TLR ligands/IL-1 (12). However, whether Trib family members regulate TLR-mediated signaling pathways under physiological conditions is still unknown.

In this study, we generated Trib1-deficient mice by gene targeting and analyzed TLR-mediated responses. Although the activation of NF-κB and MAP kinases in response to LPS was comparable between wild-type and Trib1-deficient cells, microarray analysis revealed that a subset of LPS-inducible genes was dysregulated in Trib1-deficient cells. Subsequent yeast two-hybrid analysis identified the CCAAT/enhancer-binding protein (C/EBP) family member NF-IL6 (also known as C/EBPβ) as a binding partner of Trib1, and phenotypes found in NF-IL6–deficient cells were opposite to those observed in Trib1-deficient cells. Moreover, overexpression of Trib1 inhibited NF-IL6–mediated gene expression and reduced amounts of NF-IL6 proteins. Inversely, NF-IL6 DNA-binding activity and LPS-inducible NF-IL6–target gene expression were up-regulated in Trib1-deficient cells, in which amounts of NF-IL6 proteins were increased. These results demonstrate that Trib1 plays an important role in NF-IL6–dependent gene expression in the TLR-mediated signaling pathways.

RESULTS

Comprehensive gene expression analysis in Trib1-deficient macrophages

To assess the physiological function of Trib1 in TLR-mediated immune responses, we performed a microarray analysis to compare gene expression profiles between wild-type and Trib1-deficient macrophages in response to LPS (Fig. 1 A and Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20070183/DC1). Out of 45,102 transcripts, we first defined the genes induced more than twofold after LPS stimulation in wild-type cells as "LPS-inducible genes" and identified 790 of them (Table S1). We next compared the LPS-inducible genes in wild-type and Trib1-deficient macrophages after LPS stimulation and found 59, 703, and 28 genes as up-regulated, similarly expressed, and down-regulated in Trib1-deficient cells, respectively (Table S1).

Among the up-regulated genes, several were subsequently tested by Northern blotting to confirm the accuracy. LPS-induced expression of prostaglandin E synthase (mPGES), lipocalin-2 (24p3), arginase type II, and plasminogen activator inhibitor type II, which were highly up-regulated in the microarray analysis (Table S1), was indeed enhanced in Trib1-deficient macrophages (Fig. 1 B). Furthermore, in contrast to proinflammatory cytokines such as TNF-α and IL-6, which were similarly expressed between wild-type and Trib1-deficient cells in response not only to LPS but also to other TLR ligands, IL-12 p40 was down-regulated in Trib1-deficient cells compared with wild-type cells (Fig. 1 C; Fig. S2, A–C, available at http://www.jem.org/cgi/content/full/jem.20070183/DC1; and Table S1). Thus, the comprehensive microarray analysis revealed that a subset of LPS-inducible genes is dysregulated in Trib1-deficient cells.

Previous in vitro studies demonstrate that human Trib family members modulate activation of MAP kinases and

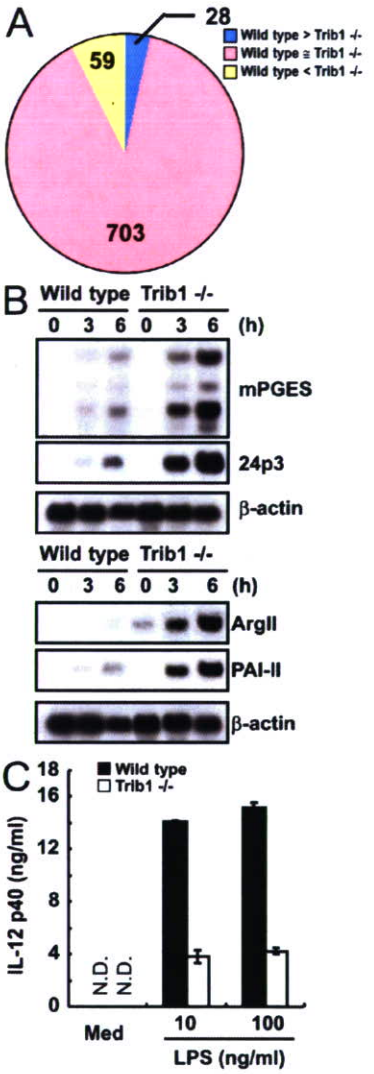

Figure 1. Dysregulation of a subset of LPS-inducible genes in Trib1-deficient cells. (A) Summary of DNA chip microarray analysis. 790 LPS-inducible genes were divided into up-regulated (yellow), similarly expressed (pink), and down-regulated (blue) groups, with the indicated amounts of each. (B) Peritoneal macrophages from wild-type or Trib1-deficient mice were stimulated with 10 ng/ml LPS for the indicated periods. Total RNA (10 μg) was extracted and subjected to Northern blot analysis for the expression of the indicated probes. (C) Peritoneal macrophages from wild-type and Trib1-deficient mice were cultured with the indicated concentrations of LPS in the presence of 30 ng/ml IFN-γ for 24 h. Concentrations of IL-12 p40 in the culture supernatants were measured by ELISA. Indicated values are means ± SD of triplicates. Data are representative of three (B) or two (C) independent experiments. N.D., not detected.

NF-κB (7–12). Both wild-type and Trib1-deficient cells showed similar levels and time courses of phosphorylation of p38, Jnk and extracellular signal-regulated kinase, and IκBα degradation (Fig. S2 D), indicating that the dysregulated

expression of LPS-inducible genes in Trib1-deficient cells might be the independent of activation of NF-κB and MAP kinases.

Interaction of Trib1 with NF-IL6

To explore signaling aspects of Trib1 deficiency other than NF-κB and MAP kinases, we performed a yeast-two-hybrid screen with the full length of human Trib1 as bait to identify a binding partner of Trib1 and identified several clones as being positive. Sequence analysis subsequently revealed that three clones encoded the N-terminal portion of a member of the C/EBP NF-IL6 (unpublished data). We initially tested the interaction of Trib1 and NF-IL6 in yeasts. AH109 cells were transformed with a plasmid encoding the full length of Trib1 together with a plasmid encoding the N-terminal portion of NF-IL6 obtained by the screening (Fig. 2 A). We next examined the interaction in mammalian cells using immunoprecipitation experiments. HEK293 cells were transiently transfected with a plasmid encoding the full length of mouse Trib1 together with a plasmid encoding the full length of mouse NF-IL6. Myc-tagged NF-IL6 was coimmunoprecipitated with Flag-Trib1 (Fig. 2 B), showing the interaction of Trib1 and NF-IL6 in mammalian cells.

TLR-mediated immune responses in NF-IL6–deficient macrophages

An in vitro study showing the interaction of Trib1 and NF-IL6 prompted us to examine the TLR-mediated immune responses in NF-IL6–deficient cells, because LPS-induced expression of mPGES is shown to depend on NF-IL6 (13). We initially analyzed the expression pattern of genes affected by the loss of Trib1 in NF-IL6–deficient macrophages by Northern blotting. LPS-induced expression of 24p3, plasminogen activator inhibitor type II, and arginase type II, as well as mPGES, was profoundly defective in NF-IL6–deficient cells (Fig. 2 C). We next tested IL-12 p40 production by ELISA. As previously reported, IL-12 p40 production by LPS stimulation was increased in a dose-dependent fashion in NF-IL6–deficient cells compared with control cells (Fig. 2 D) (14). In addition, the production in response to bacterial lipoprotein (BLP), macrophage-activating lipopeptide–2 (MALP-2), or CpG DNA was also augmented in

