). Therefore, we next examined the direct effect of IFN-γ and TNF-α in HBsAg-Tg/TNF-α−/+ and HBsAg-Tg/TNF-α−/− mice. IFN-γ administration enhanced the sALT levels in the HBsAg-Tg/TNF-α−/+ mice compared with those in the HBsAg-Tg/TNF-α−/− and non-Tg mice (Fig. 5). To confirm the hepatotoxic ability of TNF-α in HBsAg-Tg/TNF-α−/+ and HBsAg-Tg/TNF-α−/− mice, we measured the serum ALT level in HBsAg-Tg/TNF-α−/+ and HBsAg-Tg/TNF-α−/− mice after the administration of recombinant murine TNF-α. Recombinant murine TNF-α similarly induced liver injury in HBsAg-Tg/TNF-α−/+ and HBsAg-Tg/TNF-α−/− mice (data not shown). It is possible that the hepatotoxic response to TNF-α in the HBsAg-Tg/TNF-α−/+ mice was similar to that in the HBsAg-Tg/TNF-α−/− mice. IFN-γ secreted by activated CTLs may trigger the enhancement of TNF-α production by non-Ag-specific host cells. The results demonstrated the occurrence of necroinflammatory liver disease in the HBsAg-Tg/TNF-α−/+ mice that had received HBsAg-specific CTLs. In the primary culture, the hepatocytes from HBsAg-Tg mice were not destroyed by the addition of high-dose recombinant TNF-α to the culture medium (data not shown). However, it is possible that TNF-α directly exerted its effects to destroy the hepatocytes of HBsAg-Tg mice in this fulminant hepatitis model, because mRNA expression of TNFR1 was up-regulated after the CTL injection. Moreover, the kinetics of sALT levels and the mRNA expression of TNFR1 were quite similar after the CTL injection. These data indicate that TNF-α produced by non-Ag-specific cells may directly destroy the hepatocytes of HBsAg-Tg mice because of CTL-induced enhancement of TNFR1 expression on hepatocytes. However, our data suggests that TNF-α secreted by non-Ag-specific cells plays a critical role in the progression of fulminant hepatitis. In severe viral necroinflammatory liver disease, anti-TNF therapy is thought to be one of the effective treatments.

In summary, we established HBsAg-Tg/TNF-α−/− mice to investigate the role of TNF-α in liver injury caused by CTLs, and demonstrated that TNF-α produced by intrahepatic non-Ag-specific inflammatory cells is critical in the pathogenesis of acute and lethal necroinflammatory liver disease. Anti-TNF therapy may
provide therapeutic benefit to acute viral fulminant hepatitis patients. In contrast, a recent study reported hepatitis B reactivation in a chronic hepatitis B surface Ag carrier after therapy with anti-TNF Ab. Therefore, in severe viral necroinflammatory liver disease, anti-TNF therapy is thought to be an effective treatment. However, anti-TNF therapy should not be administered to patients with persistent HBV infection without careful consideration.

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