

Helper CD4⁺ T cells for IgE response to a dietary antigen develop in the liver

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Background: Although T-cell responses to food antigens are normally inhibited either by deletion, active suppression, or both of antigen-specific T cells, T helper cells for IgE response to a food antigen still develop by unknown mechanisms in a genetically susceptible host.

Objective: We determined the site at which those IgE helper T cells develop.

Methods: We administered ovalbumin (OVA) orally to DO11.10 mice and studied CD4⁺ T cells in Peyer's patches, the spleen, and the liver. Helper activity for IgE response was assessed by adoptively transferring those CD4⁺ T cells to naive BALB/c mice, followed by systemic immunization with OVA.

Results: OVA-specific CD4⁺ T cells were deleted by cell death in the liver and Peyer's patches of DO11.10 mice fed OVA. OVA-specific CD4⁺ T cells that survived apoptosis in the liver expressed Fas ligand and secreted IL-4, IL-10, and transforming growth factor β_1 . CD4⁺ T cells producing IFN- γ were deleted in the liver by repeated feeding of OVA. On transfer of CD4⁺ T cells to naive mice and systemic immunization with OVA, a marked increase in OVA-specific IgE response developed only in the mice that received hepatic CD4⁺ T cells from OVA-fed mice, the effect of which was not observed in the recipients of hepatic CD4⁺ T cells deficient in IL-4. In addition, significant suppression of delayed-type hypersensitivity and IgG₁/IgG_{2a} responses to OVA was observed in the recipients of hepatic CD4⁺ T cells, and this suppression required Fas/Fas ligand interaction.

Conclusion: Together, these results suggested that a food antigen might negatively select helper T cells for IgE response to the antigen by preferential deletion of T_H1 cells in the liver. (*J Allergy Clin Immunol* 2003;111:1375-85.)

Key words: Food allergy, IgE, IL-4, liver, apoptosis

Normally, T cell-mediated immune responses to dietary antigens are suppressed either by means of clonal deletion of antigen-specific CD4⁺ T cells through apopto-

Abbreviations used

DTH:	Delayed-type hypersensitivity
FasL:	Fas ligand
IHL:	Intrahepatic lymphocyte
OVA:	Ovalbumin
PI:	Propidium iodide
PP:	Peyer's patches
TCR:	T-cell receptor
TGF:	Transforming growth factor

sis, by cytokine-mediated active suppression, or both.^{1,2} These suppression mechanisms against dietary and microbial antigens might contribute to maintenance of the immunologic homeostasis of the gut to luminal antigens.³ In spite of these suppression mechanisms, some individuals still have IgE responses to a food antigen, which might lead to food allergy with manifestation in various organs, including the skin, lung, gut, and vascular vessels.⁴ Because IgE response requires IL-4-secreting T_H2 cells,⁵ the patients with food allergy should have those T cells that have escaped suppression mechanisms operating in the gut. In spite of several animal models for the study of pathogenesis of food allergy,⁶ it is still largely unknown where these T_H2 cells develop and escape from tolerogenic mechanisms in the gut.

Recent reports by us⁷ and others⁸ indicated that T-cell activation by an antigen migrating through the portal vein occurs in the liver. Although either T_H1 or T_H2 cells can develop in the liver depending on the type and dose of antigens used,^{7,9-11} some of the resident dendritic cells and sinusoidal endothelial cells direct naive CD4⁺ T cells preferentially to T_H2 differentiation.^{12,13} A recent clinical report indicated an association of allergic skin symptoms derived from food antigens and increased levels of serum transaminases.¹⁴ These observations altogether suggest that sensitization by food antigens can occur in the liver and lead us to investigate a possibility that the liver might be the site at which antigen-specific CD4⁺ T cells escape from suppression mechanism and differentiate into T_H2 cells. To test this hypothesis, we used DO11.10 mice that have CD4⁺ T cells bearing transgenic T-cell receptor (TCR) reacting to ovalbumin (OVA), fed them with OVA, and studied the function, as well as the population, of OVA-specific CD4⁺ T cells. Here we show the evidence that the liver is one of the sites at which T_H2 cells specific to a food antigen develop.

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METHODS

Animals and protocol for immunization

BALB/c mice were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). DO11.10 mice with T cells bearing the transgenic TCR that recognizes the 323-339 peptide fragment of OVA in the context of IA^d were kindly provided by K. M. Murphy (Department of Pathology and Immunology, Washington University School of Medicine, St Louis, Mo).¹⁵ *IL4* gene knockout mice (*IL-4*^{-/-} mice) were obtained from the Jackson Laboratory (Bar Harbor, Me). All these mice had the BALB/c background. DO11.10 mice were crossed with *IL-4*^{-/-} mice, and those with homozygous mutation in *IL4* genes (*IL-4*^{-/-} DO11.10 mice) were used. These mice were housed under specific pathogen-free conditions in the Animal Facility of Kyoto University. Male DO11.10 mice or *IL-4*^{-/-} DO11.10 mice were fed 100 mg of OVA (Sigma Chemical Co, St. Louis, Mo), 1 mg of OVA, or PBS alone every other day for a total of 5 times by intragastric intubation. In some experiments DO11.10 mice were immunized subcutaneously with 100 µg of OVA in CFA (Life Technologies, Grand Island, NY). 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.

Preparation of mononuclear cells from the spleen, Peyer patches, and the liver

Lymphocytes from the spleen, lymph nodes, and Peyer's patches (PP) were prepared as previously described.^{16,17} Intrahepatic lymphocytes (IHLs) were prepared according to the method described previously, with some modifications.¹⁸ The portal vein was cannulated with a 27-gauge needle and perfused with 5 mL of PBS, with the inferior vena cava being cut. The gall bladder was identified and then removed. The liver was mashed with a stainless mesh and a syringe plunger, and then the cells were suspended in RPMI containing 5% FCS. After washing once, the cells were resuspended in 10 mL of digestion buffer consisting of serum-free RPMI containing 0.05% collagenase and 0.002% DNase I (Nacalai tesque, Kyoto, Japan), and then they were incubated at 37°C for 40 minutes. Thirty milliliters of serum-free RPMI was added, and the cell suspension was spun at 300 rpm for 3 minutes. This made a sediment of the majority of hepatocytes but left IHLs in the supernatant. The supernatant was spun again at 1500 rpm for 10 minutes, and the pellet was resuspended in 5 mL of serum-free RPMI. Five milliliters of 24% metrizamide (Sigma) in PBS was laid beneath the cell suspension, followed by centrifugation at 2000 rpm for 20 minutes. IHLs identified at the interface were carefully aspirated and then washed with RPMI/5% FCS and spun at 1500 rpm for 10 minutes.