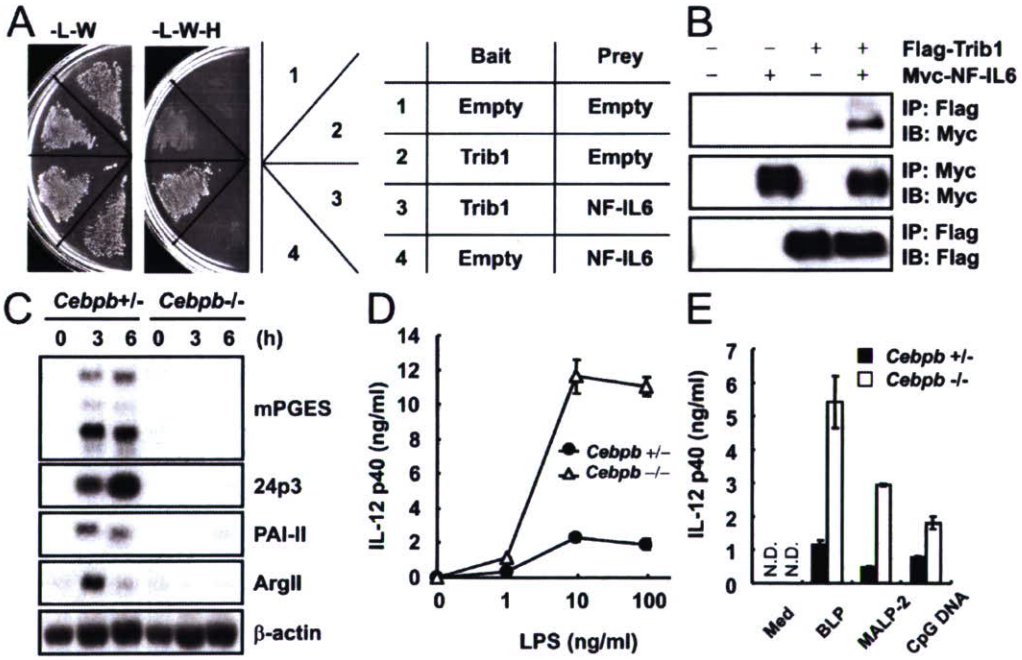

Figure 2. Association of Trib1 with NF-IL6 and TLR-mediated responses in NF-IL6–deficient macrophages. (A) Plasmids expressing human Trib1 fused to the GAL4 DNA-binding domain or an empty vector were cotransfected with a plasmid expressing NF-IL6 fused to GAL4 transactivation domain or an empty vector. Interactions were detected by the ability of cells to grow on medium lacking tryptophan, leucin, and histidine (-L-W-H). The growth of cells on a plate lacking tryptophan and leucine (-L-W) is indicative of the efficiency of the transfection. (B) Lysates of HEK293 cells transiently cotransfected with 2 μg of Flag-tagged Trib1 and/or 2 μg Myc-tagged NF-IL6 expression vectors were immunoprecipitated with the indicated antibodies. (C) Peritoneal macrophages from wild-type or NF-IL6–deficient mice were stimulated with 10 ng/ml LPS for the indicated periods. Total RNA (10 μg) was extracted and subjected to Northern blot analysis for expression of the indicated probes. (D and E) Peritoneal macrophages from wild-type and NF-IL6–deficient mice were cultured with the indicated concentrations of LPS (D) or with 100 ng/ml BLP, 30 ng/ml MALP-2, or 1 μM, CpG DNA (E) in the presence of 30 ng/ml IFN-γ for 24 h. Concentrations of IL-12 p40 in the culture supernatants were measured by ELISA. Indicated values are means ± SD of triplicates. Data are representative of three (B) and two (C–E) separate experiments. N.D., not detected.

NF-IL6–deficient cells (Fig. 2 E). Together, compared with Trib1-deficient cells, converse phenotypes in terms of TLR-mediated immune responses are observed in NF-IL6–deficient cells.

Inhibition of NF-IL6 by Trib1 overexpression

To test whether Trib1 down-regulates NF-IL6–dependent activation, HEK293 cells were transfected with an NF-IL6–dependent luciferase reporter plasmid together with NF-IL6 and various amounts of Trib1 expression vectors (Fig. 3 A). NF-IL6–mediated luciferase activity was diminished by co-expression of Trib1 in a dose-dependent manner. Moreover, RAW264.7 macrophage cells overexpressing Trib1 exhibited reduced expression of mPGES and 24p3 in response to LPS (Fig. S3 A, available at http://www.jem.org/cgi/content/full/jem.20070183/DC1). We next tested NF-IL6 DNA-binding activity by EMSA and observed less NF-IL6 DNA-binding activity in HEK293 cells coexpressing NF-IL6 and Trib1 than in ones transfected with the NF-IL6 vector alone (Fig. 3 B), presumably accounting for the down-regulation of the NF-IL6–dependent gene expression by Trib1. We then examined the effect of Trib1 on the amounts of NF-IL6 proteins by Western blotting. Although the diminution of NF-IL6 by Trib1 was marginal when excess amounts of NF-IL6 were expressed, we found that the transient expression of lower levels of NF-IL6, together with Trib1, resulted in a reduction of NF-IL6 in HEK293 cells (Fig. 3 C). Also, endogenous levels of NF-IL6 proteins in RAW264.7 cells overexpressing Trib1 were markedly less than those in control cells (Fig. 3 D). These results demonstrated that overproduction of Trib1 might negatively regulate NF-IL6 activity in vitro.

Up-regulation of NF-IL6 in Trib1-deficient cells

We next attempted to check the in vivo status of NF-IL6 in Trib1-deficient cells by comparing the NF-IL6 DNA-binding activity in Trib1-deficient macrophages with that in wild-type cells by EMSA. Although LPS-induced NF-κB–DNA complex formation in Trib1-deficient cells was similarly observed, Trib1-deficient cells exhibited elevated levels of C/EBP–DNA complex formation compared with wild-type cells (Fig. 4 A). We further examined whether the C/EBP–DNA complex in Trib1-deficient cells contained NF-IL6 by supershift assay. Addition of anti–NF-IL6 antibody into the C/EBP–DNA complex yielded more supershifted bands in Trib1-deficient cells than in wild-type cells (Fig. 4 B). In addition, the C/EBP–DNA complex was not shifted by the addition of anti-C/EBPδ (also known as NF-IL6β) antibody (Fig. S4 A, available at http://www.jem.org/cgi/content/full/jem.20070183/DC1), suggesting that NF-IL6 DNA-binding activity is augmented in Trib1-deficient cells. We then examined the amounts of NF-IL6 proteins by Western blotting (Fig. 4 C). Compared with wild-type cells, Trib1-deficient cells showed increased levels of NF-IL6 proteins. Finally, we examined NF-IL6 mRNA levels by Northern blotting and observed enhanced expression of NF-IL6 mRNA in Trib1-deficient cells (Fig. 4 D), which is consistent with the autocrine induction of NF-IL6 mRNA

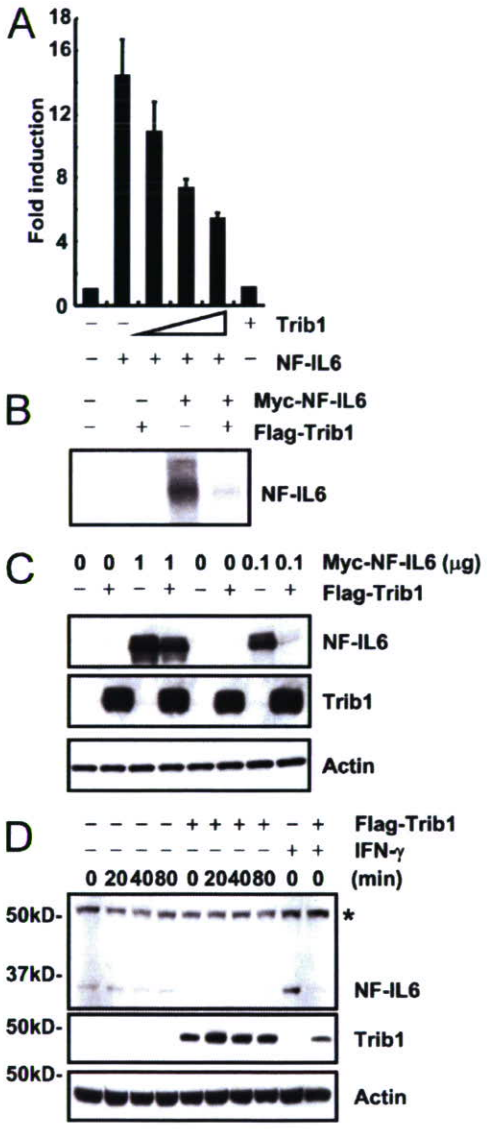

Figure 3. Inhibition of NF-IL6 activity by Trib1 overexpression. (A) HEK293 cells were transfected with an NF-IL6–dependent luciferase reporter together with either Trib1 and/or NF-IL6 expression plasmids. Luciferase activities were expressed as the fold increase over the background shown by lysates prepared from mock-transfected cells. Indicated values are means ± SD of triplicates. (B) HEK293 cells were transfected with 0.1 μg NF-IL6 expression vector together with 4 μg Trib1 expression plasmids. Nuclear extracts were prepared, and C/EBP DNA-binding activity was determined by EMSA using a probe containing the NF-IL6 binding sequence from the mouse 24p3 gene. (C) Lysates of HEK293 cells transiently cotransfected with 2 μg of Flag-tagged Trib1 alone or the indicated amounts of Myc-tagged NF-IL6 expression vectors were immunoblotted with anti-Myc or -Flag for detection of NF-IL6 or Trib1, respectively. (E) RAW 264.7 cells stably transfected with either an empty vector or Flag-Trib1 were stimulated with 10 ng/ml LPS for the indicated periods. The cell lysates were immunoblotted with the indicated antibodies. A protein that cross-reacts with the antibody is indicated (*). Data are representative of three (A and C) and two (B and D), separate experiments.

