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A Critical Role for Antigen-Specific Th1 Cells in Acute Liver Injury in Mice

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A novel liver injury model was established in mice by targeting of OVA-containing liposomes into the liver, followed by adoptive transfer of OVA-specific Th1 cells. Combined treatment of mice with OVA-containing liposomes and Th1 cell transfer caused an increase in serum transaminase activity that was paralleled with an elevation of serum IFN-γ levels. In sharp contrast, OVA-specific Th2 cell transfer resulted in an increase of serum IL-4 levels, but did not induce liver injury. Neither NK, NK T, nor CD8+ T cells were required for the Th1-induced liver injury. The liver injury was blocked by anti-IFN-γ mAb and anti-TNF-α mAb, but not by anti-Fas ligand mAb. The Fas/Fas ligand independency was also demonstrated using Fas-deficient lpr mice. These findings indicate that Th1 cells are the major effector cells in acute liver injury. The Journal of Immunology, 1999, 162: 6503–6509.

Human viral hepatitis is a disease arising from destruction of virus-infected hepatocytes by immune-mediated mechanisms (1, 2). It has been generally recognized that T cell-mediated cellular immunity is responsible for the liver damage. This conclusion was based on the finding that CD4+ T cells and CD8+ T cells have been demonstrated to infiltrate the liver tissues of hepatitis patients (3, 4), and that these infiltrating T cells can respond to viral Ags (5). To elucidate the role of T cells in liver injury, many investigators have tried to establish T cell-dependent liver injury models. To our knowledge, five T cell-dependent liver injury models have been reported to date (6–10). Although an important role for CD8+ T cells in liver injury was demonstrated using hepatitis B virus surface Ag (HBsAg) transgenic mice (8), cytokine-producing CD4+ T cells were reported as key effector cells in a Con A-induced liver injury model (11–13). Similarly, we demonstrated that IFN-γ-producing CD4+ T cells are essential for the induction of liver injury elicited by Propionibacterium acnes plus LPS (9, 14). Thus, the precise role of CD4+ T cells involved in liver injury remains unclear.

On the basis of their cytokine production profiles, CD4+ Th cells were subdivided into two distinct populations, Th1 and Th2 cells. Th1 cells, which produce IFN-γ and IL-2, play a critical role in cellular immunity, while Th2 cells producing IL-4, IL-5, IL-10, and IL-13 are essential for the regulation of humoral immunity (15, 16). The balance between Th1- and Th2-dominant immunity (Th1/Th2 balance) was suggested recently to be critically important for the outcome of various immune diseases such as inflammatory autoimmune diseases, infectious diseases, and allergy (17, 18).

In previous studies, we have found that IFN-γ-producing CD4+ T cells play a pivotal role in P. acnes plus LPS-induced liver injury (14). In this model, the onset of liver injury was completely blocked by the administration of Abs against IFN-γ or IL-12. Moreover, it was demonstrated that C57BL/6 mice with a propensity for Th1 responses were susceptible to P. acnes plus LPS-induced liver injury, whereas BALB/c mice with a propensity for Th2 responses were resistant. The important role of IFN-γ-producing CD4+ T cells was also confirmed in the Con A-induced liver injury model (13, 19). Moreover, the pathogenic effector function of long-term cultured Th1 clones was demonstrated recently using hepatitis B virus transgenic mice (10). These results strongly suggested that Ag-specific Th1 cells may be important in liver injury. However, at present, we cannot exclude the possibility that Ag-specific Th2 cells can induce liver injury, as reported for other models’ immune-mediated tissue injuries, including experimental autoimmune encephalomyelitis (20) and insulinitis (21), in which both Th1 and Th2 cells have been reported to act as effector cells.