Antigen-specific cytokine production by CD4⁺ T cells

CD4⁺ T cells were prepared from the liver, spleen, draining lymph nodes, and PP by positive selection with MACS immunobeads (Miltenyi Biotec, Bergisch Gladbach, Germany). In the liver, spleen, lymph nodes, and PP, double-positive (CD4⁺CD8⁺) T cells were less than 1% (confirmed by means of flow cytometric analysis). Purified CD4⁺ T cells (5×10^4 /well) were cultured for 3 days with 1 µg/mL OVA 323-339 peptide in the presence of irradiated splenic antigen-presenting cells (2.5×10^5 /well) from BALB/c mice in U-bottom 96-well culture plates (Sumitomo Bakelite, Tokyo, Japan). The cultures were incubated for 72 hours, and 1 µCi of tritiated thymidine was added for the final 16 hours. Cells were harvested by using a multiple cell harvester, and incorporated thymidine was determined with a Topcount microplate scintillation counter (Packard, Meriden, Conn). For cytokine assays, the culture super-

natants were collected at 48 hours for IFN-γ and IL-2 and at 72 hours for IL-4, IL-10, and transforming growth factor (TGF) β₁. Proliferation and cytokine production were evaluated in triplicate assay.

Cytokine analysis

The concentrations of IL-2 and IL-4 were determined by using the IL-2-dependent CTLL-2 and IL-4-dependent CT.4S cell lines, respectively.^{19,20} Standards for these assays consisted of human recombinant IL-2 (Takeda Pharmaceutical Co, Osaka, Japan) and murine recombinant IL-4 (PeproTech, London, England), respectively. The concentrations of IL-10 and IFN-γ were measured by means of sandwich ELISA, as described elsewhere.²¹ Paired antibodies (capture and secondary biotin-conjugated antibodies), and recombinant cytokines were purchased from Pharmingen (San Diego, Calif) and Peprotech, respectively. Color was developed by adding a substrate solution (Sigma 104, Sigma) after incubating with alkaline phosphatase-labeled anti-biotin antibody (Zymed, South San Francisco, Calif). The optical density was measured at 405 nm with a microplate reader (SOFT max; Molecular Devices, Sunnyvale, Calif). TGF-β₁ was measured with an ELISA kit (Promega, Madison, Wis).

Adoptive transfer of CD4⁺ T cells to naive BALB/c mice and antigenic challenge

Six-week old naive male BALB/c mice were injected intraperitoneally with 1×10^6 CD4⁺ T cells purified from PP, the spleen, and the liver of DO11.10 mice orally immunized with 100 mg of OVA, 1 mg of OVA, or PBS for a total of 5 times and killed 3 days after the fifth feeding. The day after the transfer, the recipient mice were immunized subcutaneously with 100 µg of OVA in CFA, followed by booster immunization with 100 µg of OVA in incomplete Freund adjuvant subcutaneously 1 week later. Delayed-type hypersensitivity (DTH) and OVA-specific antibody responses were measured 7 days after the booster immunization. OVA-specific IgG, IgG₁, and IgG_{2a} antibody levels were measured by means of ELISA, as described elsewhere.²² OVA-specific IgE antibody was measured as previously described.²³ DTH responses were performed as previously described.²⁴ Footpad swelling was measured 48 hours later with a dial-thickness gauge (Ozaki MFG, Tokyo, Japan), and DTH responses were expressed as specific footpad increments.

Administration of anti-mouse Fas ligand mAb to recipient BALB/c mice

BALB/c mice that received hepatic CD4⁺ T cells from DO11.10 mice fed 100 mg of OVA were injected intraperitoneally with 0.5 mg of anti-mouse Fas ligand (FasL) mAb (MFL4; kindly provided by Dr Hideo Yagita, Department of Immunology, Juntendo University School of Medicine)²⁵ or control hamster IgG (ICN pharmaceuticals, Inc, Aurora, Ohio) every 3 days from the first day of subcutaneous immunization. In some experiments, *IL-4*^{-/-} DO11.10 mice fed 100 mg of OVA were also used as donor mice. These animals were immunized subcutaneously with OVA, and then DTH and anti-OVA antibody responses were evaluated as described above.

Monoclonal antibodies and flow cytometry

Anti-CD4 mAb, anti-Fas mAb, and anti-FasL mAb were purchased from Pharmingen. KJ1-26 recognizing transgenic TCR specific to OVA was purchased from Caltag (South San Francisco, Calif). These antibodies were directly coupled to FITC, phycoerythrin, or biotin, in which case cells were stained with streptavidin Red670 (Life Technologies). Surface immunofluorescence was assessed as described previously²⁶ with a flow cytometer (Epics XL; Coulter Electronics, Miami, Fla). Propidium iodide (PI; 25 µg/mL, Sigma) was added at 10 µL per 1×10^6 cells just before analysis to exclude dead cells.

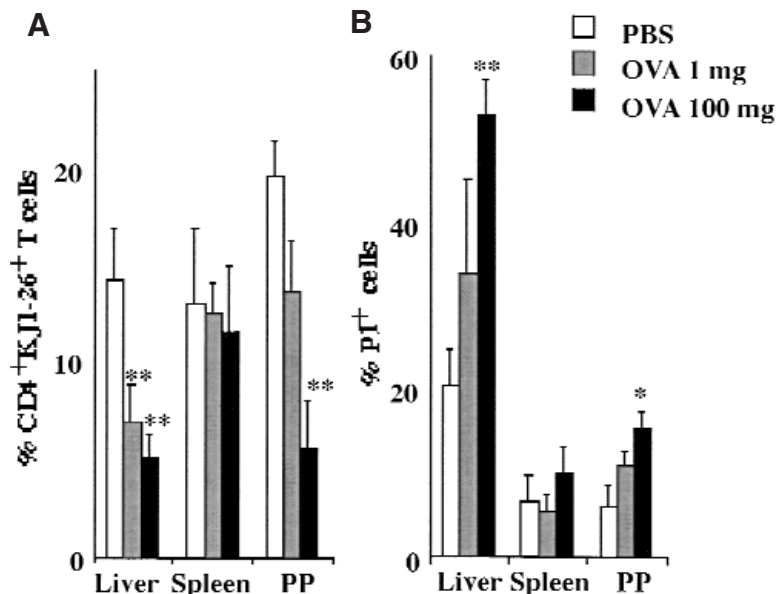


FIG 1. Percentages of OVA-specific (KJ1-26⁺) CD4⁺ T cells (**A**) and PI⁺ cells in CD4⁺KJ1-26⁺ T cells (**B**) in PP, the spleen, and the liver of DO11.10 mice fed OVA. DO11.10 mice were fed 100 mg of OVA, 1 mg of OVA, or PBS every other day for a total of 5 times. Mice were killed 3 days after the fifth feeding, and then mononuclear cells were prepared from the liver, the spleen, and PP. Cells were stained with phycoerythrin-labeled anti-CD4 mAb, FITC-labeled KJ1-26 mAb, and PI. Results were expressed as means $\pm$ SD and are representative of 3 independent experiments. * $P < .05$ and ** $P < .01$ compared with PBS control mice in each organ.