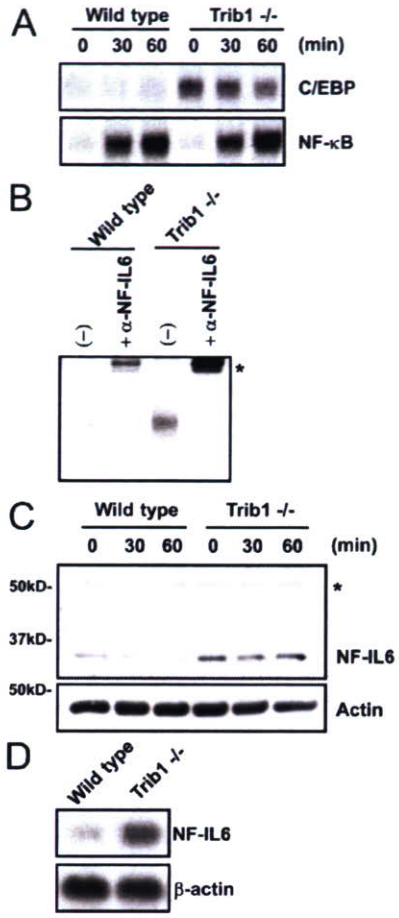

Figure 4. Up-regulation of NF-IL6 activity in Trib1-deficient cells. (A) Peritoneal macrophages from wild-type or Trib1-deficient mice were stimulated with 10 ng/ml LPS for the indicated periods. Nuclear extracts were prepared, and C/EBP DNA-binding activity was determined by EMSA using a C/EBP consensus probe. (B) Nuclear extracts of wild-type and Trib1-deficient unstimulated macrophages were preincubated with anti-NF-IL6, followed by EMSA to determine the C/EBP DNA-binding activity. Supershifted bands are indicated (*). (C) Peritoneal macrophages from wild-type or Trib1-deficient mice were stimulated with 10 ng/ml LPS for the indicated periods and lysed. The cell lysates were immunoblotted with the indicated antibodies. A protein that cross-reacts with the antibody is indicated (*). (D) Total RNA (10 μg) from unstimulated peritoneal macrophages from wild-type or NF-IL6-deficient mice was extracted and subjected to Northern blot analysis for expression of the indicated probes. Data are representative of two (A and B) and three (C and D) separate experiments.

in a previous study (15). Thus, Trib1 may negatively control amounts of NF-IL6 proteins, thereby affecting TLR-mediated NF-IL6–dependent gene induction.

DISCUSSION

In this study, we demonstrate by microarray analysis and biochemical studies that Trib1 is associated with NF-IL6 and negates NF-IL6–dependent gene expression by reducing the amounts of NF-IL6 proteins in the context of TLR-mediated responses. Especially regarding IL-12 p40, although the microarray data showed an almost twofold reduction of the mRNA in Trib1-deficient cells (Table S1), the production was three to four times lower than that in wild-type cells (Fig. 1 C), suggesting translational control of IL-12 p40 by Trib1 in addition to the transcriptional regulation. Moreover, the transcription of the IL-12 p40 gene itself may be affected by not only the amount of NF-IL6 proteins but also the phosphorylation or the isoforms such as liver-enriched activator protein and liver-enriched inhibitory protein (16–18). The molecular mechanisms of how Trib1 deficiency affects IL-12 p40 production on the transcriptional or translational levels through NF-IL6 regulation need to be carefully studied in the future.

The name Trib is originally derived from the *Drosophila* mutant strain *tribbles*, in which the *Drosophila* tribbles protein negatively regulates the level of *Drosophila* C/EBP *slbo* protein and C/EBP-dependent developmental responses such as border cell migration in larvae (19–22). It is also of interest that Trib1-deficient female mice and *Drosophila* in adulthood are both infertile (unpublished data) (18). In mammals, other Trib family members such as Trib2 and Trib3 have recently been shown to be involved in C/EBP-dependent responses (23, 24). Mice transferred with bone marrow cells, in which Trib2 is retrovirally overexpressed, display acute myelogenous leukemia–like disease with reduced activities and amounts of C/EBPα (23). In addition, ectopic expression of Trib3 inhibits C/EBP-homologous protein–induced ER stress–mediated apoptosis (24). Thus, the function of tribbles to inhibit C/EBP activities by controlling the amounts appears to be conserved throughout evolution.

Given the up-regulation of the mRNA in Trib1-deficient cells (Fig. 4 D), the reduction of NF-IL6 in Trib1-overexpressing cells (Fig. 3 C), the auto-regulation of NF-IL6 by itself (15), and the degradation of C/EBPα by Trib2 (23) and slbo by tribbles (22), the loss of Trib1 might primarily result in impaired degradation of NF-IL6 and, subsequently, in excessive accumulation of NF-IL6 via the autoregulation in Trib1-deficient cells.

In this study, we focused on the involvement of Trib1 in TLR-mediated NF-IL6–dependent gene expression. However, given that the levels of NF-IL6 proteins were increased in Trib1-deficient cells, it is reasonable to propose that other non–TLR-related NF-IL6–dependent responses might be enhanced in Trib1-deficient mice. Moreover, Trib3 is also shown to be involved in insulin-mediated Akt/PKB activation in the liver by mechanisms apparently unrelated to C/EBP, suggesting that Trib family members possibly function in a C/EBP-independent fashion (25–27). Future studies using mice lacking other Trib family members, as well as Trib1, may help to unravel the nature of mammalian tribbles in wider points of view.

MATERIALS AND METHODS

Generation of Trib1-deficient mice. A genomic DNA containing the *Trib1* gene was isolated from the 129/SV mouse genomic library and characterized by restriction enzyme mapping and sequencing analysis. The gene encoding mouse Trib1 consists of three exons. The targeting vector was constructed by replacing a 0.4-kb fragment encoding the second exon of the

Trib1 gene with a neomycin resistance gene cassette (*neo*) (Fig. S1 A). The targeting vector was transfected into embryonic stem cells (E14.1). G418 and gancyclovir doubly resistant colonies were selected and screened by PCR and Southern blot analysis (Fig. S1 B). Homologous recombinants were microinjected into C57BL/6 female mice, and heterozygous F1 progenies were intercrossed to obtain *Trib1*$^{+/-}$ mice. We interbred the heterozygous mice to produce offspring carrying a null mutation of the gene encoding Trib1. Trib1-deficient mice were born at the expected Mendelian ratio and showed a slight growth retardation with reduced body weight until 2–3 wk after birth (unpublished data). Trib1-deficient that mice survived for >6 wk were analyzed in this study. To confirm the disruption of the gene encoding Trib1, we analyzed total RNA from wild-type and Trib1-deficient peritoneal macrophages by Northern blotting and found no transcripts for Trib1 in Trib1-deficient cells (Fig. S1 C). All animal experiments were conducted with the approval of the Animal Research Committee of the Research Institute for Microbial Diseases at Osaka University.

Reagents, cells, and mice. LPS (a TLR4 ligand) from *Salmonella minnesota* Re 595 and anti-Flag were purchased from Sigma-Aldrich. BLP (TLR1/TLR2), MALP-2 (TLR2/TLR6), and CpG oligodeoxynucleotides (TLR9) were prepared as previously described (28). Antiphosphorylated extracellular signal-regulated kinase, Jnk, and p38 antibodies were purchased from Cell Signaling. Anti–NF-IL6 (C/EBPβ), C/EBPδ, actin, IκBα, and Myc-probe were obtained from Santa Cruz Biotechnology, Inc. NF-IL6–deficient mice were as previously described (29). Epitope-tagged Trib1 fragments were generated by PCR using cDNA from LPS-stimulated mouse peritoneal macrophages as the template and cloned into pcDNA3 expression vectors, according to the manufacturer's instructions (Invitrogen).

Measurement of proinflammatory cytokine concentrations. Peritoneal macrophages were collected from peritoneal cavities 96 h after thioglycollate injection and cultured in 96-well plates (10^5 cells per well) with the indicated concentrations of the indicated ligands for 24 h, as shown in the figures. Concentrations of TNF-α, IL-6, and IL-12 p40 in the culture supernatant were measured by ELISA, according to manufacturer's instructions (TNF-α and IL-12 p40, Genzyme; IL-6, R&D Systems).

Luciferase reporter assay. The NF-IL6–dependent reporter plasmids were constructed by inserting the promoter regions (−1200 to +53) of the mouse 24p3 gene amplified by PCR into the pGL3 reporter plasmid. The reporter plasmids were transiently cotransfected into HEK293 with the control *Renilla* luciferase expression vectors using a reagent (Lipofectamine 2000; Invitrogen). Luciferase activities of total cell lysates were measured using the Dual-Luciferase Reporter Assay System (Promega), as previously described (28).

Yeast two-hybrid analysis. Yeast two-hybrid screening was performed as described for the Matchmaker two-hybrid system 3 (CLONTECH Laboratories, Inc.). For construction of the bait plasmid, the full length of human Trib1 was cloned in frame into the GAL4 DNA-binding domain of pGBKT7. Yeast strain AH109 was transformed with the bait plasmid plus the human lung Matchmaker cDNA library. After screening of 10^6 clones, positive clones were picked, and the pACT2 library plasmids were recovered from individual clones and expanded in *Escherichia coli*. The insert cDNA was sequenced and characterized with the BLAST program (National Center for Biotechnology Information).

Microarray analysis. Peritoneal macrophages from wild-type or Trib1-deficient mice were left untreated or were treated for 4 h with 10 ng/ml LPS in the presence of 30 ng/ml IFN-γ. The cDNA was synthesized and hybridized to Murine Genome 430 2.0 microarray chips (Affymetrix), according to the manufacturer's instructions. Hybridized chips were stained and washed and were scanned with a scanner (GeneArray; Affymetrix). Microarray Suite software (version 5.0; Affymetrix) was used for data analysis. Microarray data have been deposited in the Gene Expression Omnibus under accession no. GSE8788.

Western blot analysis and immunoprecipitation. Peritoneal macrophages were stimulated with the indicated ligands for the indicated periods, as shown in the figures. The cells were lysed in a lysis buffer (1% Nonidet P-40, 150 mM NaCl, 20 mM Tris-Cl [pH 7.5], 5 mM EDTA) and a protease inhibitor cocktail (Roche). The cell lysates were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. For immunoprecipitation, cell lysates were precleared with protein G–sepharose (GE Healthcare) for 2 h and incubated with protein G–sepharose containing 1 μg of the antibodies indicated in the figures for 12 h, with rotation at 4°C. The immunoprecipitants were washed four times with lysis buffer, eluted by boiling with Laemmli sample buffer, and subjected to Western blot analysis using the indicated antibodies, as previously described (28).