To study the effector function of Th1 and Th2 cells in liver injury, it is necessary to establish a liver injury model that is based on freshly induced Ag-specific Th1 and Th2 cells derived from the same source of naive Th precursor cells. We therefore developed a novel Ag-specific Th1 cell-dependent liver injury model using Th1 and Th2 cells isolated from OVA-specific TCR-transgenic mice. We also developed a method for targeting of OVA Ag to the liver tissue using OVA-containing liposomes (OVA-Lip). Mice were treated with OVA-Lip and, at the same time, received OVA-specific Th1 or Th2 cells. The results indicated that Ag-specific Th1 cells, but not Th2 cells, are responsible for the onset of liver injury. Th1-induced liver injury was dependent on IFN-γ and TNF-α, but there was no absolute requirement for CD8+ T cells, NK cells, or NKT cells. Moreover, our results suggested that Th1 cells contributed directly to the liver damage using Fas/FasL-independent apoptotic cell death mechanisms.
FIGURE 1. Targeting of OVA-Lip and OVA-specific Th cells into the liver. A–D, Liposome containing FITC-labeled OVA (FITC-OVA-Lip) was injected i.v. into BALB/c mice, and after 2 h the mice were treated with adoptive transfer of PKH26-labeled Th1 (A, B) or Th2 cells (C, D). Liver tissues were collected 24 h after the cell transfer, and the fluorescence signals of FITC (A, C) or PKH26 (B, D) on frozen sections were examined by fluorescence microscopy. Arrows indicate where FITC-labeled OVA Ag and PKH26-labeled cells coexist. E, F, BALB/c mice were treated with FITC-OVA-Lip and Th1 cells. After 6 h, FITC signals on the frozen sections of the liver (E) and the kidney (F) were examined by fluorescence microscopy.

Cytokines, mAbs, and Ags

IL-12 was kindly donated by Genetics Institute (Cambridge, MA). Anti-IL-12 mAbs (C15.1 and C15.6) were kind gift from Dr. G. Trinchieri (Wistar Institute of Anatomy and Biology, Philadelphia, PA). Recombinant murine IL-4 and anti-asialo GM1 Ab were purchased from Wako Pure Chemical Industries (Osaka, Japan). Anti-IL-4 mAb (11B11) was purchased from American Type Culture Collection (Manassas, VA). Recombinant mouse IFN-γ and anti-IFN-γ mAb (R4-6A2) were purchased from PharMingen (San Diego, CA). Anti-FlNa mAb (MFL-1) and anti-TNF-α mAb (MP6-XT22) were a kind gift from Dr. H. Yagita (Juntendo University School of Medicine, Tokyo, Japan). OVA323–339 peptide was obtained from Fujiya (Hadano, Japan). rHBsAg was kindly donated by the Chemo-Pharmaceuticals (Chiba, Japan). HBsAg-specific Th1 cells were induced from CD4+ CD45RB+ naive Th cells obtained from wild-type C57BL/6 mice, which were immunized with HBsAg (100 μg/mouse) five times at 2-wk intervals. C57BL/6 or C57BL6/JByPr mice were given an i.v. injection of HBsAg-Lip (250 μl/mouse) and HBsAg-specific Th1 cells (2 × 10⁵ cells/mouse) at an interval of 2 h. Severity of the liver injury was analyzed by measuring serum transaminase activity.

Fluorescence microscopy

FITC-labeled OVA-Lip was synthesized by incubating OVA with FITC for 1 h at room temperature in 0.1 M carbonate buffer (pH 9.2), and purified by gel filtration. Liposome containing FITC-labeled OVA (FITC-OVA-Lip) was prepared according to the method for OVA-Lip described above. BALB/c mice were injected i.v. at an interval of 2 h with FITC-OVA-Lip and Th1/Th2 cells, which were labeled with the fluorescent dye PKH26 using a labeling kit (Zynaxis Cell Science, Malvern, PA), according to the manufacturer’s instructions. Tissue samples were obtained after 24 h, fixed in 1% glutaraldehyde/4% paraformaldehyde/PBS for 6 h, and frozen in liquid N₂ using OCT compound. Tissue blocks were sectioned and examined by fluorescence microscopy. PKH-26 emission peak at 567 nm was visualized using rhodamine filters.

