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Antigenic activation of Th1 cells in the gastric mucosa enhances dysregulated apoptosis and turnover of the epithelial cells☆

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Abstract

Colonization of *Helicobacter pylori* in the stomach leads to chronic gastritis with massive infiltration by Th1 cells. To assess a role played by those T cells in the remodeling of gastric epithelium, we activated gastric T cells utilizing mice with CD4 T cells bearing transgenic TCR with or without deficiency in either IL-4 or IFN-γ or IL-12. Mice developed gastritis upon injection of an antigen into gastric mucosa. While neutrophil infiltration occurred even with a control antigen, infiltration by transgenic T cells was dependent on the specific antigen. The numbers of epithelial cells undergoing apoptosis and regeneration were increased in the mice with infiltrating T cells producing IFN-γ and the alignment of those cells in the glands was markedly dysregulated. In contrast, mice deficient in Th1 response showed no increase in cell division and apoptosis of epithelial cells. Thus, Th1 type T cells infiltrating into gastric mucosa play an independent role in controlling turnover of epithelial cells.

Keywords: Immunology; *Helicobacter pylori*; Gastritis; Lymphokine; T helper 1; Pathology; Disease model; Apoptosis; Cell turnover

Helicobacter pylori (*H. pylori*) colonizes in the stomach of almost half of the world population, which invariably associates with chronic gastritis of various severities. The infection usually persists for life long unless eradicated and predisposes the patients to such diseases as peptic ulcer, gastric cancer, and MALT lymphoma [1,2]. The spectrum of the disease patients may develop varies depending on the type of gastritis they have. For instance, the patients with severe atrophy or gastritis of corpus-predominant form with intestinal metaplasia have a higher risk of gastric cancer than those without [2]. This then leads to a question as to what determines the diversity of gastritis in *H. pylori* infection. In this regard, studies done in some countries reported that certain bacterial virulence factors, such as VacA and CagA genes, predict for gastric ulceration and malignancies, however, this is not applicable to the patients in other geographical localization [3–6]. Although these gene products can directly interact with gastric epithelium and potentially cause tissue damage [7,8], they can also do so via immune and inflammatory responses by the hosts [9]. Since bacterial virulence and host immune/inflammatory responses are intertwined with each other, it is difficult to determine whether host immune response per se plays an independent role in tissue damage and remodeling of the epithelium. Given the fact that the epithelial cells of gastric glands are continuously turning over in the physiological condition, and that patients with *H. pylori* infection have massive infiltration of T cells producing proinflammatory cytokines [10], it is important to determine whether local immune response per se is involved in and plays an independent role in the pathogenesis and prognosis of *H. pylori* gastritis [9,11]. In this regard, we created a new gastritis model and studied the role gastric T cells may play in the regeneration and apoptosis of gastric epithelial cells.

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Materials and methods

Animals. BALB/c mice, 8–12 weeks old, were purchased from CLEA Japan (Osaka, Japan) and used as recipients for adoptive cell-transfer studies. DO11.10 mice with T cells bearing the transgenic TCR that recognizes the 323–339 peptide fragment of OVA were described previously [12]. DO11.10 mice were crossed to Rag2$^{-/-}$, IL-4$^{-/-}$, IFN-$\gamma^{-/-}$, and IL-12$^{-/-}$ mice, and those with homozygous mutations in respective lymphokine genes were used in this study. All mice were housed under specific pathogen-free conditions in the Animal Facilities of Kyoto University's Department of Medicine. All animal experimentation was performed in accordance with our institutional guidelines and ethical permission for this study was granted by the Review Board of Kyoto University.

Protocol for the induction of gastritis. OVA (50 mg/ml) in phosphate-buffered saline (PBS) was boiled for 10 min. Fifty microliters of heat denatured OVA (HDO) was injected at multiple sites of gastric mucosa of the mice with an incision of abdominal wall under diethyl ether anesthesia. Likewise, heat-denatured BSA (HDB) was injected in control mice. The condition for injection was optimized by preliminary studies utilizing HDO mixed with ethylene blue dye and submucosal injection was confirmed by staining the tissue with rabbit anti-OVA antibody (Cappel, Flanders, NJ). After the procedures, the rectus sheath and the skin were sutured, respectively. For adoptive transfer experiments, BALB/c mice were injected intraperitoneally with 1×10^7 of splenic T cells obtained from DO11.10/Rag-2$^{-/-}$ mice a day previous to the antigenic challenge. Oral immunization was done by feeding 1 mg of soluble OVA and 10 μg cholera toxin (LIST Biological Laboratories, Campbell, CA) four times for 2 weeks prior to intragastric challenge. Histology of gastritis was evaluated according to the updated Sydney System [13].

Immunohistochemistry. Proliferating epithelial cells were labeled by injecting bromodeoxyuridin (BrdU; Nacalai Tesque, Kyoto, Japan) 50 mg/kg 3 h before sacrifice. Seven days after the antigenic challenge, the stomach was removed from each mouse and half of it was fixed in 10% buffered formalin and embedded in paraffin. Five-micrometer sections were made and stained with hematoxylin and eosin (H.E.) after deparaffinization. Sections in series were stained with rabbit polyclonal anti-CD3 antibody (1:500 dilution; DAKO, Hamburg, Germany) or mouse anti-BrdU antibody (1:100; BD Pharmingen, San Diego, CA). After incubation for an overnight at 4 °C, the antibodies bound were detected with biotinylated anti-rabbit immunoglobulin antibody and biotinylated anti-mouse immunoglobulin antibody, respectively, for 30 min at room temperature. Next, the sections were incubated with avidin–biotin complex (Vector Laboratories, Burlingame, CA, USA) for 30 min and then incubated with DAB solution (Vector Laboratories) to develop the color. Finally, the nuclei were counterstained with Methyl Green (DAKO Japan, Kyoto, Japan). TUNEL staining was carried out utilizing the in situ Apoptosis Detection Kit (Takara Bio, Shiga, Japan). The result was evaluated by counting cells stained positive to anti-CD3, anti-BrdU, and TUNEL in the area within 1 mm distance from squamo-columnar junction of hind-stomach under a magnification of 1:200. For the staining of OVA specific T cells, clonotypic antibody KJ1.26 (Caltag, Burlingame, CA) was used as described previously [14].