EMSA and supershift assay. 2×10^6 peritoneal macrophages were stimulated with the indicated stimulants for the indicated periods, as shown in the figures. 2×10^6 HEK293 cells were transfected with 0.1 μg Myc–NF-IL6 and/or 4 μg Flag-Trib1 expression vectors. Nuclear extracts were purified from cells and incubated with a probe containing a consensus C/EBP DNA-binding sequence (5′-TGCAGATTGCGCAATCTGCA–3′; Fig. 4, A and B) or mouse 24p3 NF-IL6 binding sequence (sense, 5′-CTTCCTGTTGCTCAACCTTGCA-3′;antisense,5′-TGCAAGGTTGAGCAACAGGAAG–3′; Fig. 3 B), electrophoresed, and visualized by autoradiography, as previously described (28, 30). When the supershift assay was performed, nuclear extracts were mixed with the supershift-grade antibodies indicated in the figures before the incubation with the probes for 1 h on ice.

Online supplemental material. Fig. S1 showed our strategy for the targeted disruption of the mouse *Trib1* gene. Fig. S2 showed the status of proinflammatory cytokine production in response to various TLR ligands and LPS-induced activation of MAP kinases and IκB degradation. Fig. S3 showed decreased expression of NF-IL6–dependent gene in Trib1-overexpressing cells. Fig. S4 showed that the C/EBP-DNA complex in Trib1-deficient cells contained NF-IL6, but not C/EBPδ. Table S1 provides a complete list of the LPS-inducible genes studied. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20070183/DC1.

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Periportal and sinusoidal liver dendritic cells suppressing T helper type 1-mediated hepatitis

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HEPATITIS

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Background: Recently, we found that portal vein tolerance is associated with generation of Th2 cells and apoptosis of Th1 cells in the liver, which is regulated by antigen (Ag)-presenting dendritic cells (DCs) in the periportal area and sinusoids.
Aim: In this study, we tested whether the periportal and sinusoidal DCs, which were loaded with an Ag in vivo, can inhibit liver injury caused by Th1 cells activated by the Ag administered systemically.
Methods: Ag-specific hepatitis model was created by adoptively transferring ovalbumin (OVA)-specific $CD4^+$ T cells to BALB/c mice and venous injection of OVA-containing liposomes. Liver $CD11c^+$ cells obtained from mice fed OVA were then transferred into these mice.
Results: The transfer of liver $CD11c^+$ cells from OVA-fed mice completely inhibited hepatic injury, which was associated with apoptosis of OVA-specific $CD4^+$ T cells and emergence of Th2 cells in the liver. Transfer of $CD11c^+$ cells and subcutaneous OVA challenge led to enhancement of OVA-specific IgE Ab as well as Th2 cytokine responses in the recipient mice.
Conclusions: Periportal and sinusoidal DCs loaded with an Ag in the portal vein can induce Th2 response in the liver and prevent hepatic injury caused by Th1 cells.

Although portal blood flow contains various antigens (Ags) derived from foods and intestinal microflora, the liver normally evades such inflammatory responses that would lead to tissue injury. This immunological hyporesponsiveness, which is called portal vein tolerance,[1 2] explains several immunological properties of the liver. First, the liver is such a tolerogenic organ that transplantation of an allogenic liver sometimes requires little immunosuppressive therapy.[3 4] Second, surgical diversion of portal blood away from the liver abrogates oral tolerance.[5] Third, pancreatic islet cells transplanted via the portal vein evades rejection by the host and cures insulin-dependent diabetes.[6] Thus, portal vein tolerance can establish hyporesponsiveness to Ags migrating to the liver through portal blood flow.

Regarding the induction mechanisms of portal vein tolerance, Ag-presenting cells (APCs), such as Kupffer cells, liver sinusoidal endothelial cells (LSECs) and dendritic cells (DCs), are known to play important roles in the liver.[7] For example, Ag-presentation by LSECs and DCs preferentially leads to development of Th2 cells producing anti-inflammatory cytokines.[8–10] In contrast to these tolerogenic responses, some Ag-presentations in the liver can result in the production of tissue-damaging T cells, which leads to autoimmune liver diseases such as autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC). In these latter situations, a marked increase of Th1 type cytokines is known to play an important role in the establishment of the diseases.[11–13] This was corroborated by the studies of experimental hepatitis and cholangitis, which has revealed a pathogenic role played by Th1 cytokines.[14 15]

Recently, we found that an Ag administered orally can activate Ag-specific $CD4^+$ T cells in the liver and increase the number of Th2 cells, which associates with Fas-mediated apoptosis of Th1 cells.[16] We reported that Ag-capturing $CD11c^+$ cells in the liver are defective in IL12 secretion and responsible for the generation of Th2 cells.[8]

Based on these findings, we speculated that DCs play pivotal roles in the induction of portal vein tolerance. Here we show that adoptive transfer of liver $CD11c^+$ DCs loaded with an Ag in the portal vein can suppress Th1-mediated liver injury in the recipient mice.

METHODS

Animals and protocol for immunisation

DO11.10 mice with T cells bearing the transgenic T cell receptor (TCR) that recognises the 323–339 peptide fragment of ovalbumin (OVA) in the context of IA^d were crossed to Rag_2–/– mice.[8] BALB/c and Rag_2–/–DO11.10 mice were housed under specific pathogen free conditions in the Animal Facility of Kyoto University. Male BALB/c mice were fed 100mg of OVA (Sigma Chemical Co., St Louis, Missouri, USA) or PBS alone, every other day for a total of five times by intragastric intubation. All animal experiments were performed in accordance with institutional guidelines and ethical permission for this study was granted by the review board of Kyoto University.

Histological analysis

Liver sections were stained with biotinylated anti-CD11c (Pharmingen, San Diego, California, USA), anti-IA^d (Pharmingen), and anti-F4/80 Ab (Serotec Ltd, Oxford, England) as described previously.[17] For the detection of apoptotic hepatocytes, TdT-mediated dUTP nick-end labelling (TUNEL)-staining was performed using a commercial kit (apoTACS-DAB, Trevigen, Gaithersburg, Maryland, USA).

Preparation of mononuclear cells from the spleen, the lymph nodes, and the liver

Lymphocytes from the spleen and draining lymph nodes (dLNs) were prepared as previously described.[8] Intrahepatic lymphocytes (IHLs) were prepared following the method

Abbreviations: Ag, antigen; APC, antigen presenting cell; LSEC, liver sinusoidal endothelial cell; DC, dendritic cell; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; TUNEL, TdT-mediated dUTP nick-end labelling; IHL, intrahepatic lymphocyte; PI, propidium iodide; AST, aspartate aminotransferase; ALT, alanine aminotransferase; dLN, draining lymph nodes