Statistical analysis

The Student *t* test was used to evaluate the significance of the differences. Statistical analysis was performed with the Stat View version 4.5 program (Abacus Concepts, Berkeley, Calif). A *P* value of less than .05 was regarded as statistically significant.

RESULTS

Deletion of T cells specific to a dietary antigen occurs in the liver and PP

To avoid untoward reaction to food antigens, T cells with TCR reactive to those antigens should be deleted or actively suppressed in the lymphoid tissue, where the presentation of those antigens occurs. To test this hypothesis, we administered OVA of 100 mg, 1 mg, or PBS as a control to the DO11.10 mice that have CD4⁺ T cells bearing TCR specific to OVA and then assessed the population and function of OVA-specific (KJ1-26⁺) CD4⁺ T cells in PP, the spleen, and the liver. As shown in Fig 1, A, oral administration of OVA decreased the percentage of CD4⁺KJ1-26⁺ T cells both in PP and in the liver. Oral administration of OVA did not change the percentage of splenic CD4⁺KJ1-26⁺ T cells. The decrease in the percentage of hepatic OVA-specific CD4⁺ T cells was due to cell death because OVA feeding increased the percentage of PI⁺ cells in hepatic KJ1-26⁺ T cells (Fig 1, B). We next examined Fas and FasL expression on OVA-specific T cells because Fas-mediated apoptosis plays an important role in clonal deletion of T cells. As shown in Fig 2, Fas⁺FasL^{low}KJ1-26⁺ T cells were observed only in the livers of control mice fed

PBS. Both low-dose and high-dose feeding of OVA generated Fas⁺FasL^{high}KJ1-26⁺ T cells only in the liver. The expression level of FasL was much stronger in the livers of mice fed 100 mg of OVA than in the livers of the mice fed 1 mg of OVA. Because there were marked differences in the expression level of Fas and FasL between PP and the liver, we studied the time course of deletion in PP and the liver. As shown in Fig 3, the percentage of hepatic CD4⁺KJ1-26⁺ T cells was gradually decreased during the period from the first through the fifth feeding, which was in parallel to a gradual increase of the percentage of PI⁺ cells in hepatic KJ1-26⁺ T cells. In contrast, the percentage of CD4⁺KJ1-26⁺ T cells in PP was rapidly decreased after the first feeding, and the percentage stayed at the low level thereafter, which was associated with a rapid increase of the percentage of PI⁺ cells in PP KJ1-26⁺ T cells soon after the first feeding. No significant difference was observed in the total number of IHLs or PP cells at any time point investigated (IHLs $\times 10^6$ per mouse: before feeding, 1.6 ± 0.6 ; after the first feeding, 1.8 ± 0.7 , after the third feeding, 1.4 ± 0.4 , after the fifth feeding 1.3 ± 0.5 ; PP $\times 10^6$ per mouse: before feeding, 3.3 ± 0.6 ; after the first feeding, 2.9 ± 0.7 ; after the third feeding, 3.1 ± 0.3 ; after the fifth feeding, 2.9 ± 1.0). Thus deletion of CD4⁺ T cells specific to OVA occurred in PP and the liver with the kinetics different between the 2 organs. In the liver the deletion was gradual, incomplete, and associated with emergence of Fas⁺FasL^{low}KJ1-26⁺ cells and Fas⁺FasL^{high}KJ1-26⁺ cells in the mice with OVA administered at a dose of 1 and 100 mg, respectively.