Results

Establishment of a Th1-dependent liver injury model

To evaluate the role of Th cells in the onset of liver injury, we established a novel liver injury model in mice by adoptive transfer of OVA-specific Th1 or Th2 cells, following the targeting of OVA Ag into the liver. Since i.v. administered liposomes are known to accumulate into the liver (26), we decided to target OVA Ag into the liver using OVA-encapsulated liposomes. As shown in Fig. 1, i.v. injection of liposomes containing FITC-bound OVA (FITC-OVA-Lip) resulted in a scattered distribution of fluorescence signals in the liver (Fig. 1, A and C). Kupffer cells appeared to be strongly stained by FITC (Fig. 1E). However, no significant uptake was observed in the kidney (Fig. 1F) and in parenchymal cells of the liver (Fig. 1G). In addition, liver sections stained with anti-FasL mAb and anti-IFN-γ mAb showed no staining in the liver, indicating that FasL and IFN-γ were not induced by the liposome treatment.

Induction of liver injury

BALB/c mice were treated with i.v. injection of OVA-Lip (200 μl) 2 h before cell transfer. Cultured Th1 and Th2 cells were washed, resuspended in PBS at 1 × 10⁸ cells/ml, and injected i.v. at a volume of 200 μl (2 × 10⁷ cells/mouse). Mice were sacrificed after 24 h, and the liver injury was assessed by measuring serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, as described previously (14). Sera were also tested for IFN-γ and IL-4 levels using ELISA kits (Amersham International, Buckinghamshire, U.K.). Tissue samples were fixed in 10% formalin-PBS and embedded in paraffin, and sections were stained with hematoxylin and eosin. Tissue samples were also snap frozen in liquid N₂ using OCT compound (Sakura Finetechnical, Tokyo, Japan), sectioned, and examined for hematoxylin-eosin staining, TUNEL method (24), or peroxidase staining (25). In some experiments, BALB/c mice were treated i.p. with 500 μg/mouse of anti-FasL mAb, anti-IFN-γ mAb, anti-TNF-α mAb, or rat IgG (control) 24 and 1 h before the injection of OVA-Lip.

HBsAg-specific Th1 cell-dependent liver injury

Liposomes containing HBsAg (HBsAg-Lip) were prepared with 5 mg/ml HBsAg and Coatsome EL-A-01 by the same methods used for the preparation of OVA-Lip. Resulting liposomal suspension included 400 μg/ml of HBsAg. HBsAg-specific Th1 cells were induced from CD4+ CD45RB+ naive Th cells obtained from wild-type C57BL/6 mice, which were immunized with HBsAg (100 μg/mouse) five times at 2-wk intervals. C57BL/6 or C57BL6/JByPr mice were given an i.v. injection of HBsAg-Lip (250 μl/mouse) and HBsAg-specific Th1 cells (2 × 10⁵ cells/mouse) at an interval of 2 h. Severity of the liver injury was analyzed by measuring serum transaminase activity.

Preparation of OVA-Lip

Freeze-dried liposomes (Coatsome EL-A-01; kindly donated by NOF, Tokyo, Japan) were hydrated with 50 mg/ml OVA (Sigma, St. Louis, MO). Coatsome EL-A-01 is a negatively charged liposome consisting of dipalmitoylphosphatidylcholine/cholesterol:dipalmitoylphosphatidylglycerol = 3:4:3. This liposomal suspension was treated with three cycles of freeze (1 min in liquid N₂) and thaw (2 h at 4°C), and then washed twice by centrifugation at 100,000 × g for 20 min. Precipitated liposomes were resuspended in PBS and extruded through sterile membrane of 0.45 μm pore size (Sartorius, Gottingen, Germany). Protein concentration in the resulting OVA-Lip suspension contained ~800 μg/ml of OVA, according to Lowry’s method (23).

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controls. 

The elevation of serum transaminases was significant at 2 × 10^7 of transferred cells. Therefore, in all of the following experiments, Th1-dependent liver injury was induced by transfer of 2 × 10^7 cells/mouse. The serum transaminase levels reached a peak 24 h after cell transfer and declined thereafter (data not shown). In contrast, transfer of Th2 cells did not cause any significant changes in serum transaminase levels.