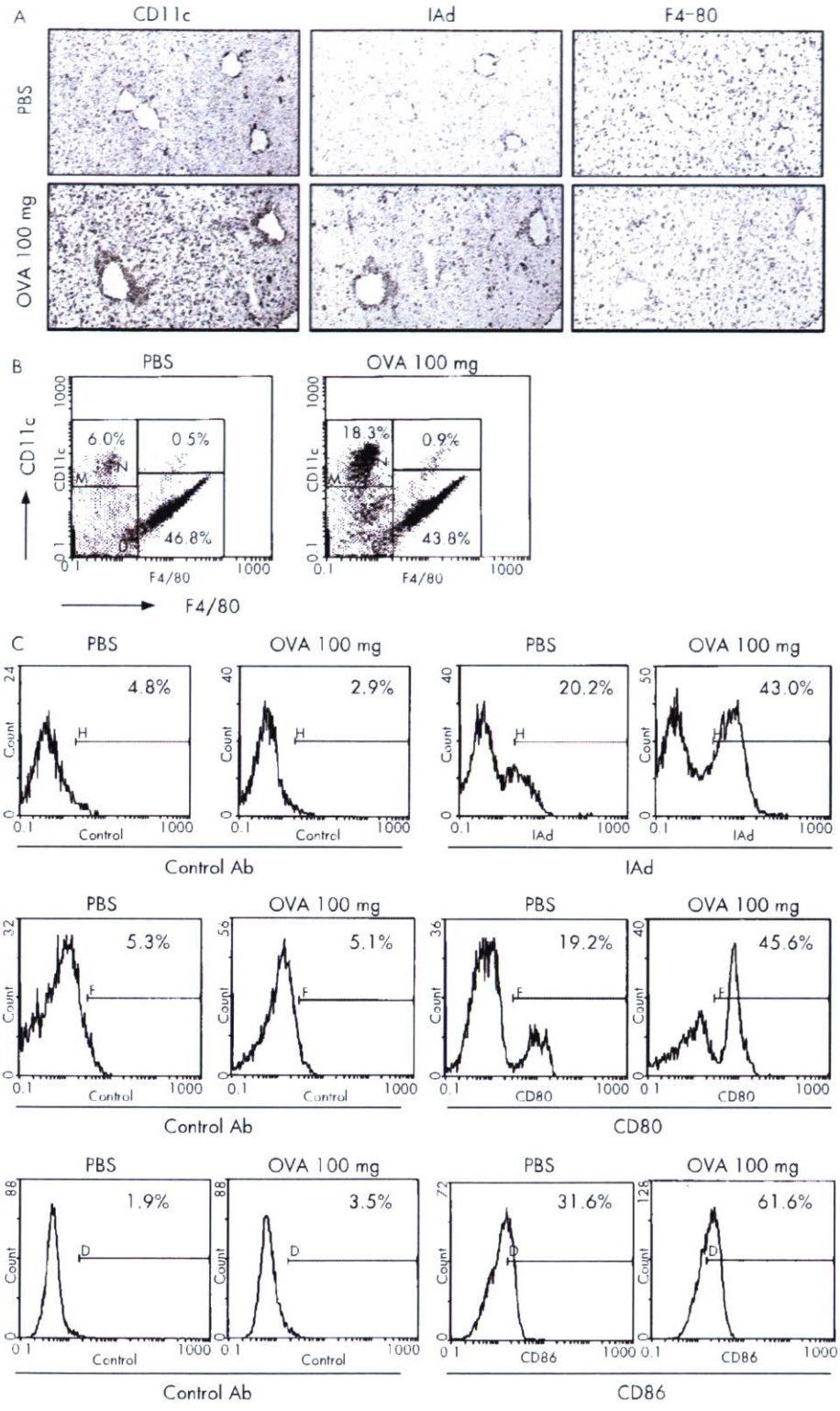

Figure 1 Characterisation of CD11c$^+$ cells in the liver of BALB/c mice fed OVA. BALB/c mice were fed 100mg of OVA or PBS every other day for a total of five times and killed 3 days after the fifth feeding. (A) Localisation of CD11c$^+$, IA^{d+}, F4/80$^+$ cells in the liver. Frozen liver sections were stained with anti-CD11c mAb, anti-IA^d mAb, or anti-F4/80 mAb. (B) Flow-cytometric analysis of non-parenchymal low density cells in the liver. Cells were stained with PE-conjugated anti-CD11c mAb and biotin-conjugated anti-F4/80 mAb followed by incubation with streptavidin-FITC. Dead cells were excluded by propidium iodide (PI) staining. The number shows the percentage of cells in each region. (C) CD11c$^+$ cells are isolated from the liver of mice fed PBS or OVA. These cells were stained with PE-conjugated control Ab, anti-IA^d mAb, anti-CD80 mAb, and anti-CD86 mAb. The results shown are representative one of two experiments (n = 4 in each group).

described previously.[16] Hepatic low density non-parenchymal cells were also obtained as described previously.[8]

Adoptive transfer of CD11c$^+$ cells to naive BALB/c mice

Splenic and hepatic CD11c$^+$ cells were purified by positive selection using autoMACS (Miltenyi Biotec, Bergisch Gladback, Germany) as described previously.[8] The recovered cells were more than 90% CD11c$^+$ (confirmed by flow cytometric analysis). Six-week old BALB/c mice were injected intravenously with 5×10^5 CD11c$^+$ cells purified from the spleen or the liver of BALB/c mice fed OVA or PBS. The next day after the transfer, the recipient mice were immunised subcutaneously with complete Freund's adjuvant (CFA, GIBCO BRL, Grand Island, New York, USA) and OVA (1mg/ml). OVA-specific IgG, IgG1, IgG2a, IgE Ab were measured by ELISA as described elsewhere.[18]

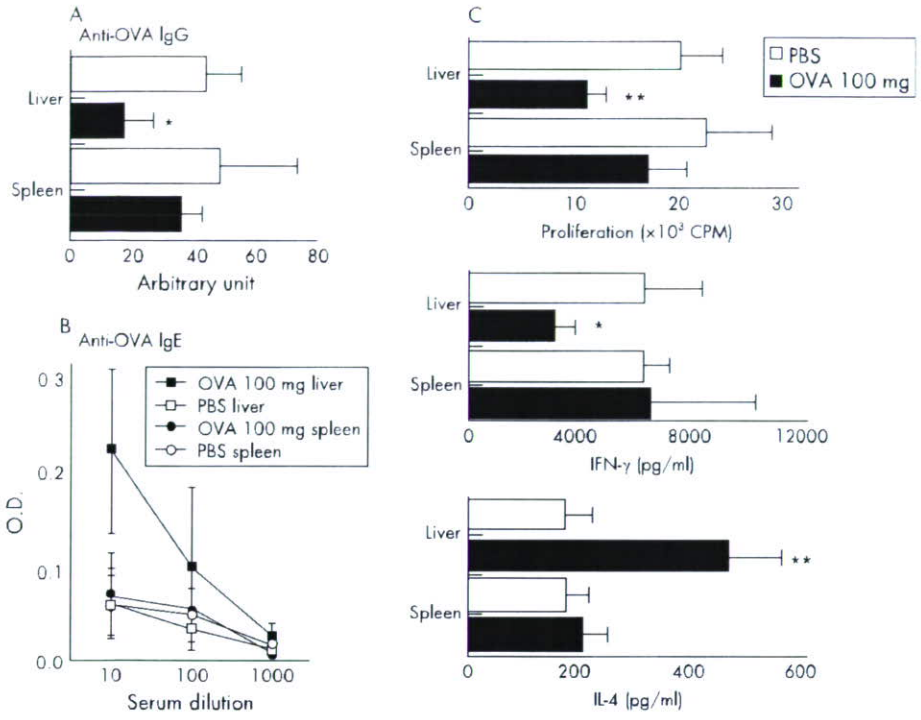

Figure 2 OVA-specific Ab and cytokine responses in the mice transferred with hepatic CD11c$^+$ cells. Donor BALB/c mice were fed 100mg of OVA or PBS every other day for a total of five times and killed 3 days after the fifth feeding. Hepatic or splenic CD11c$^+$ cells of the donor mice were transferred into recipient BALB/c mice via intravenous route (5×10^5/mouse). The day after the transfer, recipient mice were immunised subcutaneously with 100μg of OVA. Serum OVA-specific IgG Ab (A) and OVA-specific IgE Ab responses (B) of the recipient mice were measured 14 days after the subcutaneous immunisation. Pooled immune sera obtained from BALB/c mice that received repeated immunisation with OVA were used as control, and the Ab titre equivalent to 2^{10} dilution of immune sera was defined as one arbitrary unit (A). (C) Proliferative responses, IFN-γ secretion, and IL4 secretion by dLN-CD4$^+$ T cells were measured 10days after the subcutaneous immunisation. CD4$^+$ T cells (1×10^5/well) were stimulated by OVA (0.1mg/ml) presented by irradiated splenocytes (5×10^5/well) from BALB/c mice. For proliferative responses, the cultures were incubated for 72 hours and 1μCi of [^{3}H] thymidine was added for the final 16hours. For cytokine analysis, culture supernatants were collected at 48 hours for IFN-γ production and at 72 hours for IL4 production. The results are expressed as meanSD. *$p<0.05$, **$p<0.01$ versus PBS control. The results shown are representative one of two independent experiments (n = 4 in each group).

Induction of Ag-specific hepatic injury

Six-week old BALB/c mice were injected intravenously with 5×10^5 CD11c$^+$ cells purified from the liver of BALB/c mice fed OVA or PBS as described above. OVA-specific liver injury using OVA-liposome was induced in these recipient mice as reported previously with some modifications.[14] Briefly, splenocytes from Rag_2–/–DO11.10 mice were stimulated for 3days with $OVA_{323-339}$ peptide (1μg/ml) and then activated OVA-specific CD4$^+$ T cells were purified by positive selection using autoMACS (>90% KJ1-26$^+$, confirmed by flowcytometric analysis). BALB/c mice that received CD11c$^+$ cells were treated with intravenous injection of OVA-containing liposome (15mg, NOF corporation, Tokyo, Japan) followed by the transfer of activated splenic CD4$^+$KJ1-26$^+$ T cells 2hours later (2.5×10^6/mouse). Mice were killed 16hours later and then serum, IHLs, and splenocytes were prepared. The liver injury was assessed by serum aspartate aminotransferase (AST) and alanine animotransferase (ALT) activities by a commercial kit (Wako, Osaka, Japan).

Stimulation of draining lymph nodes (dLN)-cells, spleen cells, and IHLs

CD4$^+$ T cells were purified from the spleen, dLN, and the liver by positive selection using autoMACS (Miltenyi Biotec, Bergisch Gladback, Germany). These CD4$^+$ T cells (1×10^5/well) were stimulated in vitro with OVA (0.1mg/ml) or OVA-peptide (1μg/ml) presented by irradiated splenocytes (5×10^5/well) from BALB/c mice. The cultures were incubated for 72hours and 1μCi of [^{3}H] thymidine was added for the final 16hours. Proliferation and cytokine production were evaluated as described previously.[10] In some experiments, splenic OVA-specific CD4$^+$ T cells (1×10^5/well) were stimulated with OVA-peptide (1μg/ml) presented by hepatic CD11c$^+$ cells (1×10^5/well) in the presence of mouse IL12 (10ng/ml, Peprotec), rat IgG (10μg/ml, Pharmingen), or neutralising anti-IL12p40 mAb (10μg/ml, Pharmingen).

mAbs and flow cytometry

FITC-conjugated KJ1-26 recognising transgenic TCR specific to OVA and biotin-conjugated F4/80 mAb were purchased from Caltag (San Francisco, California, USA). FITC or PE-conjugated anti-mouse CD11c mAb, PE-conjugated anti-mouse CD4, IA^d, CD80, CD86 mAb and biotin-conjugated Annexin V were purchased from Pharmingen. Streptavidin-RED670 was obtained from Life Technologies. Surface immunofluorescence was assessed as described previously.[16]

Statistical analysis

Student's t-test was used to evaluate the significance of the differences. Statistical analysis was performed with the Stat View v.4.5 program (Abacus Concepts, Berkeley, CA). A p value <0.05 was regarded as statistically significant.