Isolation of gastric lymphocytes and flowcytometric analysis. The stomach was washed with PBS and cut into small pieces. The tissue was placed in 5 ml serum-free RPMI containing 1.5 mg/ml dispase (GODO SHUSEI, Tokyo, Japan) and 300 μg/ml collagenase (Wako, Osaka, Japan) in a conical flask and incubated for 30 min at 37 °C with stirring. The medium containing cells were removed and replaced with new medium. This procedure was repeated three times and pooled cells were resuspended in serum-free RPMI. Lymphocytes were purified by Percoll (Pharmacia, Uppsala, Sweden). Cells were stained with anti-CD4 PE (Pharmingen) and anti-KJ1-26 FITC (Caltag South San Francisco, CA, USA). Propidium iodide was added to the sample to gate out dead cells before loading to a flowcytometer, Epics XL (Coulter Electronics, Miami, USA).

Statistical analysis. Results are expressed as means ± SD. Statistical analysis was done using Student's *t* test, the Mann–Whitney *U* test for non-parametric data, and the linear regression analysis. p values <0.05 were considered significant. (StatView 5.0, SAS Institute, Cary, NC, USA).

Results and discussion

Establishment of a gastritis model based on immune response to a defined antigen

Recently, we demonstrated that T cells predominate in number over other cell-infiltrates in the gastric biopsy obtained from patients with *H. pylori* infection and that those T cells produce a large amount of IFN-γ and very little amount of IL-4 on antigenic stimulation [10]. Although this skewed polarization to Th1 type immune response in the stomach [15] was not exclusively caused by the infecting *H. pylori* [10], IFN-γ produced by Th1 cells is essential to establish gastritis [16]. In contrast, the active immunization which protects from *H. pylori* colonization also results in the increase of T cell infiltration, which is called post-vaccination gastritis [17,18]. Moreover, adoptive transfer of Th1 type T cells to mice with *Helicobacter felis* infection leads to severer gastritis [19]. In human gastritis, chronic inflammation leads to mucosal atrophy which is characterized by massive T cell infiltration and decreases of the number and height of gastric glands. To assess how antigenic activation of gastric T cells may affect the epithelial cell turnover, we created a non-infection gastritis model by injecting heat-denatured OVA into the gastric mucosa of the BALB/c mice that took adoptive transfer of T cells monospecific tc OVA or into those of mice with T cells bearing transgenic TCR specific to OVA with or without deficiency either in IFN-γ or IL-4 or IL-12. As shown in Fig. 1, this method elicited gastritis and allowed us to enumerate T cells specific to the injected antigen by staining with clonotypic antibody KJ1-26 in histochemistry and flowcytometry. In mice which took an adoptive transfer of CD4 T cells obtained from Rag2$^{-/-}$/DO11.10 mice and injected with heat-denatured OVA, OVA specific T cells were localized mainly in the submucosa and beyond the muscularis mucosae into interglandular space. In contrast, control mice which received an identical cell fraction and injection of heat-denatured BSA, gastritis was very mild and no KJ1-26 positive T cells were seen in the gastric mucosa. Thus, T cell infiltration occurred in antigen-dependent manner and that this model allowed us to enumerate antigen specific T cells infiltrating into the stomach.

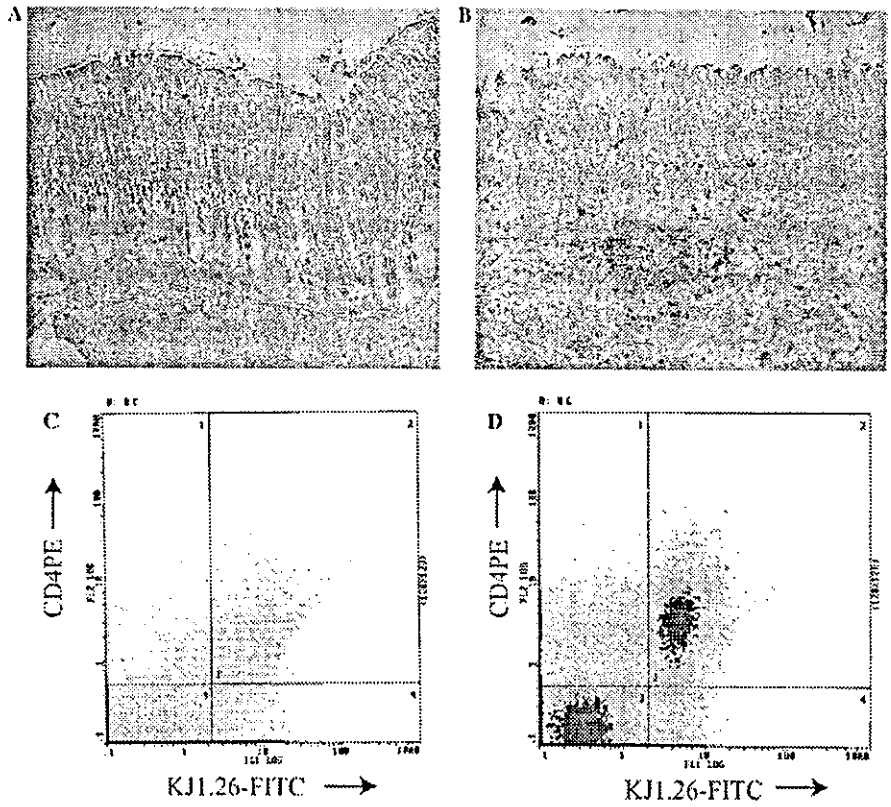

Fig. 1. Localization and quantitative analysis of gastric T cells specific to a gastritogenic antigen. Heat-denatured ovalbumin (HDO) or bovine serum albumin (HDB) was directly injected into the gastric mucosa of BALB/c mice that were adoptively transferred of CD4 T cells prepared from Rag2$^{-/-}$/DO11.10. Cryosections of the stomach were made from mice injected with HDB (A) and HDO (B) seven days after the injection and stained with KJ1.26, a clonotypic antibody of CD4 T cells specific to OVA. KJ1.26-positive cells localized mainly in the submucosa and beyond the muscularis mucosae of the mice with HDO injection. Occasionally the T cell infiltration extended into the interglandular space. To quantitatively evaluate the cells infiltrating into the gastric mucosa by flowcytometry, mononuclear cells were purified from the gastric tissue and stained with PE-labeled anti-CD4 and FITC-labeled KJ1.26. The figures in the histogram indicate percentages of the CD4 T cells specific to OVA in the gastric mononuclear cells of the mice with HDB (A) and HDO (B) injection.