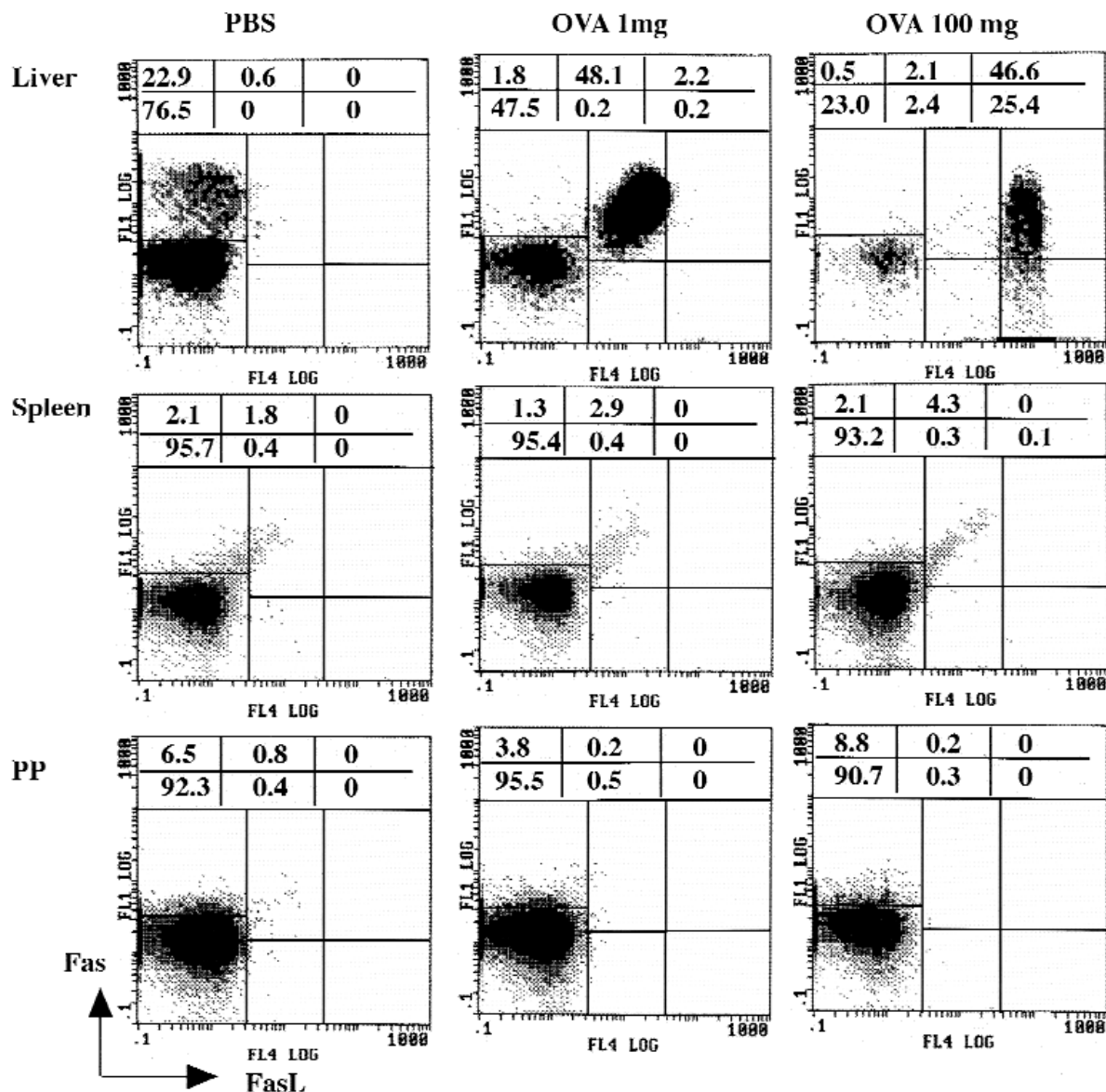


FIG 2. Fas/FasL expression in KJ1-26⁺ T cells after oral administration of OVA. Mice were orally immunized as described in Fig 1 and killed 3 days after the fifth feeding. Mononuclear cells were prepared from the liver, the spleen, and PP. Cells were stained with phycoerythrin-conjugated KJ1-26 mAb, FITC-conjugated anti-Fas mAb, and biotinylated anti-FasL mAb, followed by streptavidin-RED670. An analysis gate was set on KJ1-26⁺PI⁻ cells. The results shown are representative of 3 independent experiments.

Oral administration of OVA generates CD4⁺ T cells producing IL-4 in the liver

We next asked whether the deletion of OVA-specific CD4⁺ T cells might associate with differences in the profiles of cytokines produced by CD4⁺ T cells. As shown in Fig 4, hepatic CD4⁺ T cells, after feeding with 100 mg of OVA, secreted a large amount of IL-4 on restimulation with OVA peptide. To the contrary, IL-2 secretion was markedly suppressed, and a significant increase was seen in IL-10 and TGF- β_1 secretion by those CD4⁺ T cells. IFN- γ secretion by hepatic CD4⁺ T cells was slightly suppressed by OVA feeding. Thus clonal deletion in the

liver after feeding of OVA to DO11.10 mice led to alteration in cytokine production by OVA-specific CD4⁺ T cells, and there was an increase of T_H2-type cytokines, IL-4 in particular. This alteration in the profiles of cytokines was dependent on the route of OVA administration because there was no significant change in IL-4 or IFN- γ secretion by hepatic CD4⁺ T cells in the mice immunized with OVA/CFA subcutaneously (PBS/CFA vs OVA/CFA: IFN- γ in the liver, 14.0 ± 2.6 vs 21.8 ± 5.2 ng/mL and IFN- γ in the lymph node, 23.6 ± 1.0 vs 62.6 ± 23.7 ng/mL [$P < .010$]; IL-4 in the liver, 138 ± 52 vs 165 ± 31 pg/mL and IL-4 in the lymph node, 182 ± 22 vs 41 ± 17 pg/mL [$P < .01$]). Thus development of OVA-

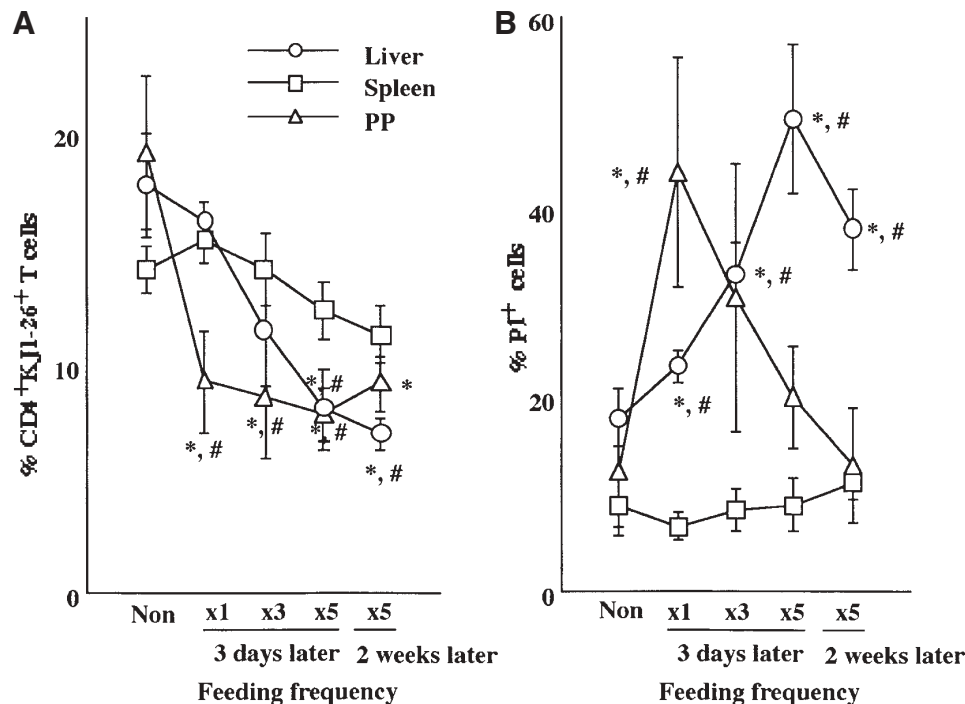


FIG 3. Time course of the changes in the percentage of CD4⁺KJ1-26⁺ T cells (A) and PI⁺ cells in CD4⁺KJ1-26⁺ T cells (B). DO11.10 mice were fed 100 mg of OVA every other day for a total of 5 times. Before feeding; 3 days after the first, third, and fifth feeding; and 2 weeks after the fifth feeding, mice were killed. Mononuclear cells were prepared from the liver, the spleen, and PP and stained as described in Fig 1. Results were expressed as means $\pm$ SD and are representative of two independent experiments. **P* < .05 compared with nonfeeding mice in each organ; #*P* < .05 compared with the spleen.

specific T_H2 cells was not observed in the livers of mice that received systemic sensitization. We next studied the time course of the alteration in the profiles of cytokines produced by hepatic CD4⁺ T cells. As shown in Fig 5, A, proliferative responses specific to OVA by hepatic CD4⁺ T cells was markedly enhanced after the first feeding of OVA, which was followed by a significant decrease after the third and fifth feedings. As shown in Fig 5, B and C, feeding of OVA led to a significant increase in IFN- γ production by hepatic CD4⁺ T cells after the first feeding, which was followed by a rapid decrease to less than the level of control mice. To the contrary, there was a significant increase in IL-4 production by hepatic CD4⁺ T cells from the first through the fifth feeding. The time course of IFN- γ production by PP CD4⁺ T cells was similar to that of hepatic CD4⁺ T cells. However, such an increase of IL-4 production as seen in the liver, was not observed in PP. Thus, an initial surge of IFN- γ production preceded the decrease of the number of OVA-specific CD4⁺ T cells, which was associated with an increase of IL-4 production by hepatic OVA-specific CD4⁺ T cells in the mice fed OVA for a total of 5 times. Given the fact that the number of CD4⁺KJ1-26⁺ T cells decreased after repeated feeding of OVA, this increase of IL-4 indicated deletion of T_H1 cells and negative selection of T_H2 cells in the liver. Some of the DO11.10 mice fed OVA repeatedly had diarrhea and showed ruffling of body hairs, sug-

gesting that those T_H2 cells in the liver might have caused allergic reactions in the intestines.