During the onset of liver injury, mice that had received Th1 cells exhibited marked elevation of serum IFN-γ levels in parallel with an increase in serum transaminase activity (Figs. 2, A and B). On the other hand, mice that had received Th2 cells revealed a marked elevation of serum IL-4 levels, but no increase in serum transaminase activity was observed (Figs. 2, A and B). These results demonstrated that Th2 cells could respond to liver-targeted OVA Ag in vivo to produce IL-4, but that these cells did not induce liver damage.

As shown in Fig. 2C, it was also demonstrated that Th1-dependent liver injury was induced only when the mice were treated with OVA-specific Th1 cells after i.v. injection with OVA-Lip, but not with BSA-containing liposomes, empty liposomes, or OVA solution. Furthermore, anti-CD3 mAb-activated Ag-nonspecific CD4+ T cells failed to replace OVA-specific Th1 cells for the induction of liver injury in mice pretreated with OVA-Lip. These data clearly indicated that the Th1-dependent liver injury in this model was highly dependent on Ag-specific T cell recognition.

**Histologic analysis of Th1-dependent liver injury**

Histologic examination demonstrated the induction of necroinflammatory foci with infiltration of lymphocytes and neutrophils in Th1 cell-dependent liver injury (Fig. 3A). In addition, some degenerating hepatocytes were observed in the liver (Fig. 3A). Since kidney and lung from the same mouse showed no histologic abnormalities (Fig. 3, B and C), we concluded that this tissue injury was tissue specific. No evidence for liver damage was observed in mice that had received Th2 cells (data not shown).

Although, based on their ability to produce cytokines (Fig. 2B), both Th1 and Th2 cells responded to the Ag, only Th1 cells induced liver injury. This may be due to the different effector functions of Th1 and Th2 cells in the local environment of the liver. To provide evidence for this hypothesis, we performed a histologic analysis of the liver for the presence of apoptotic cells by TUNEL method. As shown in Fig. 4, apoptotic cells were prevalent in the liver tissue from mice that had received Th1 cells. The strong overlap between TUNEL-positive areas and necroinflammatory sites (Fig. 4, A and B) strongly indicated that degeneration of hepatocytes was induced by Th1 cell-mediated apoptotic cell death mechanisms. In contrast, no TUNEL-positive cells were observed in the liver of mice that had received Th2 cells (Fig. 4, E and F). In the liver of mice that had received Th2 cells, there was an infiltration of eosinophils that stained black by peroxidase, where such infiltrates were absent in mice that had received Th1 cells (Fig. 4, C, D, G, and H). In mice that had received Th2 cells, eosinophils were clustered around the portal vein and midzonal area of the liver lobule. These results suggested that Th2 cells could cause an inflammation with eosinophil infiltration, but not liver injury.

**Th1 cells directly induce liver injury**

Next we examined whether Th1 cells damaged the liver directly by their effector function or indirectly through the activation of other cells such as CD8+ T cells, NK cells, or NKT cells. To elucidate this issue, we examined the occurrence of liver injury in T cell-deficient BALB/c nude mice (Fig. 5A) and BALB/c RAG2−/− mice (Fig. 5B). As compared with wild-type BALB/c mice, both immunodeficient mouse lines showed similar increases in serum
transaminase levels, suggesting that Th1 cells could induce liver injury without the involvement of the recipient’s CD8+ CTL and NKT cells.

To study the role of NK cells, we depleted these cells from BALB/c nude and RAG2−/− mice by an i.p. injection of anti-asialo GM1 mAb. Anti-asialo GM1 treatment completely abrogated NK activity against YAC-1 cells (data not shown). Both anti-asialo GM1-treated nude and RAG2−/− mice showed a strong increase in the serum transaminase levels compared with control mice, although this increase was not as dramatic as in mice that had not received anti-asialo GM1 (Fig. 5). Therefore, in our established liver injury model, Th1 cells appeared to be directly involved in liver injury without significant cooperation with other cells, including CD8+ T cells, NKT cells, and NK cells.