Sensitized hosts develop severe gastritis with metaplasia

We next investigated what type of gastritis is developed in the host who has T cells specific to OVA at high frequency. For this purpose, we either immunized mice by feeding of OVA with an adjuvant or adoptively transferring OVA-specific T cells into naïve mice prior to antigenic challenge. We evaluated the grade of gastritis seen at the area within 1 mm distance from squamo-columnar junction in the gastric body. Gastritis developed in both immunized mice and cell-transplanted mice was severe in grade and associated with hyperplasia and mucous-gland metaplasia of gastric glands (Figs. 2B and C). In these mice, the numbers of epithelial cells labeled with BrdU were increased (Figs. 2F and G). Thus, these findings altogether suggested that high rate of epithelial cell turnover is occurring in the mice that have T cells specific to a gastritogenic antigen at high frequency.

Th1 cells accelerate the rate of cell turnover of gastric epithelium

In the next series of experiments, we explored what type of immune response, in addition to antigen-specificity, would regulate turnover and apoptosis of gastric epithelium. For this purpose, we took DO11.10 mice deficient in either IL-4 or IFN-γ or IL-12. In DO11.10 mice, injection of heat-denatured OVA led to gastritis with hyperplasia of the glands, which was often associated with mucous-gland metaplasia (Fig. 3A). Number of cells labeled with BrdU was increased, which were localized in wide area in lower half of the gastric glands (Fig. 3E). Overlapping to this area, TUNEL-stain positive cells were localized along the entire glands, which was normally aligned in restricted area above the proliferative zone in upper half of the glands (Fig. 3I). In IL-4 deficient mice, which are deficient in Th2 response, injection of heat-denatured OVA also led to hyperplasia

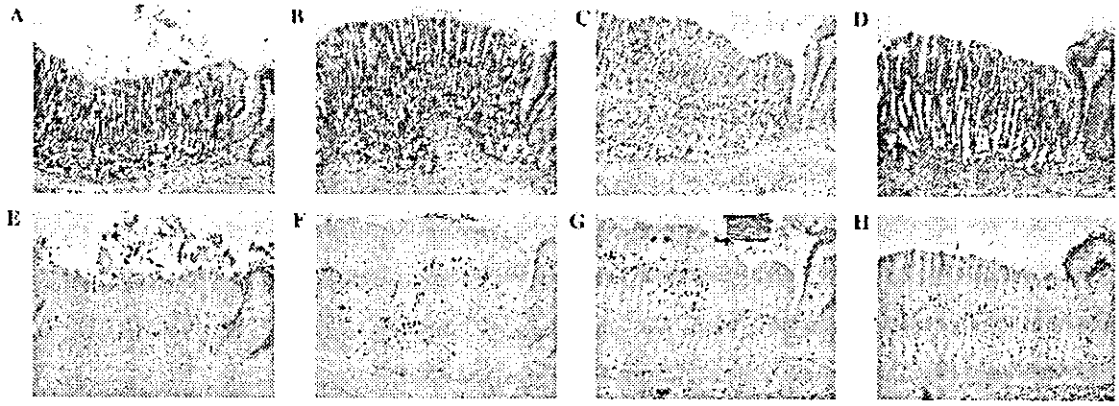

Fig. 2. Gastritis caused by HDO injection to BALB/c mice (A,E), BALB/c mice immunized orally with OVA (B,F), BALB/c mice that were adoptively transferred of CD4 T cells obtained from Rag2$^{-/-}$/DO11.10 (C,G), and DO11.10 mice (D,H). Sections of gastric corpus including fore- and hind-stomach junction were stained with H&E (A–D) and anti-BrdU antibody (E–H). Mice were killed 7 days after HDO injection. Massive infiltration by mononuclear cells and eosinophils, and elongation of gastric gland epithelium were seen in immunized mice (B) and in mice with OVA specific T cells (C,D), which had prominent mucous-gland metaplasia. These changes were hardly seen and cells pulsed with BrdU were well aligned in the middle of gastric glands of control mice (A,E). Cells pulsed with BrdU were increased in number and they localized in wide area of gastric gland in non-aligned manner in the mice with OVA specific T cells (G,H).