Function of hepatic CD4⁺ T cells generated in the mice after feeding of OVA

Having obtained the results that feeding of OVA to DO11.10 mice selected CD4⁺ T cells expressing FasL and secreting IL-4 in the liver, we studied *in vivo* function of those CD4⁺ T cells by transferring them to a naive host, followed by systemic challenge with OVA. As shown in Fig 6, the mice that received hepatic CD4⁺ T cells showed a marked increase in anti-OVA IgE response, which was associated with a marked decrease in total IgG, IgG₁, IgG_{2a}, and DTH responses to OVA. In contrast, significant changes of these responses were not seen in the mice that received CD4⁺ T cells obtained from the spleen or PP. Thus, CD4⁺ T cells that developed in the liver of DO11.10 mice after OVA feeding had regulatory function *in vivo* and helper activity for systemic IgE response to OVA.

Regulatory function by hepatic CD4⁺ T cells depends on IL-4 and FasL

Because enhancement of IgE response was seen only in the recipients of hepatic CD4⁺ T cells, we examined the roles played by FasL and IL-4 in those cells. To do this, we used neutralizing antibody to FasL and DO11.10 mice

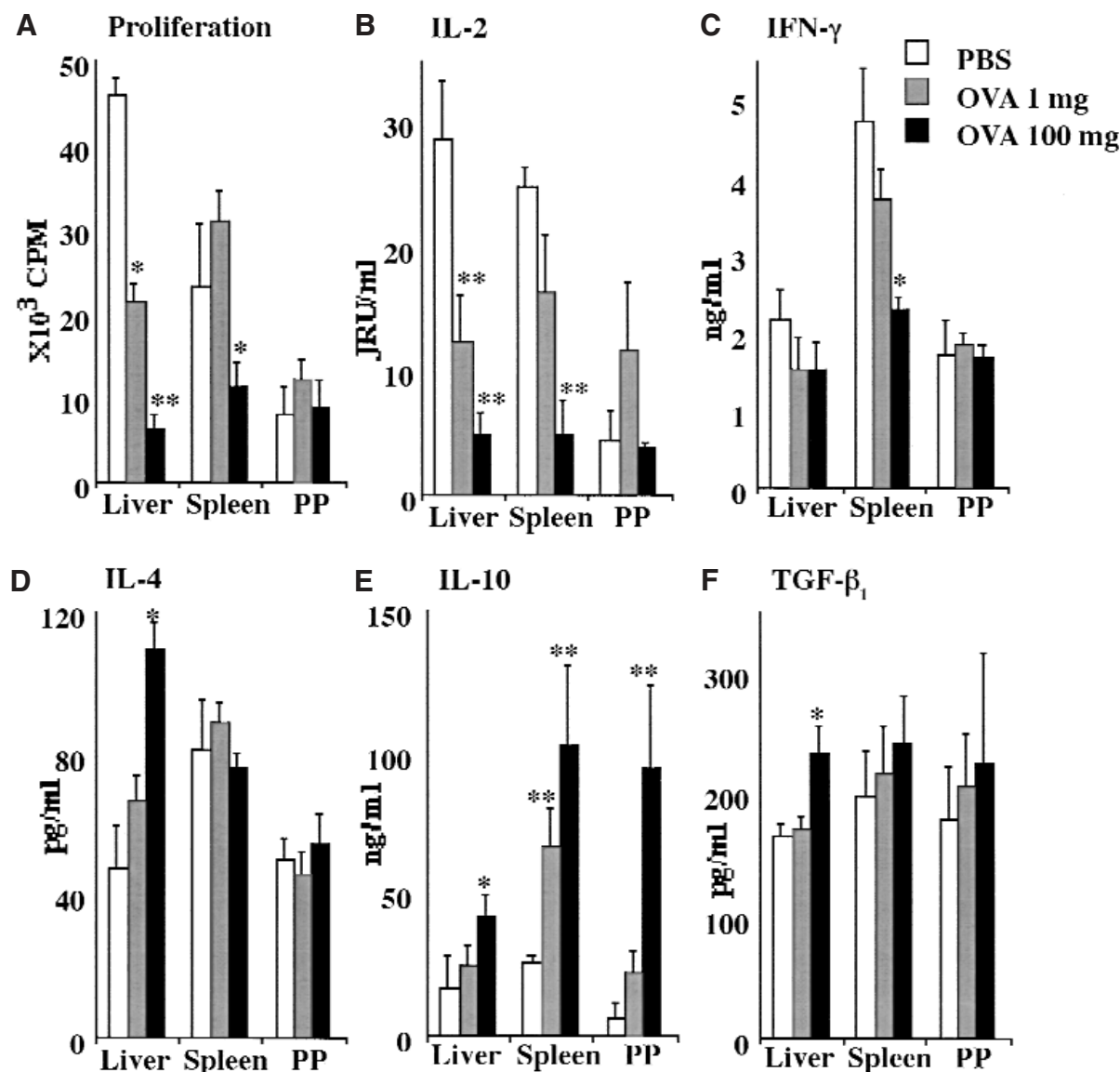


FIG 4. Antigen-specific proliferation and cytokine production by CD4⁺ T cells in DO11.10 mice fed OVA. Mice were orally immunized as described in Fig 1 and killed 3 days after the last feeding. CD4⁺ T cells (5×10^4 /well) were stimulated for 3 days in vitro with OVA peptide (1 μ g/mL) presented by irradiated syngeneic spleen cells of BALB/c mice (2.5×10^5 /well). For proliferative responses (A), 1 μ Ci of tritiated thymidine was added for the final 16 hours. Culture supernatants were collected at 48 hours for IL-2 (B) and IFN- γ (C) and at 72 hours for IL-4 (D), IL-10 (E), and TGF- β_1 (F). * $P < .05$ and ** $P < .01$ versus PBS control mice in each organ. The results shown are representative of 3 independent experiments.