RESULTS

Activation of liver DCs upon oral administration of an Ag

As our previous study indicated that a part of OVA administered orally is carried to the liver and co-localise with class II (IA^d)$^+$ cells in the periportal area,[8] we analysed tissue localisation of DCs in the liver by staining class II and CD11c antigens (fig 1A). Class II$^+$ and CD11c$^+$ cells were mainly localised in the periportal area of the liver of PBS-fed mice. In mice fed OVA, numbers of class II$^+$ and CD11c$^+$ cells increased not only in the periportal area but also in the sinusoidal area. No increase was seen in F4/80$^+$ Kupffer cells that localised mainly sinusoidal area. To analyse quantitatively and to discriminate between DCs and Kupffer cells, we did dual staining flow-cytometric analysis by using hepatic low density

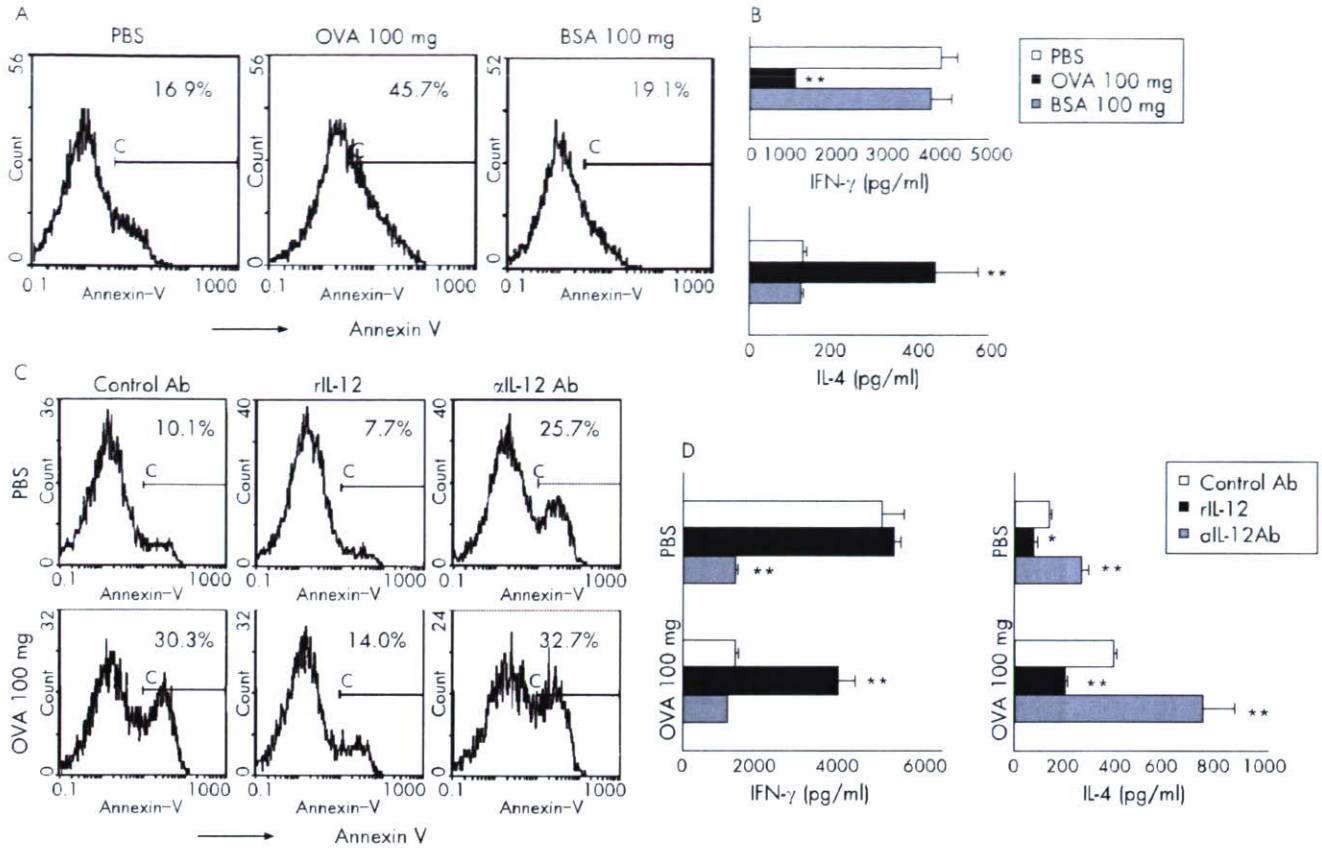

Figure 3 IL12 deficiency in liver DCs is responsible for Th2 responses and apoptosis. Naïve OVA-specific $CD4^+$ T cells (1×10^5/well) isolated from the spleen of Rag_2−/−DO11. Ten mice were stimulated with OVA-peptide (1μg/ml) in the presence of hepatic $CD11c^+$ cells (1×10^5/well) isolated from mice fed PBS, BSA, or OVA. (A) Cells were cultured for 48 hours and the percentage of Annexin V^+ cells in OVA-specific $CD4^+KJ1\text{-}26^+$ T cells was determined. Cells were stained with FITC-conjugated Annexin V and PE-conjugated KJ1-26 mAb. Analysis gate was set on $KJ1\text{-}26^+$ cells. The number in each panel shows the percentage of Annexin V^+ cells. (B) Production of IFN-γ and IL4 by OVA-specific $CD4^+$ T cells. Culture supernatants were collected at 48 hours for IFN-γ production and at 72 hours for IL4 production. (C, D) Effects of IL12 signalling on induction of apoptosis and generation of Th2 responses by hepatic $CD11c^+$ cells. Anti-IL-12p40 mAb (10μg/ml), control Ab (10μg/ml), or mouse IL12 (10ng/ml) were added to the culture. The percentage of Annexin V^+ cells in OVA-specific $CD4^+KJ1\text{-}26^+$ T cells (C) and production of IFN-γ and IL4 (D) were determined as described above. $^*p<0.05$, $^{**}p<0.01$ versus PBS control (B) or Control Ab (D). The results shown are representative one of two independent experiments (n = 3 in each group).

non-parenchymal cells (fig 1B). Most $CD11c^+$ cells were negative for F4/80 staining, suggesting that these $CD11c^+$ cells are DCs rather than Kupffer cells. Consistent with the results of tissue staining, the percentage of $CD11c^+$ cells was markedly increased in the liver of OVA-fed mice. As shown in fig 1C, the percentages of $CD11c^+$ DCs expressing class II, CD80, and CD86 were all increased in the liver by oral administration of OVA. Thus, these flow-cytometric and immunohistochemical analyses indicated that OVA feeding associates with activation of DCs in the liver.

Systemic Th2 response elicited by liver DCs

Next, we assessed the functional properties of in vivo Ag-loaded $CD11c^+$ cells by adoptively transferring them into naïve recipient mice. For this purpose, hepatic or splenic $CD11c^+$ cells from donor BALB/c mice fed OVA or PBS were transferred into the recipient BALB/c mice, which were immunised subcutaneously with OVA/CFA after the transfer. As shown in fig 2A, OVA-specific IgG Ab responses were significantly reduced in the recipient mice which received hepatic $CD11c^+$ cells of OVA-fed mice while the ratio of OVA-specific IgG_1 Ab/IgG_{2a} Ab was not altered (data not shown). In contrast, OVA-specific IgE Ab responses were significantly enhanced in the recipient mice transferred with hepatic $CD11c^+$ cells from OVA-fed mice (fig 2B), suggesting that Th2 response occurred in the recipient mice. This was exactly the case as IL4 secretion by dLN-$CD4^+$ T cells was markedly increased while OVA-specific proliferative responses and secretion of IFN-γ were significantly decreased in those mice (fig 2C). The effect on cytokine production was Ag-specific as anti-CD3 stimulation showed no differences (data not shown). In contrast to the $CD11c^+$ cells in the liver, those taken from the spleen of mice fed either OVA or PBS did not alter cytokine or Ab responses. Taken together, $CD11c^+$ DCs in the liver, not in the spleen, of mice administered OVA orally were sufficient to transfer Th2 response to OVA in the recipient mice.