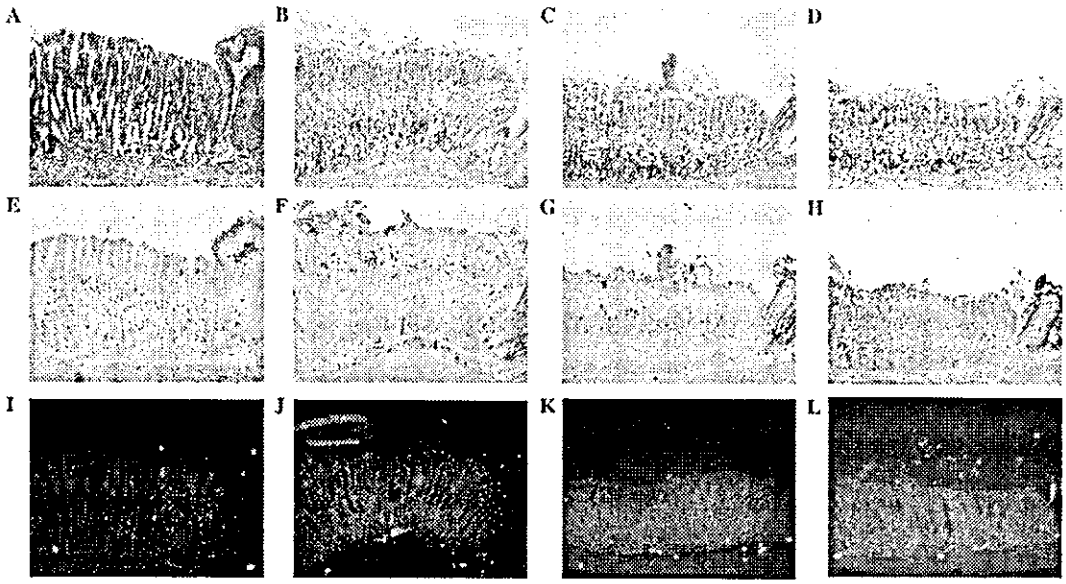

Fig. 3. Serial sections of stomach were made from DO11.10 mice (A,E,I), DO11.10/IL-4$^{-/-}$ mice (B,F,J), DO11.10/IL-12$^{-/-}$ mice (C,G,K), and DO11.10/IFN-$\gamma^{-/-}$ mice (D,H,L) seven days after the HDO injection. H&E, anti-BrdU, and TUNEL staining was done and photographs were taken at 100× magnification. To note that elongation of gastric glands and mucous-gland metaplasia was not seen and that numbers of anti-BrdU and TUNEL-stain positive cells were markedly reduced in DO11.10/IL-12$^{-/-}$ and DO11.10/IFN-$\gamma^{-/-}$ mice. Localization of anti-BrdU and TUNEL positive cells was well aligned in the gastric glands.

of gastric glands (Fig. 3B). However, distribution of BrdU and TUNEL positive cells was well aligned at upper half of the glands (Figs. 3B, F, and J). Interestingly, cell infiltration and elongation of gastric glands were very mild or none in mice deficient in IL-12 (Fig. 3C) and in IFN-γ (Fig. 3D), which are deficient in Th1 type response. In these mice, distributions of BrdU labeled cells were aligned at the middle part of the glands (Figs. 3G and H) and cells stained by TUNEL were very few. Thus, regeneration and death of

epithelial cells were associated with the infiltration by Th1 cells into the gastric mucosa.

Correlation of epithelial cell turnover and apoptosis to the type of T cells infiltrating in the stomach

We next accessed the correlation between the number of CD3 positive cells infiltrated and that of cells stained positive to anti-BrdU and TUNEL staining. As shown in Fig. 4A, number of CD3 positive cells in the tissue and that of BrdU positive cells are correlated ($R = 0.568$, $p = 0.0016$). This was also the case for the correlation between TUNEL positive cells and BrdU positive cells ($R = 0.697$, $p < 0.0001$). Thus, the greater the T cell infiltration, the more cell regeneration and death occurred. Interestingly, if we compared the numbers of cells by the group of mice with each lymphokine deficiency, we noticed that mice deficient in Th1 type immune response (DO11.10/IL-12$^{-/-}$, DO11.10/IFN-$\gamma^{-/-}$) have significantly less BrdU labeled cells ($p < 0.01$) and TUNEL positive cells ($p < 0.01$), which was also associated with less T cell infiltration in the tissue ($p < 0.01$) than those seen in DO11.10 and DO11.10/IL-4$^{-/-}$ mice. The latter phenomenon may reflect the facts that IFN-γ enhances antigen presentation by augmenting class II expression in antigen-presenting cells and induces more T cells primed by antigen and infiltrating to the tissue than in those mice deficient in IFN-γ. Consonant to this notion, effect of IL-12 deficiency can be mediated by insufficient induction of IFN-γ. Alternatively, decreased T cell infiltration in DO11.10/IL-12$^{-/-}$ mice may due to the fact that IL-12 can prevent apoptosis of T cells in inflamed mucosa [20]. With regard to the effect on cell turnover and death of epithelial cells by IFN-γ and IL-12, we speculate that responsiveness of epithelial cells to growth stimuli or to survival factors might be regulated by cytokines Th1 cell may release, which requires further investigation. The effect of IL-4 deficiency was very little and there was no significant difference in BrdU/TUNEL positive cells between DO11.10 mice and DO11.10/IL-4$^{-/-}$ mice. This is likely due to the fact that vast majority of gastric T cells are Th1 cells so that the effect of IL-4 deficiency was hardly seen because of the predominant presence of Th1 cells in the stomach [10]. Since neutrophils have various cytocidal mechanisms, we compared the number of

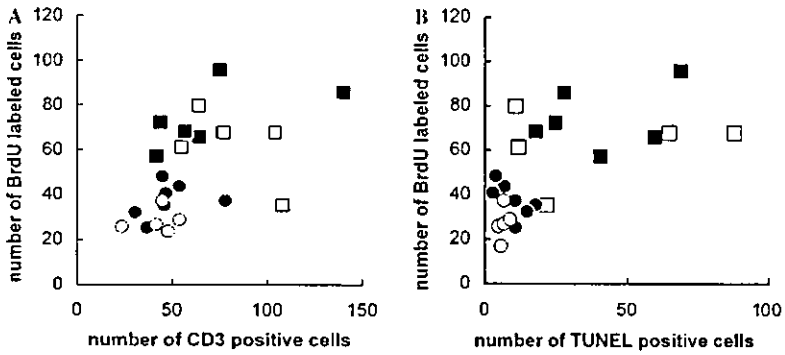

Fig. 4. After the enumeration of stained cells in each sections, numbers of anti-BrdU, anti-CD3, and TUNEL staining positive cells were plotted as indicated (A,B). Closed squares, DO11.10 mice; open squares, DO11.10/IL-4$^{-/-}$ mice; open circle, DO11.10/IFN-$\gamma^{-/-}$ mice; and closed circle, DO11.10/IL-12$^{-/-}$ mice. DO11.10/IL-12$^{-/-}$ and DO11.10/IFN-$\gamma^{-/-}$ mice have less T cell infiltration into the stomach ($p < 0.01$), less proliferating epithelial cells ($p < 0.01$) (A), and less cells positive to TUNEL-staining ($p < 0.01$) (B) compared to DO11.10 and DO11.10/IL-4$^{-/-}$ mice.