deficient in IL-4. As shown in Fig 7, A and B, blockade of Fas/FasL interaction by means of anti-FasL antibody treatment in the recipient mice after the antigenic challenge restored DTH, total IgG, IgG₁, and IgG_{2a} responses specific to OVA. In contrast, these responses were still significantly suppressed in the mice transferred with hepatic CD4⁺ T cells of IL-4^{-/-} DO11.10 mice. In the recipient mice transferred with hepatic CD4⁺ T cells from OVA-fed mice, anti-FasL treatment decreased the OVA-specific IgG₁/IgG_{2a} ratio to the level of that in the mice transferred with hepatic CD4⁺ T cells from PBS-fed mice

(Fig 7, C). Enhancement of IgE response in the recipient mice was completely suppressed by transferring CD4⁺ T cells of IL-4^{-/-} DO11.10 mice, whereas the effect of anti-FasL antibody treatment on IgE response was small and not significant. Thus IL-4 and FasL play nonredundant roles in the regulatory function of hepatic CD4⁺ T cells.

DISCUSSION

In this article, we showed that helper T cells for IgE response to a food antigen develop in the liver but not in

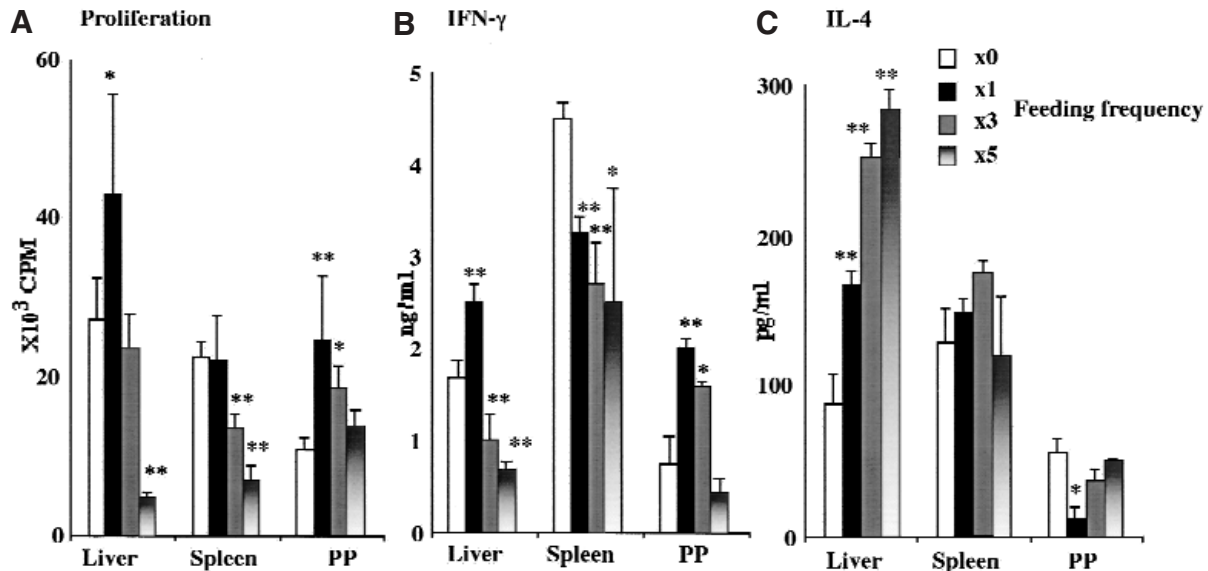


FIG 5. Time-course study of proliferative responses (A) and productions of IFN- γ (B) and IL-4 (C) by OVA-specific CD4⁺ T cells. DO11.10 mice were fed 100 mg of OVA every other day for a total of 5 times. Mice were killed before feeding and 3 days after the first, third, and fifth feeding, and then CD4⁺ T cells were prepared from the liver, the spleen, and PP. CD4⁺ T cells (1×10^5 /well) were stimulated for 3 days in vitro with OVA peptide (1 μ g/mL) presented by irradiated syngeneic spleen cells of BALB/c mice (5×10^5 /well). Proliferative responses and production of cytokines were analyzed as described in Fig 4. Results were expressed as means $\pm$ SD and are representative of 2 independent experiments. * $P < .05$ and ** $P < .01$ compared with nonfeeding control mice in each organ.

PP or spleens of the mice that have CD4⁺ T cells specific to the antigen. We also showed that on transfer to naive mice, those helper T cells developed in the liver suppress antigen-specific IgG₁, IgG_{2a}, and DTH responses by a mechanism nonredundant to that which augments IgE response to the same antigen.

Production of antigen-specific IgE can underlie the development of allergic reactions to food antigens. In spite of the development of animal models for the hypersensitivity to various food antigens,²⁷⁻²⁹ the induction site for IgE helper T cells to those antigens is largely unknown. In this regard, Li et al^{30,31} reported that repeated oral administration of peanut or cow's milk leads to antigen-specific IgE-mediated hypersensitivity, as well as predominant T_H2 responses in the spleen. However, the model used by the authors raises theoretic problems in that antigens are administered with cholera toxin, which is a strong adjuvant for T_H2 response, and in that C3H/HeJ mouse strain used in the study has a defect in LPS-induced cytokine production. Here, we provided evidence that feeding of an antigen can lead to development of the antigen-specific T_H2 cells in the liver, suggesting that the liver might function as a generator, as well as a reservoir, for those T_H2 cells that predispose the host to the development of food allergy. This notion is consistent with the fact that DBA/2 mice fed a continuous casein diet produced high levels of casein-specific IgE accompanied by an increase of T_H2 cells in the liver.²³ Furthermore, mast cells that require IL-4 for their survival³² and play an

important role in IgE-mediated hypersensitivity develop in the liver after feeding of an antigen.³³ More interestingly, a report described a case in which peanut allergy was transferred to the recipient of a donor liver and not to the recipient of the kidney and pancreas of the same donor.³⁴ On the basis of these reports and together with our findings, we speculate that IgE helper T cells for ingested antigens are generated in the liver of the individual who has CD4⁺ T cells with TCRs cross-reactive to those antigens when the antigen is delivered at a dose high enough to reach the liver in an immunogenic form.

Previously, we showed that OVA-specific FasL⁺ T_H2 cells develop in the liver after feeding of OVA, which was associated with deletion of OVA-specific CD4⁺ T cells by Fas-mediated apoptosis.⁷ Clonal deletion in the liver occurred more gradually and continuously than that in PP. We postulate that Fas-mediated apoptosis of OVA-specific CD4⁺ T cells in the liver is incomplete under the conditions in this study. Furthermore, some of the T_H2 cells remain undeleted, which is compatible with the fact T_H2 cells are more resistant to Fas-mediated apoptosis than T_H1 cells.^{35,36} Alternative to this notion, we recently found that CD11c⁺ cells in the liver downregulate IL-12 and upregulate IL-18 secretion on OVA feeding, which collectively indicates preferential survival of T_H2 cells in the liver (T. Watanabe and Y. Wakatsuki, unpublished observations). In this context the background of the mice (BALB/c) used in this study might also have affected T_H response, which possibility we do not preclude.