The mechanism of Th1-dependent liver injury

To determine what molecules are crucial for the induction of liver injury by Th1 cells, we studied the effects of mAbs against FasL, IFN-γ, and TNF-α (Fig. 6). Neutralization of IFN-γ or TNF-α strongly inhibited Th1 cell-induced elevation of serum transaminase levels. Although Th1 cells expressed FasL and lysed Fas-positive target cells (data not shown), FasL on Th1 cells was not essential for Th1 cell-dependent liver injury, because anti-FasL mAb pretreatment resulted only in a negligible decline of serum transaminase levels. These data strongly suggested that Fas/FasL interactions are not involved in this model of Th1-dependent liver injury. To provide conclusive evidence that Th1 cells could induce liver injury in a Fas/FasL-independent manner, we investigated whether Th1 cells could induce liver damage in Fas-deficient lpr mice. Since commercially available lpr mice are on a C57BL/6 background, we used Th1 cells induced from spleen cells of wild-type C57BL/6 mice immunized with hepatitis B virus surface Ag (HBsAg). As for OVA-specific liver injury, the HBsAg was targeted into the liver of the mice using liposomes (HBsAg-Lip) and then HBsAg-specific Th1 cells were i.v. transferred. As shown in Fig.

FIGURE 3. Histochemical analysis of Th1 cell-dependent liver injury. Mice were treated as described in the legend to Fig. 2B to induce Th1-dependent liver injury. After 24 h, liver (A), kidney (B), and lung (C) were isolated from the mice, fixed in 10% Formalin-PBS, sectioned, and stained with hematoxylin/eosin. The arrow in A indicates the degenerating hepatocytes.

FIGURE 4. Th1 cells induce apoptotic liver cell death, while Th2 cells induce eosinophil recruitment in the liver. Th1 (A–D) or Th2 (E–H) cells were i.v. transferred into the mice after OVA-Lip treatment. Fresh frozen liver tissues were dissected and stained by hematoxylin/eosin (A, C, E, and G), TUNEL method (B and F), or peroxidase staining (D and H). The arrow in B shows the apoptotic cell death of hepatocytes induced by Th1 cells. The arrow in H indicates eosinophil infiltration into the liver induced by Th2 cells.
7, HBsAg-specific Th1 cells induced liver injury in C57BL/6 mice pretreated with HBsAg-Lip, but not with OVA-Lip. This HBsAg-specific Th1-dependent liver injury was not blocked by the administration of mAb against FasL. Moreover, this HBsAg-specific Th1-dependent liver injury was also induced in Fas-deficient lpr mice. Taken together, we concluded from these results that our novel model for Th1-dependent liver injury could be induced in a Fas/FasL-independent manner.

Discussion

Dysregulation of the Th1/Th2 balance can dramatically influence the outcome of autoimmune inflammatory diseases; however, the relevance of the Th1/Th2 balance to liver injury induced by viral hepatitis remains unclear. Several mouse hepatitis models suggested an important role for CD4+ T cells producing IFN-γ and TNF-α in causing liver injury (11, 12, 27). We have previously proposed a pivotal role for IL-12 and IL-12-activated Th1 immunity in P. acnes plus LPS-induced liver injury (14). Furthermore, in human hepatitis patients, accumulation of IFN-γ-producing cells was detected in the inflamed lobe (28), and mRNA expression of IFN-γ and IL-2 in the liver was correlated with histologic inflammation (29). IL-12 was also detected in viral hepatitis patients (30). These observations therefore suggest a critical role for Th1-type cytokines such as IFN-γ and IL-12, in the pathogenesis of hepatitis.

Although these studies suggested that Ag-specific Th1 cells are the main effector cells in causing liver injury, they do not rule out the possibility that Th2 cells can contribute to liver injury. Because of the lack of an appropriate Ag-specific Th cell-dependent liver injury model, this issue has been difficult to address directly. The T cell-dependent mouse liver injury models that are commonly used are established by inducing effector T cells by the administration of polyclonal activators such as Con A or LPS, which stimulate many kinds of cells and make it difficult to analyze the direct roles of Ag-specific Th1 and Th2 cells in the liver injury. To resolve this problem, we established a novel Ag-specific liver injury model by cell transfer of OVA-specific Th1 or Th2 cells derived from the same source of naive Th cells. As described previously (31), adoptive transfer of Th1 or Th2 cells is a reliable method for immunodeviation of the Th1/Th2 balance. To introduce OVA Ag