A mechanism of Th2 differentiation by liver DCs

As we knew that liver $CD11c^+$ cells loaded an Ag in vivo can induce Th2 differentiation of naïve $CD4^+$ T cells by Fas-mediated apoptosis of Th1 cells,[8] we assessed whether Ag-specific killing was occurring in our model. For this purpose, hepatic $CD11c^+$ cells isolated from mice fed PBS, BSA, or OVA were cultured with naïve OVA-specific $CD4^+$ T cells from Rag_2−/−DO11.10 mice. We saw an increase of Annexin V^+ OVA-specific $CD4^+$ T cells in the culture with DCs of OVA-fed mice, which was associated with a decrease of IFN-γ and an increase of IL4 production (fig 3A, B). In contrast, the liver DCs of BSA-fed mice did not alter the percentage of Annexin V^+ cells or cytokine production. Thus, direction of Th2 differentiation by

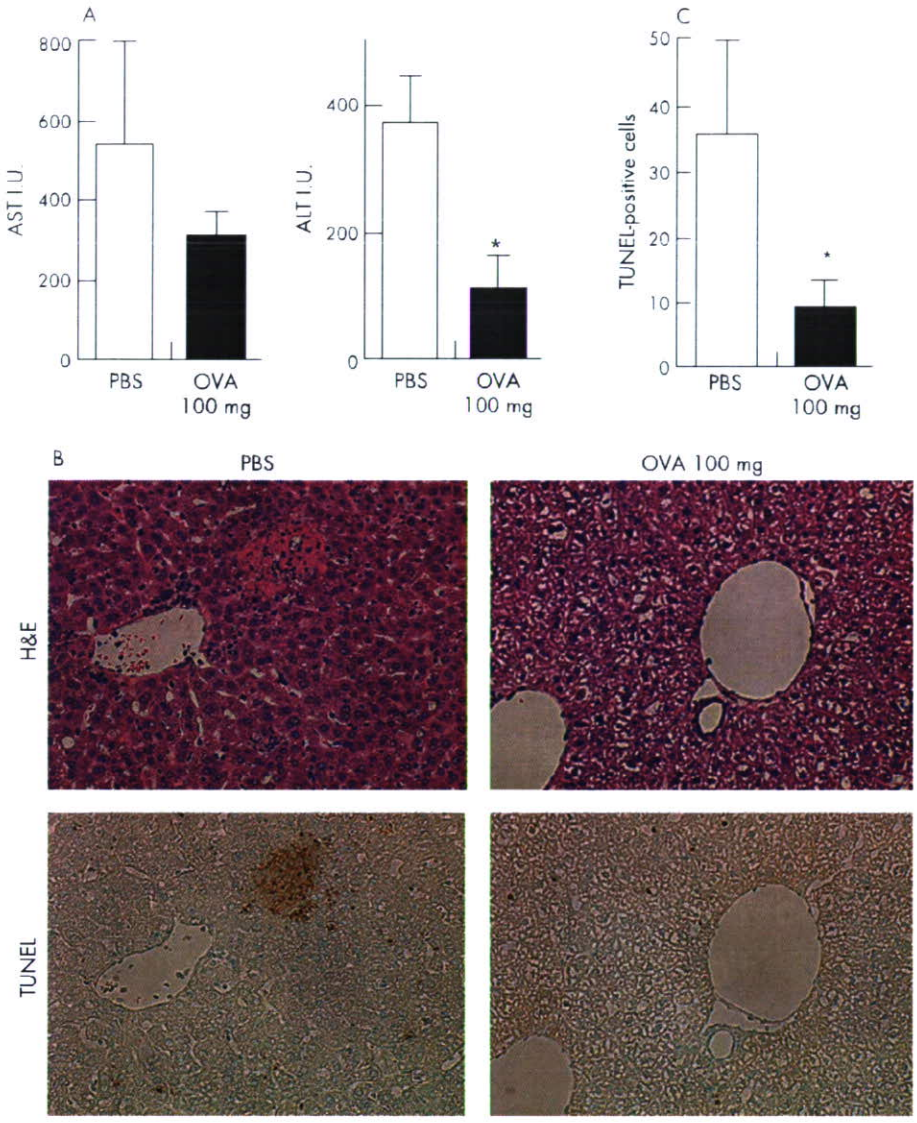

Figure 4 Inhibition of development of OVA-specific hepatitis by adoptive transfer of hepatic CD11c$^+$ cells. Donor BALB/c mice were fed 100mg of OVA or PBS every other day for a total of five times and killed 3 days after the fifth feeding. Hepatic CD11c$^+$ cells of donor mice were transferred into the recipient BALB/c mice via intravenous route (5×10^5/mouse). The day after the CD11c$^+$ cell transfer, recipient mice were treated with intravenous injection of 0.3ml of 50mg/ml OVA-containing liposome and in vitro activated OVA-specific CD4$^+$ T cells (2.5×10^6/mouse). Mice were killed 16 hours after the induction of hepatitis. (A) Serum levels of AST and ALT. (B) Liver sections staining with H&E and TUNEL in the mice transferred with hepatic CD11c$^+$ cells of PBS or OVA-fed mice. (C) The number of TUNEL$^+$ hepatocytes. TUNEL$^+$ cells are counted in high power fields. *$p<0.05$, versus PBS control. The results are expressed as mean SD. The results shown are representative one of two independent experiments (n = 4 in each group).

liver DCs involves Ag-presentation and apoptosis of Ag-specific CD4 T cells.

To assess how the above phenomenon relates to the property of DCs, which are deficient in IL12 secretion and cause apoptosis,[8] we neutralised and restored IL12 signalling by anti-IL12p40 mAb and recombinant IL12, respectively. The percentage of Annexin V$^+$ OVA-specific CD4$^+$ T cells and IL4 production were markedly increased when anti-IL12 mAb was added to the culture containing liver DCs of PBS-fed mice (fig 3C, D). In contrast, the percentage of apoptotic cells and IL4 production were decreased when IL12 was added to the culture containing liver DCs of OVA-fed mice. Thus, blockade of IL12 signalling in liver DCs of PBS-fed mice had Th2-inducing function similar to that of DCs of OVA-fed mice. Conversely, restoration of IL12 signalling in liver DCs of OVA-fed mice abrogated Th2 induction. Taken together, these data suggest that deficiency in IL12 secretion by liver DCs of OVA-fed mice is responsible for Th2 differentiation and apoptosis of naïve OVA-specific CD4$^+$ T cells.

Inhibition of hepatitis by adoptive transfer of liver DCs

In our final series of experiments, we assessed the in vivo function of liver DCs in the OVA-specific hepatitis model which utilises intravenous injection of OVA-liposome and adoptive transfer of pre-activated OVA-specific CD4$^+$ T cells.[14] In the mice that received hepatic CD11c$^+$ cells of control mice, elevations of serum AST and ALT levels were seen (fig 4A). Compatible with this, H&E and TUNEL staining revealed mononuclear cell infiltration in the periportal region and focal necrosis of hepatocytes in the liver of these mice (fig 4B, C). In contrast, elevation of serum levels of transaminases or necrosis of hepatocytes was not seen in the mice which received cells obtained from OVA-fed mice (fig 4A, C). Thus, adoptive transfer of liver DCs from OVA-fed mice abolished the development of hepatitis in the recipient mice. The prevention of hepatitis was associated with OVA-specific Th2 responses in the liver. As shown in fig 5A, OVA-specific IL4 production by hepatic CD4$^+$ T cells was markedly increased in mice received liver CD11c$^+$ cells of OVA-fed mice. In contrast, OVA-specific proliferative response and IFN-γ production were significantly decreased in these mice. Finally, we studied the population of hepatic CD4$^+$KJ1-26$^+$ T cells that were specific to OVA and causing hepatitis. As shown in fig 5B, compared to control, the percentage of CD4$^+$KJ1-26$^+$ T cells in the liver was remarkably reduced in the mice that received hepatic CD11c$^+$ cells of OVA-fed mice. The total number of IHLs was significantly reduced in