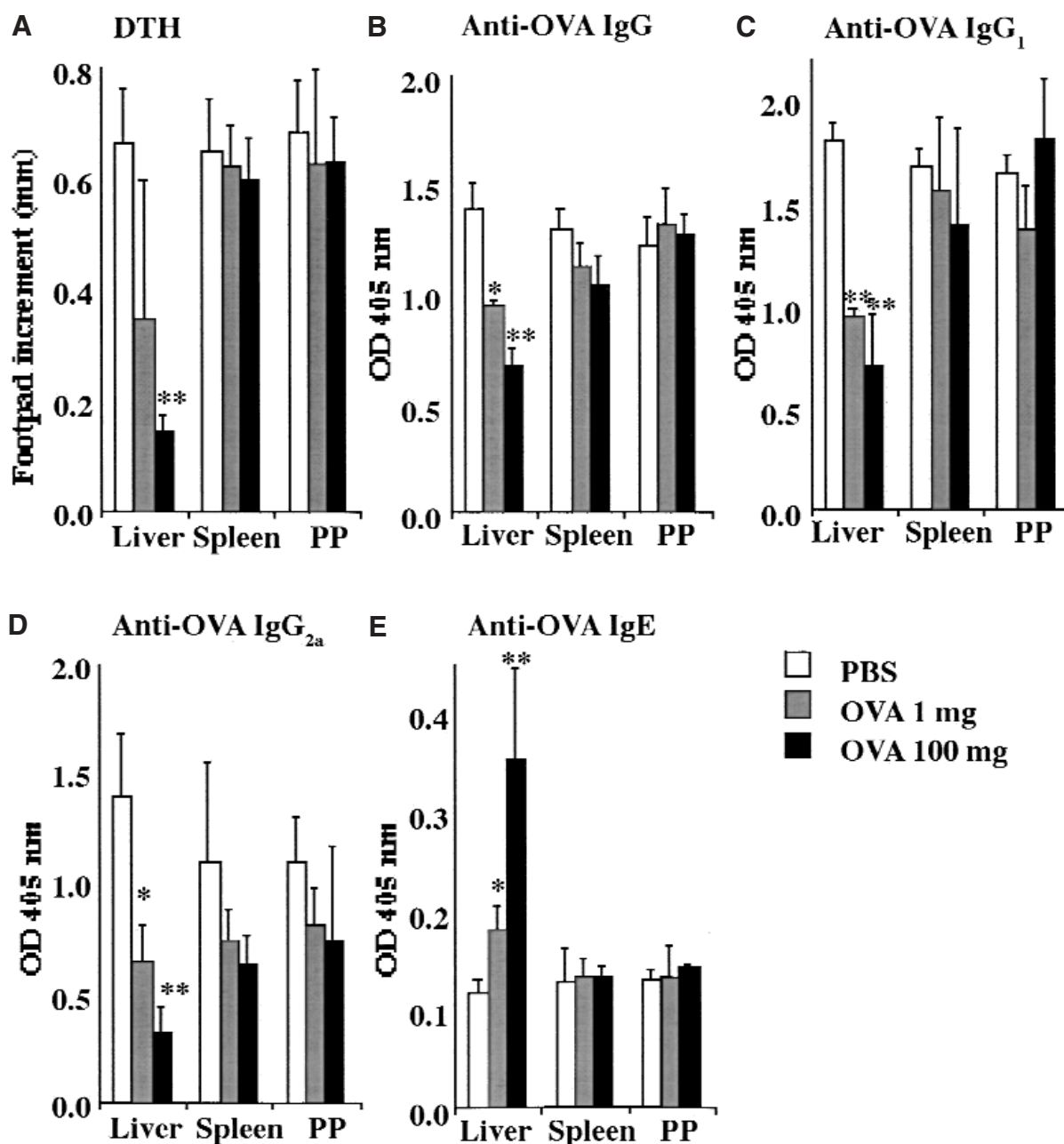


FIG 6. DTH and immunoglobulin subclass responses specific to OVA in the recipient mice transferred with hepatic, splenic, or PP CD4⁺ T cells from mice fed OVA or PBS. Donor DO11.10 mice were fed 100 mg of OVA, 1 mg of OVA, or PBS every other day for a total of 5 times and killed 3 days after the fifth feeding. CD4⁺ T cells (1×10^6 /one mouse) in the livers, PP, or spleens of the donor mice were purified and transferred into naive BALB/c mice. The day after the transfer, the recipient mice were immunized subcutaneously with 100 μ g of OVA followed by booster immunization with 100 μ g of OVA 1 week later. DTH (A), anti-OVA IgG (B), IgG₁ (C), IgG_{2a} (D), and IgE (E) responses in the recipient mice were measured 2 weeks after the first immunization. * $P < .05$ and ** $P < .01$ compared with PBS control mice in each organ. The results are expressed as means $\pm$ SD.

Fas/FasL interaction is known to play an important role in immunoglobulin class switching, as well as in terminal B-cell differentiation.³⁷⁻³⁹ The former effect in vivo is seen in our model in that IgG₁ and IgG_{2a} response to OVA was suppressed in the recipient of the hepatic CD4⁺ T cells, an effect that was fully abolished by the

anti-FasL treatment. This might have resulted from the fact that Fas⁺ B cells are killed by hepatic CD4⁺ T cells expressing FasL. Alternatively, FasL expressed in the hepatic CD4⁺ T cells might also be directed to Fas⁺ T_H1 cells, which are more sensitive to Fas-mediated apoptosis than T_H2 cells.^{35,36} In fact, Fas-deficient MRL lpr/lpr