![FIGURE 5](http://www.jimmunol.org/)

Neither NK, NKT cells, nor host T cells are required for Th1-dependent liver injury. Wild-type BALB/c mice and BALB/c-background nude mice (A) or BALB/c-background Rag2−/− mice (B) were treated with OVA-Lip and Th1 cells (2 × 10^7 cells), and 24 h later their serum transaminase levels (AST, ■; ALT, □) were determined. Some mice were pretreated with anti-asialo GM1 Ab to deplete NK cells. Data shown are representative of three (A) or four (B) experiments, and are mean ± SE of three mice. *, p < 0.05 vs respective controls.

![FIGURE 6](http://www.jimmunol.org/)

IFN-γ and TNF-α, but not FasL, are key molecules in Th1-dependent liver injury. BALB/c mice were treated with (OVA-Lip + Th1 cells) or without (None) OVA-Lip plus Th1 cell transfer. Twenty-four hours before the treatment with OVA-Lip and Th1 cells, the mice were pretreated with i.p. injection of 500 µg/mouse of mAb against FasL, IFN-γ, or TNF-α. As control, the mice were also treated with saline (no Ab) or rat Ig. Serum transaminase levels of the mice were determined 24 h after Th1 cell transfer. AST, ■; ALT, □. The data depict mean ± SE of three mice and are representative of three comparable experiments. *, p < 0.05; **, p < 0.01 vs group with use of rat Ig.

![FIGURE 7](http://www.jimmunol.org/)

Fas/FasL interaction is not involved in Th1-dependent liver injury. Wild-type C57BL/6 mice were treated with or without HBsAg-containing liposomes. Some of these animals were further treated with cell transfer of HBsAg-specific Th1 cells. To determine the role of Fas/FasL interaction in this Th1-dependent liver injury, the mice pretreated with anti-FasL mAb or Fas-deficient C57BL/6 lpr mice were used for the induction of liver injury using HBsAg-Lip and HBsAg-specific Th1 cells. AST, ■; ALT, □. The data depict mean ± SE of three mice and are representative of three comparable experiments. *, p < 0.05 vs respective controls.
into the liver, liposomes were used as a carrier, because i.v. injected liposomes have been reported to be preferentially distributed to the liver (26). Moreover, liposome-entrapped Ags can interact with MHC class II molecules (32), which facilitate the activation of Th cells reactive against class II-bound OVA323–339 peptide.

Using this novel strategy, both OVA Ag and OVA-reactive Th1 or Th2 cells were successfully targeted into the liver (Fig. 1) and it was clearly demonstrated that OVA-specific Th1 cells induced Ag-specific liver injury in mice in which OVA-Lip was targeted into liver (Figs. 2A, 2C, 3, and 4). The entrapment of Ag into liposomes is essential for the targeting of Ag into the liver because OVA solution was not sufficient to induce liver injury (Fig. 2C). In contrast to Th1 cells, Th2 cells were unable to induce liver injury, despite the fact that they responded to targeted OVA Ag in vivo by inducing IL-4 production in the serum (Fig. 2B) and inducing eosinophil recruitment into the liver (Fig. 4).

The distinct pathogenic effect of Th1 and Th2 cells appeared to be correlated with their distinct cytokine production profiles. IFN-γ and TNF-α are key cytokines involved in our established Th1-dependent liver injury model, as shown by blocking experiments using mAbs (Fig. 6). These results are consistent with previous results showing that IFN-γ and TNF-α are important for liver injury induced by hepatitis B virus, Con A, or P. acnes plus LPS (10, 14, 33). As reported previously (34, 35), IFN-γ and TNF-α may synergistically act to induce liver injury because the blockage of either cytokine suppressed liver injury.