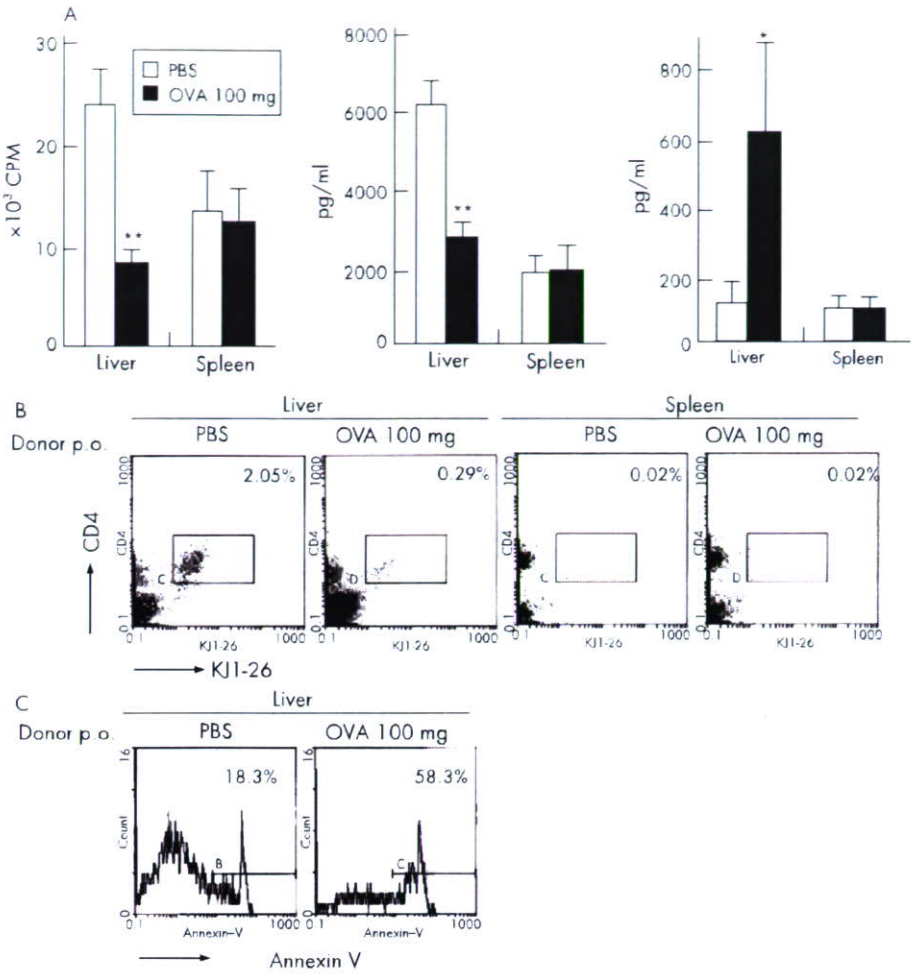

Figure 5 Cytokine production and apoptosis of OVA-specific $CD4^+$ T cells in the liver. OVA-specific hepatic injury was induced in BALB/c recipient mice transferred with hepatic $CD11c^+$ cells as described in fig 4. (A) Proliferative responses (left), IFN-γ secretion (midde), and IL4 secretion (right) by hepatic $CD4^+$ T cells were measured 16 hours after the induction of hepatitis. Hepatic or splenic $CD4^+$ T cells (1×10^5/well) were stimulated with OVA-peptide (1 μg/ml) presented by irradiated splenocytes (5×10^5/well) from BALB/c mice. (B, C) Flow cytometric analysis of IHLs or splenocytes in the mice transferred with hepatic $CD11c^+$ cells from mice fed PBS or OVA followed by the induction of OVA-specific hepatitis. IHLs or splenocytes were stained with KJ1-26 FITC mAb, anti-CD4 PE mAb, and biotinylated Annexin V followed by streptavidin-RED670. An analysis gate was set on $CD4^+KJ1\text{-}26^+$ cells for Annexin V binding assay. The number in each panel shows the percentage of $CD4^+KJ1\text{-}26^+$ T cells (B) or Annexin V^+ cells in $CD4^+KJ1\text{-}26^+$ T cells (C). *$p<0.05$, **$p<0.01$ versus PBS control. The results are expressed as meanSD. The results shown are representative one of two independent experiments (n = 3 in each group).

those mice (PBS versus OVA, 3.81 (SD 8) $\times10^6$/mouse versus 1.90 (SD 6)$\times10^6$), suggesting that adoptive transfer of hepatic $CD11c^+$ cells from OVA-fed mice inhibited clonal expansion of OVA-specific $CD4^+$ T cells. Furthermore, this inhibition was caused by apoptosis as the percentage of Annexin V^+ cells in $CD4^+KJ1\text{-}26^+$ T cells was significantly increased in the liver of mice received hepatic $CD11c^+$ cells of OVA-fed mice (fig 5C). Taken together, these data suggest that adoptive transfer of liver DCs loaded of OVA in vivo deleted Th1 cells and suppressed hepatitis in recipient mice.

DISCUSSION

Utilising an experimental hepatitis model, we show here that the dual blood flow system, systemic and portal, serves to maintain immunological homeostasis in the liver. This was indicated by the fact that an inflammatory response elicited by a systemic OVA challenge is counter-regulated by another response to OVA migrating via the portal blood flow. This counter-regulation was achieved by the function of periportal and sinusoidal $CD11c^+$ cells.

Three populations of APCs are known to reside in the liver; Kupffer cells, DCs, and LSECs. Depending on the type of APCs, immune responses elicited are different in their spectra of inflammation in the liver. One such example is the case with DCs derived from GM-CSF stimulated progenitors in the liver. The DCs, upon injection to allogenic recipients, activate T cells secreting anti-inflammatory cytokines such as IL4 and IL10.[10] Another report shows that liver-derived $DEC205^+B220^+CD19^-$ DCs induce apoptosis of activated T cells.[20] Thus, induction of Th2 responses, rather than Th1, and apoptosis of activated T cells seem to be shared properties of some hepatic DCs. This notion fits well to our previous finding in that $CD11c^+$class II^+ cells in the periportal and sinusoidal areas take a soluble Ag (OVA) administered orally and present the Ag to Ag-specific $CD4^+$ T cells, which finally generates regulatory Th2 cells expressing Fas ligand.[8 16] Thus, some liver DCs, capturing dietary Ags and being activated by materials contained in portal blood flow, generates tolerogenic Th2 cells. The similar property was shared by LSECs which can induce Th2 responses in vitro.[9] However, LSECs have an additional function and induce tolerogenic responses by $CD8^+$ T cells.[21 22] In contrast to these tolerogenic APCs, Kupffer cells present Ags and lead to development of Th1 type inflammatory response in the liver. In fact, Kupffer cells are known to capture OVA-liposome and elicit hepatitis by presenting OVA to $CD4^+$ T cells in our model.[14]

As to a mechanism how some liver DCs are involved in the generation of Th2 cells, our knowledge has been very limited. We previously showed that DCs deficient in IL12 secretion play an important role.[8] It was also seen in this study that neutralisation of IL12 signalling enhanced apoptosis and IL4 production by the naïve $CD4^+$ T cells that were presented an Ag by hepatic $CD11c^+$ cells of PBS-fed mice. By contrast, restoration of IL12 signalling abolished apoptosis and IL4 production when an Ag was presented by hepatic $CD11c^+$ cells of OVA-fed mice (fig 3). Furthermore, we showed that Fas–Fas ligand interaction between Ag-activated $CD4^+$ T cells deletes Th1 cells and results in survival of Th2 cells which are less

dependent on IL12 and more resistant to Fas-mediated apoptosis than Th1 cells.[8] Therefore, $CD11c^+$ cells deficient in IL12 secretion enhance not only Th2 differentiation but also apoptosis of Th1 cells. This property of DCs explains the results that transfer of hepatic DCs of OVA-fed mice rendered recipients resistant to hepatitis and gave rise to Th2 responses and apoptosis of $CD4^+$ T cells in the liver. Thus, in the absence of these periportal and sinusoidal DCs, OVA presentation by Kupffer cells alone would have resulted in clonal expansion of OVA-specific Th1 cells and caused hepatitis.[14]

Having stated above conclusion, we consider the pathogenic roles DCs might play in some disease conditions. For instance, some functions of DCs are impaired in the patients with AIH or PBC.[23] DCs from patients with PBC present pyruvate dehydrogenase complex and cause proliferation of patients' T cells.[24] Considering that Th1 cytokines are associated with development of hepatitis, one would ask the role of IL12 produced by liver DCs in the pathogenesis of above diseases. In fact, Kanto *et al* reported increase of IL12 secretion by DCs of the patient with HCV-hepatitis when pulsed with HCV core-Ag.[25] However, we should take note that all studies cited above utilised DCs and T cells in peripheral blood, so that immune responses seen in vitro do not necessarily reflect those occurring in the liver. Holding above reservation and assuming that pathogenic DCs invariably produce IL12, the function of DCs described here and in our previous studies[8] could suggest a distinct subset of liver DCs. As shown in this study, the DCs transfer the resistance to hepatitis caused by Th1 cells. Thus, the periportal and sinusoidal DCs, which take Ags in the portal vein, might physiologically tilt the immune response to Th2 to balance excessive Th1 responses elicited by systemic Ags in the liver.

Finally we consider another aspect of the role these liver DCs might play. We showed that adoptive transfer of hepatic DCs of OVA-fed mice leads to an increase of IgE Abs in the sera of recipient mice after systemic OVA challenge. This could imply a role liver DCs might play in food allergy. As most of the food allergy cases associate with an increase of IgE responses to Ags digested,[26] food Ag-specific Th2 cells are essential for the pathogenesis. However, the generation of those Th2 cells has not been known well. Our result here suggests that the liver is the site wherein those Th2 cells can develop. In fact, some animal studies suggest that the liver is the organ where the induction of systemic IgE response occurs,[27 28] In human studies, a case report described that peanut allergy was transferred to the recipient of the liver transplant, not to the recipient of the kidney and pancreas of the same donor.[29] Compatible to these, we previously reported that helper T cells for IgE response to a dietary Ag develop in the liver, but not in Peyer's patches or the spleen.[18] We show here that periportal and sinusoidal DCs loaded with OVA in vivo are sufficient to transfer systemic Th2 response to OVA in recipient mice. Taken together, we argue that dietary Ags can develop liver DCs which direct naïve Ag-specific $CD4^+$ T cells to Th2. We speculate that if frequency of the Ag-specific $CD4^+$ T cells is high and the amount of IL4 accumulated is large enough, then IgE produced as a result would reach to a pathogenic level. In a physiological context, the presence of DCs, which can generate Th2 response to portal Ags and prevent liver injury caused by Th1 cells specific to systemic Ags, might indicate a counter-regulatory mechanism in the liver. In a theoretical extension, this might suggest that Th1-mediated liver disease can be treated by targeting pathogenic Ags to portal blood flow, with careful control of allergic reactions.

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