Table 1
Evaluation of histological changes of gastric glands and grade of cell infiltration

	BALB/c	DO11.10	DO11.10/IL-4$^{-/-}$	DO11.10/ IL-12$^{-/-}$	DO11.10/ IFN-$\gamma^{-/-}$
Height of gland*	0.31 ± 0.036	0.38 ± 0.060[a]	0.36 ± 0.045	0.27 ± 0.060[c]	0.27 ± 0.062[b]
Width of proliferative zone*	0.069 ± 0.016	0.12 ± 0.029[a]	0.066 ± 0.033[b]	0.045 ± 0.022[c]	0.034 ± 0.017[c]
Neutrophil infiltration	1.00 ± 0.70	0.33 ± 0.52	0.40 ± 0.55	0.29 ± 0.49	0.17 ± 0.40
Mononuclear cell infiltration	0.80 ± 0.45	1.17 ± 0.41	1.00 ± 0.71	0.43 ± 0.54	0.50 ± 0.55
Eosinophil infiltration	1.80 ± 0.84	2.17 ± 0.75	1.00 ± 0.71[b]	1.43 ± 0.79	1.67 ± 1.21

Serial sections made from the mice injected with HDO were evaluated by blind manner by two pathologists independently. Height of gastric glands and width of proliferative zone were measured utilizing grid attached to eyepiece of microscopy. The grade of infiltration by mononuclear cells, neutrophils, and eosinophils in each section was classified according to the updated Sydney system as: 0, normal; 1, mild; 2, moderate; and 3, marked. Figures in the table indicate mean ± SD of each value measured.

[a] Compared to BALB/c control ($p < 0.05$).

[b] Compared to DO11.10 control ($p < 0.05$).

[c] Compared to DO11.10 control ($p < 0.01$).

* The results in the table indicate the height and width expressed in millimeters.

neutrophils infiltrated among five groups of mice (Table 1). We found that there was no significant difference in the number of infiltrating neutrophils in the stomach, at the time point studied. Collectively, the data in this study indicated that the grade of T cell infiltration and lymphokines they produce on activation by an antigen regulate the rate of regeneration and death of gastric epithelial cells. In the presence of antigen-activated T cells, epithelial cell regeneration often associates with metaplasia. Although the mechanism how this was achieved by activated T cells is yet to be known, the finding obtained in this study has two clinical implications. First, even in the absence of the bacterial virulence factors, the host T cell response per se can regulate cell death and regeneration of gastric epithelium. Second, diversity of *H. pylori* gastritis, which is caused by continuous apoptosis, regeneration, and metaplasia of epithelial cells, can be determined by the frequency of T cells specific to immunodominant antigens of *H. pylori* and the type of cytokines they produce. This may lend support for an idea that, depending on the host immune response, i.e., frequency of T cells specific to those antigens and the profile of lymphokines they produce, remodeling of gastric epithelium would be different even among the patients infected with virulent types of strains, which is the case for most of the Japanese patients. This may also suggest that an alteration of immune response to *H. pylori* along with aging of the host may impact on the regeneration of gastric epithelium. Finally, with regard to the magnitude of immune response seen in this study, we estimate 10–14% of the total cell infiltrates are OVA specific T cells (Fig. 1 and Table 1). In gastric biopsy of the patients, *H. pylori* infection caused increase of T cell infiltration by 10–13% of the total cell infiltrates [10], indicating that antigen-dependent migration of T cells into gastric mucosa in our model was comparable to that occurring in human stomach.

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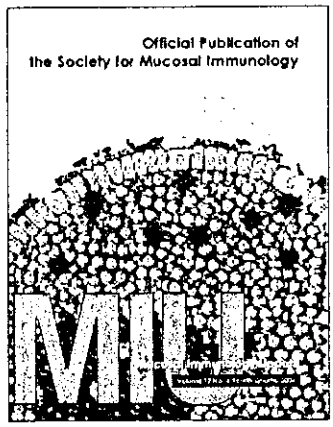

Cover Art: Schematic representation of the dome of a Peyer's Patch. CD11c+ DC are implicated in uptake of antigen from beneath M cells or via luminal sampling between the follicle-associated epithelial cells before migrating to areas of specialized antigen presentation. Prepared by Janine Bilsborough.

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Preface

In this issue of Mucosal Immunity Update three aspects of immune function affecting or affected by the liver are presented. The first aspect, discussed by Watanabe et al. concerns the role of the liver in the mechanism of oral tolerance, a function that has long been considered by poorly understood. The second and third aspects concern the role of T cell responses in causing autoimmune hepatitis, infectious hepatitis or primary biliary cirrhosis.

I. Regulatory T cells in the Liver (Contributed by: Watanabe, T., Chiba, T., and Wakatsuki, Y.)

An often over-looked fact in discussions of oral tolerance is that the development of tolerance depends, at least to some extent, on tolerogenic processes occurring in the liver. As pointed out in the accompanying review of Watanabe and his colleagues this is supported by the observation that surgical diversion of portal blood away from the liver leads to impaired oral tolerance.

Watanabe and his colleagues have studied the mechanism of oral tolerance mediated by the liver in OVA-specific TCR transgenic (Tg) mice given large amounts of oral antigen (OVA). In this modd of high dose oral tolerance they found that feeding induced apoptosis of the Ova-specific cells in all organs tested including the liver, but in the latter organ the apoptosis was incomplete. This apoptosis was Fas/FasL induced since was associated with the appearance (only in the liver) of activated CD4+ cells that were also dual-positive Fas/FasL-bearing cells or single positive FasL-bearing cells. In addition, it was prevented by administration of anti-FasL. Importantly, sorting studies revealed that the FasL-bearing cells secrete IL-4 as well as IL-10 and TGF-β upon activation.

Recognizing that Th1 cells are more susceptible to Fas-mediated apoptosis than Th2 cells, these results led Watanabe and his colleagues to postulate that the FasL-bearing cells cause preferential deletion of Th1 cells and the emergence of a predominantly Th2 T (apoptosis resistant) cell population in the liver. This view was supported by the observation that transfer of OVA-specific TCR Tg hepatic CD4+ T cells (i.e., liver regulatory cells) to naïve BALB/c mice followed by antigen challenge led to prevention of Th1 responses in the recipient and such prevention could be reversed by administration of anti-FasL. These *in vivo* studies were amplified by *in vitro* studies that showed that the regulatory cells act via the Fas pathway and not the secretion of suppressor cytokines.