In addition to Th cells, CTL were reported to be involved in the induction of hepatitis in HBsAg-transgenic mice (8), and the essential role of NK cells was also demonstrated in Con A-induced liver injury (36). To exclude the possibility that the recipient’s T cells and NKT cells played a role in our established Th1-dependent liver injury model, we examined the liver injury in BALB/c-background nude mice and RAG2−/− mice. These immunodeficient mice, upon treatment with OVA-Lip and Th1 cells, showed the same levels of liver injury as those observed in wild-type BALB/c mice (Fig. 5). Therefore, adoptively transferred OVA-specific Th1 cells appear to induce liver injury in the absence of the recipient’s CD4+ T cells, and NKT cells. Furthermore, the finding that NK cell depletion by anti-asialo GM1 administration resulted in only a partial inhibition of the liver injury suggested that NK cells are not major effector cells in our established Th1-dependent liver injury (Fig. 5). Taken together, these results indicated that Th1 cells damaged liver cells directly. However, we do not exclude the possibility that Ag-specific Th1 cells together with Ag-specific CD8+ T cells or other cytokine-activated effector cells may accelerate the onset of liver injury, because the magnitude of liver damage induced by Th1 cells was always lower than that induced by P. acnes plus LPS. In the latter model, various effector cells other than CD4+ T cells are involved (37).

Three distinct cytotoxic mechanisms (perforin, Fas/FasL, and TNF-α) were considered to be involved in the direct liver injury mediated by Th1 cells. From the histologic study using TUNEL method, it was demonstrated that liver cells are destroyed by apoptotic mechanisms (Fig. 4). Hepatocytes are known to express Fas molecule on their surface, and Fas/FasL interaction was reported to be an essential process in the liver injury (38, 39). However, in our model, anti-FasL mAb exhibited only marginal inhibitory effect on Th1-dependent liver injury (Figs. 6, 7), despite the fact that the same mAb completely blocked Fas/FasL-dependent cytotoxicity by anti-CD3 mAb-activated Th1 cells (data not shown). Moreover, we confirmed that HBsAg-specific Th1 cells can induce liver injury even in Fas-deficient lpr mice (Fig. 7). We concluded from these results that Fas/FasL interaction is not essential for our established Th1 cell-dependent liver injury. These results were consistent with previous results showing that Fas/FasL interaction was not essential for Con A-induced liver damage (19, 40). In the CTL-induced liver injury model, Nakamoto et al. (41) examined the relative contribution of different death pathways, and showed that neither FasL nor perforin was required for the liver injury; however, conflicting data were reported by Kondo et al. (38), who suggested that soluble Fas treatment blocked CTL-induced liver injury. However, it remains unclear whether perforin is the only death pathway involved in Th1-mediated liver injury. We have evidence that pretreatment of Th1 cells with the perforin-inhibitor concanamycin A (42) partially inhibits liver injury (data not shown). To resolve this issue more directly, we are now trying to determine whether Th1 cells derived from perforin knockout mice can induce liver injury in Fas-deficient lpr mice.

The precise mechanism of the acute liver injury mediated by Th1 cells has not been clarified completely. However, we speculate that the following series of events may be induced during Th1-dependent liver injury: 1) Ag-specific Th1 cells enter into the liver and recognize Ag that was processed and presented by non-parenchymal cells in the liver; 2) triggering of Th1 cells with MHC-bound OVA peptide causes the secretion of inflammatory cytokines; 3) Th1 cells themselves or Th1-type cytokine-activated macrophages induce liver injury in Fas/FasL-independent manner; 4) secreted cytokines recruit inflammatory cells to amplify the liver injury, and high concentrations of IFN-γ and TNF-α may lead to cytokine-induced liver cell death.

As previously reported (14, 43), the susceptibility to Th1-dependent liver injury is genetically controlled by unknown factors, which may play an important role in the regulation of the Th1/Th2 balance in mice (44–48). Such genetically controlled differences in Th1/Th2 balance regulation remain unclear in the human system but, based on the present data, it is possible to speculate that the bias toward Th1-type immunity might become a risk factor for human acute hepatitis. We are currently investigating the relevance of the Th1/Th2 balance in acute hepatitis using clinical samples.

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References