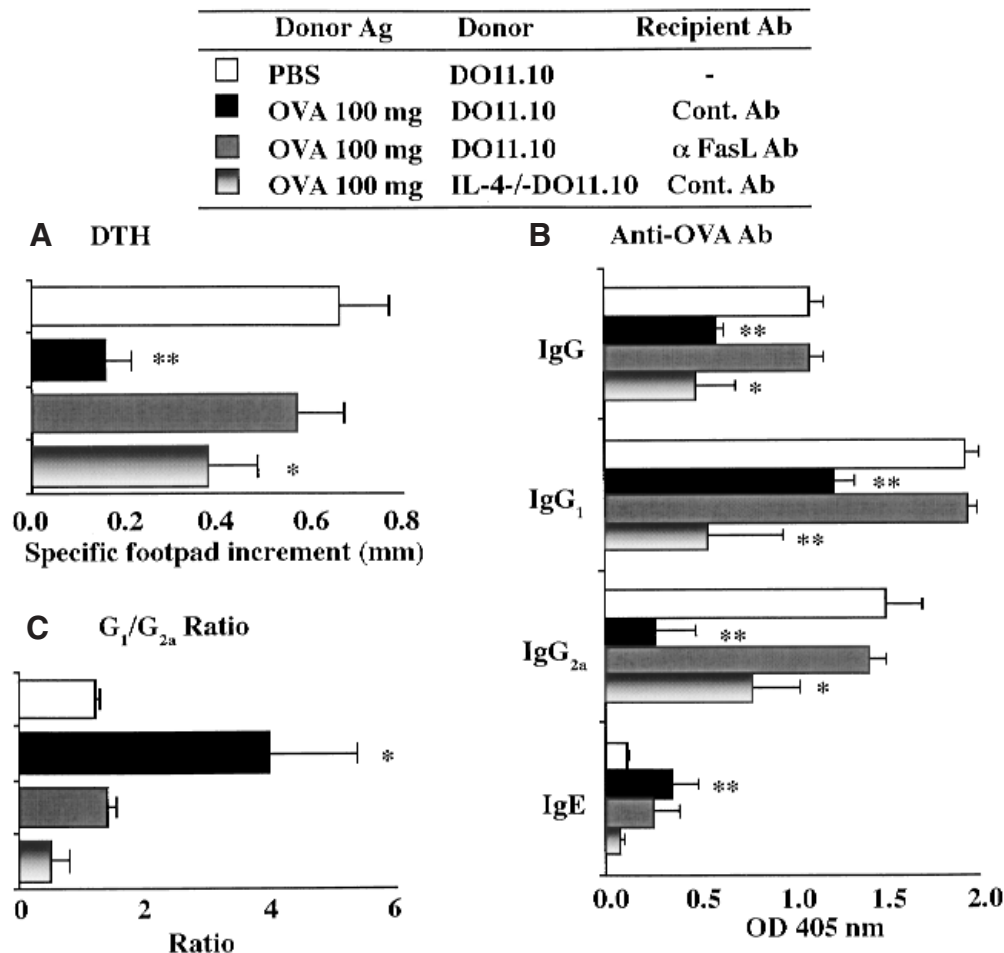


FIG 7. DTH and immunoglobulin subclass responses specific to OVA in the recipient mice transferred with hepatic CD4⁺ T cells from mice fed OVA and treated with anti-FasL antibody or in the recipient mice transferred with hepatic CD4⁺ T cells from IL-4-deficient mice fed OVA. Donor DO11.10 mice or IL-4^{-/-} DO11.10 mice were fed 100 mg of OVA or PBS every other day for a total of 5 times and killed 3 days after the fifth feeding. CD4⁺ T cells (1×10^6 /one mouse) in the liver of the donor mice were purified and transferred into recipient BALB/c mice, followed by immunization as described in Fig 6. Mice transferred with hepatic CD4⁺ T cells were injected intraperitoneally with 0.5 mg of anti-mouse FasL antibody or control hamster IgG every 3 days from the first day of immunization. DTH response (**A**), OVA-specific antibody response (**B**), and OVA-specific IgG₁/IgG_{2a} ratio (**C**) in the recipient mice were measured 2 weeks after the first immunization. **P* < .05 and ***P* < .01 compared with PBS control mice. The results are expressed as means $\pm$ SD.

mice show enhancement in antigen-specific IgG_{2a} response, indicating enhancement of T_H1 response.⁴⁰ In our model, IFN- γ secretion by splenic CD4⁺ T cells is significantly suppressed in the mice transferred with hepatic CD4⁺ T cells from the mice fed 100 mg of OVA,⁷ which is associated with an increase in the OVA-specific IgG₁/IgG_{2a} antibody ratio. Moreover, this significant decrease of IFN- γ secretion by splenic CD4⁺ T cells in the recipient is restored by anti-FasL treatment (T. Watanabe and Y. Wakatsuki, unpublished observations). Thus, these findings together indicate that hepatic CD4⁺ T cells of mice fed OVA might direct immune response to the T_H2 type not only by means of IL-4 secretion but also by means of FasL-mediated apoptosis. However, this still does not explain the different effect of anti-FasL

antibody treatment on IgG₁ and IgE response. This can be related to the fact that IgG₁ and IgE responses have differential requirements for IL-4 in their class switching; although IL-4 is an absolute requirement for IgE response,⁵ IL-4-independent IgG₁ responses also exist.⁴¹ In this regard, we should also consider the fact that IL-4 is involved in the resistance to Fas-mediated apoptosis,⁴² which might compound the effect of IL-4 and FasL on class-switch differentiation in the mice transferred with hepatic CD4⁺ T cells.

We should consider the frequency of CD4⁺ T cells to a dietary antigen in the liver when we extrapolate these findings to the patients with food allergy. In this regard, we showed that clonal expansion of CD4⁺ T cells reactive to OVA precedes the deletion in the liver of BALB/c mice

that received adoptive transfer of DO11.10 CD4⁺ T cells and then were fed OVA.⁷ In this case, feeding of OVA to those recipient mice also led to the emergence of OVA-specific CD4⁺FasL⁺ T cells in the liver,⁷ which was associated with an enhancement in the secretion of IL-4 (73 ± 18 vs 149 ± 25 pg/mL, $P < .05$) and IL-10 (535 ± 215 vs 969 ± 297 pg/mL) by hepatic CD4⁺ T cells. Moreover, antigens causing food allergy are usually multivalent and activate multiple T-cell clones. Thus, although not directly applicable to human food allergy, we infer that individuals who have CD4⁺ T-cell clones cross-reactive to dietary antigens might harbor Th₂ cells reactive to the antigen in the liver by the mechanism alluded to above.

Finally, both enhancement of antigen-specific IgE and suppression of antigen-specific DTH responses were less in the mice that received hepatic CD4⁺ T cells from mice fed 1 mg of OVA than those in the mice that received hepatic CD4⁺ T cells from mice fed 100 mg of OVA. This is likely due to the fact that levels of FasL expression and IL-4 secretion were lower in the former cells than in the latter cells, indicating that more efficient stimulation of OVA-specific CD4⁺ T cells occurs in the liver on feeding with 100 mg of OVA than in the case of feeding with 1 mg of OVA.

In conclusion, we showed that IgE helper CD4⁺FasL⁺ T cells for a food antigen can develop by antigenic selection in the livers of mice that have CD4⁺ T cells specific to the antigen at high frequency.

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