In further studies, Watanabe and his colleagues showed that the development of the regulatory FasL-bearing Th2 cells in the liver were induced by antigen-presenting CD11c+ cells that produced relatively low amounts of IL-12 and high amounts of IL-18, a profile that favors Th2 development. These antigen-presenting cells could function ectopically, since their transfer along with naïve CD4+ T cells to recipient mice led to apoptosis of transferred cells in the lymph nodes of the recipients together with emergence of Th2 cells at this site.

These interesting studies add another dimension to our knowledge of oral tolerance and, for the first time, provide a mechanism for the role of the liver in this phenomenon. Whereas in prior studies of oral tolerance regulatory cells that mediate tolerance in the GI tract appeared to operate through the secretion of TGF-β and/or IL-10, the regulatory cells that mediate tolerance in the liver operate via Fas/FasL. Finally, since the regulatory cells developing in the liver as a result of oral feeding can operate in other tissues, it may be possible to devise ways of enhancing oral tolerance induction via the ability to induce the dissemination of these cells to non-hepatic tissues.

II. The Role of IL-27 and Related Cytokines in Liver Inflammation and Ulcerative Colitis (Contributed by: Yoshida, H., Hamano, S., and Miyazaki, Y.)

Studies of infectious and autoimmune hepatitis in this issue of MIU focus on the role of a new cytokine, IL-27, in these diseases. As succinctly reviewed by Yoshida, et. al., IL-27 is a member of the IL-12 family of cytokines which includes IL-12, IL-23 and IL-27 as well as two other less well characterized cytokines. These are all heterodimeric molecules composed of an IL-12p40 chain linked to an p35 chain (IL-12), an IL-12p40 chain linked to a p19 chain (IL-23) or an IL-12p40-related chain (EBI3) linked to a p35-related chain (p28) (IL-27). They each interact with different receptors that are also heterodimeric and structurely inter-related; in the case of IL-27, the receptor is composed of the WSX-1 chain having homology to IL-12Rα chain linked to a gp130 of IL-6R.

As members of the IL-12 family of cytokines, these molecules are all involved in the generation of Th1 T cell responses, albeit in somewhat different ways. In the case of IL-27, the role appears to be that of a cytokine that "jump-starts" Th1 T cell differentiation in that it is produced very early by antigen-presenting cells and induces early expression of T-bet, a molecule necessary for Th1 differentiation. However, its effect on T-bet expression is only partly dependent on Stat1, meaning that it does not necessarily affect T-bet through the induction of IFN-γ. In fact, IL-27 is a poor inducer of IFN-γ as shown by the fact that mice lacking p40 and unable to produce either IL-12 or IL-23 have low IFN-γ levels. In addition, mice lacking IL-27 signaling infected with L. major organisms have impaired early IFN-γ responses but these increase over time, presumably when the IL-12-mediated response "kicks in."

The above described positive aspect of IL-27 function on early Th1 responses is paradoxically accompanied by a marked negative effect on later Th1 responses. This is revealed in studies of T. cruzi and T. gondii infection of WSX-1 KO mice that lack the IL-27 receptor and therefore lack IL-27-induced effects. In this situation, one sees greatly increased production of IFN-γ and massive liver necrosis rather than the minimal liver injury seen in wild type mice. This is accompanied by production of many inflammatory cytokines, not just IFN-γ, as well as by greatly increased activation of CD4+ T cells as well as NKT cells. A similar situation occurs in

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ConA-induced hepatitis that is mediated by NKT cells producing increased amounts of both IFN-γ and IL-4. Thus, the check on cytokine responses by IL-27 is not limited to Th1 responses. At the moment, the mechanism of this Janus-like quality of the IL-27 response remains unexplained, but the fact remains that inflammation of the liver (and presumably other organs) is unexpectedly regulated by a heretofore unknown cytokine.

III. Immunology of Primary Biliary Cirrhosis (Contributed by: Ishibashi, H., Ichiki, Y., Kamihira, T., and Shimoda, S.)
In a third contribution to this issue of MIU, Ishibashi and his colleagues review the current status of our knowledge of the immunopathogenesis of primary biliary cirrhosis (PBC), a progressive autoimmune hepatic inflammation seen primarily in women that frequently results in hepatic failure. Historically, this disease was identified as an autoimmune disease by the identification of highly disease-specific autoantibodies with specificity for mitochondrial antigens (AMA). These are now known to be a family of antibodies specific for members of the 2-oxo-acid dehydrogenase complexes (2-OADC) that in each case centers on an epitope of each of the 2-OADC enzymes located around the lypoic acid bound to lysine in the lipoyl domain. Additional evidence that PBC is an autoimmune disease lies in its association with other autoimmune diseases. That it is also a mucosal disease is inherent in the fact that patients manifest inflammation of the salivary and lacrimal glands and produce IgA AMA at these sites.

T cells with specificity for one of the sume antigens evoking the AMA antibodies (PDC-E2) can be found in the liver and the circulation of patients and have been implicated in the damage to the biliary epithelium characteristic of the disease. Ishibashi and his collegues review the fine specificity of these cells and point out that the Vβ and Jβ usage of the autoreactive cells are heterogeneous, but display certain commonalities in the CDR3 region. An important characteristic of the autoreactive cells in PBC is that a sub-fraction are CD4+/CD28-negative and therefore co-stimulation independent. Ishibashi and his colleagues suggest that such cells are not induced to become anergic by biliary epithelial antigens and therefore maintain the autoimmune state.

CD8+ T cells have been shown to be present in PBC lesions but their role in disease pathogenesis was unclear. Recently, however, it has been demonstrated that peptides derived from PBC autoantigens can be used to generated CD8+ CTL's. These cells have specificity for PDC-E2 peptides that were restricted by a particular HLA-A type and are thus presumed to act as cytotoxic cells causing tissue injury in the disease. NKT cells are another type of T cell found in areas of tissue injury and have been shown to produce both IFN-β and IL-4. These cells bind the NKT cell-specific antigen, alpha-galactocylceramide, and thus bear invariant NKT cell T cell receptors. Thus, therefore may participate in tissue injury by responding to release self-antigens.

Taken together, these studies show that the antibodies and T cells found in PBC recognize epitopes found within overlapping regions of autoantigens in the liver mitochondria. However, it is likely that the T cells rather than the antibodies are responsible for the pathology of the disease. The challenge of future work in this area is to define the genetic factors that lead to this injurious autoimmune response.

