Periportal and sinusoidal liver dendritic cells suppressing T helper type 1-mediated 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, 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. Second, surgical diversion of portal blood away from the liver abrogates oral tolerance. Third, pancreatic islet cells transplanted via the portal vein evades rejection by the host and cures insulin-dependent diabetes. 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. For example, Ag-presentation by LSECs and DCs preferentially leads to development of Th2 cells producing anti-inflammatory cytokines. 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. This was corroborated by the studies of experimental hepatitis and cholangitis, which has revealed a pathogenic role played by Th1 cytokines.

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. We reported that Ag-capturing CD11c+ cells in the liver are defective in IL12 secretion and responsible for the generation of Th2 cells.

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 IAα were crossed to Rag2−/− mice. BALB/c and Rag2−/−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-IAα (Pharmingen), and anti-F4/80 Ab (Serotec Ltd, Oxford, England) as described previously. 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. 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

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described previously. Hepatic low density non-parenchymal cells were also obtained as described previously.

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

Spleenic and hepatic CD11c⁺ cells were purified by positive selection using autoMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously. The recovered cells were more than 90% CD11c⁺ (confirmed by flow cytometric analysis). Six-week old BALB/c mice were injected intravenously with 5 x 10⁵ 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.
Induction of Ag-specific hepatic injury

Six-week old BALB/c mice were injected intravenously with 5 × 10⁶ 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. Briefly, splenocytes from BALB/c mice that received CD11c+ T cells were purified by positive selection using autoMACS (>90% KJ1-26+ cells) followed by the transfer of activated OVA-specific CD4+ T cells 2 hours later (2.5 × 10⁶/mouse). Mice were killed 16 hours later and then serum, IHLs, and splenocytes were prepared. The liver injury was assessed by serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities by a commercial kit (Wako, Osaka, Japan). Surface immunofluorescence localisation of DCs in the liver by staining class II and CD11c+ T cells in the periportal area, as our previous study indicated that a part of OVA administered orally is carried to the liver and co-localise with class II antigens (fig 1A). Class II+ cells in the periportal area, as our previous study indicated that a part of OVA administered orally is carried to the liver and co-localise with class II antigens (fig 1A). Class II+ cells in the periportal area, 8 we analysed tissue

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 Gladbach, Germany). These CD4+ T cells (1 × 10⁶/well) were stimulated in vitro with OVA (0.1 mg/ml) or OVA-peptide (1 μg/ml) presented by irradiated splenocytes (5 × 10⁵/well) from BALB/c mice. The cultures were incubated for 72 hours and then 1 μCi of [3H] thymidine was added for the final 16 hours. Proliferation and cytokine production were evaluated as described previously. In some experiments, splenic OVA-specific CD4+ T cells (1 × 10⁵/well) were stimulated with OVA-peptide (1 μg/ml) presented by hepatic CD11c+ cells (1 × 10⁵/well) in the presence of mouse IL12 (10 ng/ml, Peprotec), rat IgG (10 μg/ml, Pharmingen), or neutralising anti-IL12p40 mAb (10 μg/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, 1A4, 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.

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 (IAd)+ cells in the periportal area, 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
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 IgG₁/ IgG₂a 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⁺ KJ1-26⁺ T cells which were immunised subcutaneously with OVA/CFA after the transfer. As shown in fig 3A, 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.

Figure 3  IL12 deficiency in liver DCs is responsible for Th2 responses and apoptosis. Naïve OVA-specific CD4⁺ T cells (1 × 10⁵/well) isolated from the spleen of Rag₂⁻/⁻DO11.10 mice were stimulated with OVA-peptide (1 μg/ml) in the presence of hepatic CD11c⁺ cells (1 × 10⁵/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⁺KJ1-26⁺ T cells was determined. Cells were stained with FITC-conjugated Annexin V and PE-conjugated KJ1-26 mAb. Analysis gate was set on KJ1-26⁺ 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 (10 μg/ml), control Ab (10 μg/ml), or mouse IL12 (10 ng/ml) were added to the culture. The percentage of Annexin V⁺ cells in OVA-specific CD4⁺KJ1-26⁺ 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).
Liver dendritic cells involving 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, 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. 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 these 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
and IL10. Another report shows that liver-derived cells secreting anti-inflammatory cytokines such as IL4. The DCs, upon injection to allogenic recipients, activate T cells. FCs derived from GM-CSF stimulated progenitors in the liver. Inflammation in the liver. One such example is the case with immune responses elicited are different in their spectra of Kupffer cells, DCs, and LSECs. Depending on the type of APCs, 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 the systemic OVA challenge is counter-regulated by another induction of OVA-specific hepatitis. LSECs 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\(^+\)KJ1-26\(^+\) cells for Annexin V binding assay. The number in each panel shows the percentage of CD4\(^+\)KJ1-26\(^+\) T cells (B) or Annexin V cells in CD4\(^+\)KJ1-26\(^+\) T cells (C). \(p<0.05, \quad **p<0.01\) versus PBS control. The results are expressed as mean\(\pm\)SD. The results shown are representative one of two independent experiments (n = 3 in each group).

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. Another report shows that liver-derived DEC205\(^+\)B220\(^+\)CD19\(^+\)DCs induce apoptosis of activated T cells. 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 periporal 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. 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 apoptosis of activated T cells and results in survival of Th2 cells which are less sensitive to CD12 signalling.

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. It was also seen in this study that neutralisation of IL12 signalling enhanced apoptosis and IL4 production by the naive 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. 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. 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 recipient resistant to hepatitis and gave rise to Th2 responses and apoptosis of CD4+ T cells in the liver. Thus, in the absence of these perportal and sinusoidal DCs, OVA presentation by Kupffer cells alone would have resulted in clonal expansion of OVA-specific Th1 cells and caused hepatitis.

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.23,24 DCs from patients with PBC present pyruvate dehydrogenase complex and cause proliferation of patients’ T cells.25,26 Considering that Th1 cytokines are associated with development of hepatitis, one would ask the role of IL12 produced by 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 vivo 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 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 perportal 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,27 food Ag-specific Th2 cells are essential for the pathogenesis. However, the generation of those Th2 cells has not been well known. 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.28,29 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.30 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.31 We show here that perportal 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 naive 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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