– Warren Strober and Yoshio Wakatsuki

Portal Vein Tolerance and Development of Regulatory CD4 T Cells in the Liver

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The liver is an immunologically privileged organ which has to deal with copious amount of antigens (Ags) migrating from both the systemic and portal blood flow. To the Ags derived from food and bacteria in the intestine, the liver achieves hypo-responsiveness called *portal vein tolerance* (1). Although the mechanism responsible for this phenomenon is not fully understood, it explains various immunological reactions involving the liver. For example and firstly, patients with insulin-dependent diabetes have been successfully treated by transplantation of islet cells via the portal vein with long survival of the transplanted cells (2). Secondly, creation of portosystemic shunt, whereby portal venous flow is diverted directly into the systemic circulation, impairs the induction of systemic tolerance to dietary Ags in rats (3), indicating that the liver is indispensable for establishing oral tolerance. Thirdly, the liver itself is such a tolerogenic organ that the rate of spontaneous acceptance of the liver allograft both in humans and animals are very high compared with the case of other organs (4).

Recently, we found that administration of an Ag per oral route at the dose range which normally induces "high dose oral tolerance" generates a new type of immunoregulatory CD4 T cells in the liver

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(5)(6)(7). In this article, we review how these regulatory T cells are generated in the liver and comment on clinical implication these T cells may have.

Fas-mediated apoptosis selects Fas ligand$^+$ regulatory T cells in the liver

The first question we asked was whether a part of an Ag, such as ovalbumin (OVA), might be absorbed in an immunogenic form upon administration per oral route at a high dose. Indeed, this was the case and we detected immunoreactive OVA of a significant amount in the portal vein after feeding of 100 mg of OVA. This finding raised a question as to whether Ags administered orally at a high dose would activate Ag-specific CD4$^+$ T cells in the liver and how this activation is correlated to the induction of systemic tolerance.

We administered 100 mg of OVA to $I\!A^b$-restricted $OVA_{323-339}$-TCR transgenic mice (DO11.10) for a total of five times. First, we examined the population of OVA-specific (KJ1-26$^+$) CD4$^+$ T cells in the liver, spleen, and Peyer's patches (PP) since clonal deletion by apoptosis plays an important role in the induction of high dose oral tolerance (8). After the last feeding, the total number and percentage of OVA-specific CD4$^+$ T cells were remarkably reduced not only in PP but also in the liver. However, time course study using Annexin V and Propidium iodide revealed that the deletion of OVA-specific CD4$^+$ T cells was gradual and incomplete in the liver whereas those of PP was rapid and complete (5)(6). This apoptotic cell death of CD4$^+$ T cells in the liver was dependent on Fas/Fas ligand (FasL) interaction since systemic administration of anti-FasL antibody (Ab) prevented the apoptosis. Another finding in this time course study was that feeding of OVA was associated with an appearance of Fas and FasL dual positive CD4$^+$ T cells specific to OVA. Before feeding, 20-40 % of hepatic CD4$^+$KJ1-26$^+$ T cells is Fas single positive and FasL positive cells were barely seen. After the feeding, Fas single positive cells disappeared and Fas/FasL double positive (45-55 %) and FasL single positive (20-25 %) cells appeared in the population of hepatic CD4$^+$KJ1-26$^+$ T cells (5). These OVA-specific CD4$^+$FasL$^+$ T cells coexpressed CD25, CD44, and CD69. In the spleen or PP, FasL+ population was not detected at any time point during the feeding. Moreover, emergence of CD4$^+$FasL$^+$ T cells was not observed in the liver of DO11.10 mice treated with a neutralizing anti-FasL Ab, suggesting that an emergence of OVA-specific CD4$^+$FasL$^+$ T cells requires functional Fas/FasL interaction. In the adoptive transfer experiments using BALB/c and DO11.10 mice as a recipient and a donor, respectively, feeding of OVA led to clonal expansion of OVA-specific CD4$^+$ T cells followed by clonal deletion in the liver, which is coupled with the emergence of FasL$^+$ cells. In vitro study disclosed that hepatic CD4$^+$ T cells from mice fed OVA secreted IL-10, TGF-β_1, and a large amount of IL-4 upon stimulation with $OVA_{323-339}$ peptide. Cell sorting analysis showed that IL-4 high producer cells in the liver were OVA-specific CD4$^+$ T cells expressing FasL (5). Thus, oral administration of OVA at a high dose led to the development of OVA-specific CD4$^+$FasL$^+$ T cells secreting IL-4, IL-10 and TGF-β1 in the liver. It should be noted that development of these unique T cells does not require gut-associated lymphoid tissue since systemic injection of OVA also generated OVA-specific CD4$^+$FasL$^+$ T cells with similar cytokine profile in the liver.

Since Th1 cells are more sensitive to Fas-mediated apoptosis than Th2 cells, (9)(10) and IL-4 prevents apoptosis of T cells (11), we speculate that Fas$^+$Th1 cells are preferentially deleted by Fas-mediated apoptosis in the liver and as a result of this, FasL$^+$Th2 cells are selected (Figure 1). As expected from the cytokine profile and cell surface ligand expression, hepatic CD4$^+$ T cells in DO11.10 mice fed OVA suppressed the proliferation of by-stander naive T cells in an Ag-nonspecific manner. This type of suppressor effect was mainly mediated by Fas/FasL interaction, and treatment with anti-IL-4, IL-10, and TGF-β, Abs showed a marginal effect. Adoptive transfer of hepatic CD4+ T cells from DO11.10 mice fed OVA into naïve BALB/c mice and subsequent antigenic challenge led to suppression of proliferative, Th1, and DTH responses specific to OVA in the recipient mice, which effects were abrogated by the treatment of anti-FasL antibody (5). These data suggested that, on administration of an Ag at a high dose, Fas-mediated clonal deletion of Th1 cells and generation of regulatory FasL+ Th2-like cells were not independent but were synergistic and interdependent events in the liver (Figure 1).

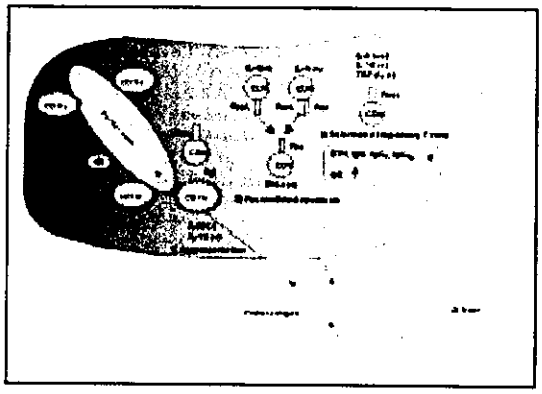

Figure 1. Model of a developmental mechanism of regulatory CD4+FasL+ T cells in the liver. See conclusion section for explanation.

Ag-presenting cells in the liver are required to achieve portal vein tolerance

Kupffer cells, dendritic cells (DCs), and sinusoidal endothelial cells (LSECs) can potentially present Ags to CD4$^+$ T cells in an MHC class II-restricted manner (12)(13). Therefore, we asked whether Ag-presentation by LSECs or DCs can drive naïve CD4$^+$ T cells to differentiate into Th2-like cells (14) (15). Firstly, we confirmed that

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FITC-labeled OVA administered orally was co-localized with class II$^+$ CD11c$^+$ Ag-presenting cells (APCs) in the periportal areas of the liver, indicating that a part of Ags administered was transported to the liver and taken up by hepatic APCs. Secondly, we assessed cytokines produced by CD11c$^+$ cells in the liver of mice fed OVA. Upon stimulation with agonistic anti-CD40 Ab, IL-12 production by liver CD11c$^+$ cells was remarkably decreased, whereas IL-18 production was increased. No significant changes in the secretion of these cytokines were seen in splenic CD11c$^+$ cells (7).

Since both Th1 and Th2 responses are enhanced by IL-18 depending on the presence of IL-12 (16)(17), we assessed whether Ag-presentation by these APCs in vitro could drive CD4$^+$ T cells to differentiate as seen in vivo (5). Indeed, production of both IL-4 and IL-10 by naïve $OVA_{323-339}$ specific CD4$^+$ T cells from the spleen of Rag2-/- DO11.10 mice were remarkably enhanced by coculture with hepatic CD11c$^+$ cells from BALB/c mice fed OVA. Moreover, presentation of $OVA_{323-339}$ peptide by these hepatic CD11c$^+$ cells led to Fas-mediated apoptosis of naïve OVA-specific CD4$^+$ T cells as well as the emergence of FasL$^+$ cells (7). Again IL-12 was also involved in this selection process of FasL$^+$Th2-like cells since addition of IL-12 prevented apoptosis of Th1 cells. Systemic generation of Th2-like cells by interaction with hepatic CD11c$^+$ cells was also confirmed by in vivo study, in which we transferred naïve OVA-specific CD4$^+$T cells together with hepatic CD11c$^+$ cells from mice fed OVA and systemically immunized with OVA. We saw development of Th2 cells as well as apoptosis of the transferred CD4$^+$ T cells in the lymph nodes of the recipient mice (7). These data altogether suggested that presentation of an ingested Ag occurs in the liver and that this Ag-presentation by hepatic APCs was sufficient for selection of regulatory FasL$^+$Th2-like cells in the liver (Figure 1).

Food allergy and hepatic CD4+ T cells

Not all but most of the patients with food allergy have increased IgE response to dietary Ags. The mechanisms which induce and maintain this type of IgE response are unknown. To explore possible involvement of hepatic CD4$^+$ T cells in this response, we transferred hepatic CD4$^+$ T cells from DO11.10 mice fed OVA into naïve BALB/c mice, followed by systemic immunization with OVA. In the recipient mice, serum anti-OVA IgE Ab response was remarkably increased, which was associated with decrease of anti-OVA IgG, IgG_1, IgG_{2a} Ab responses. No difference in IgE response was seen in the mice which received PP or splenic CD4$^+$ T cells. In this study, we found that FasL and IL-4 played non-redundant role in this anti-OVA Ab response; blockade of Fas/FasL interaction in the recipient mice selectively restored anti-IgG, IgG_1,IgG_{2a} responses whereas transfer of hepatic CD4+ T cells from IL-4-/-DO11.10 mice normalized anti-IgE response (6). Thus, our study indicated that if a dietary Ag stimulates a significant number of CD4$^+$ T cells in the liver, then the Ag- primed cells could be helper cells, which induce and maintain IgE response to the food Ag. Compatible to this idea, there was a case-report describing transfer of peanut allergy from the donor to the recipient of the liver transplant, not to the other recipient of the kidney and pancreas from the same donor (18).

Conclusion

Based on the findings as alluded above, we propose that the liver plays an important role as an organ generating regulatory CD4$^+$FasL$^+$ T cells which share some of the properties of regulatory T cells described previously (19) (20) (21). We showed that generation of this type of regulatory T cells was a concomitant event with massive deletion of Th1 cells, which was mostly mediated by Ag-presenting CD11c$^+$ cells in the periportal areas (Figure 1). The property of hepatic CD4$^+$FasL$^+$ T cells seems beneficial to the liver as an organ which encounters various Ags and microbial toxins generated in the gastrointestinal tract. We conclude that hepatic CD4$^+$FasL$^+$ T cells participate in portal vein tolerance and that they may also be involved in the pathogenesis of food allergy depending on a frequency and dose of Ags migrating via the portal vein.

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Analysis of 388 cases of primary sclerosing cholangitis in Japan Presence of a subgroup without pancreatic involvement in older patients

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Abstract

We analyzed 388 cases of primary sclerosing cholangitis (PSC) in Japan, according to a questionnaire sent to gastroenterologists. There was male predominance (59%), and interestingly there were two peaks in the age distribution as seen in the previous study. Jaundice and itching, major symptoms in PSC patients included in the diagnostic criteria, were observed only 28 and 16%, respectively. Alkaline phosphatase level was less than twofold of the upper limit of the normal range in 35%. In this regard, the diagnostic criteria in 2003 from Mayo Clinic, including cholestatic symptoms and two to three-fold increases in serum alkaline phosphatase, should be modified in Japan. Inflammatory bowel diseases were complicated in 37%, and autoimmune pancreatitis (AIP) in 7.2%. PSC cases with inflammatory bowel diseases were younger than the average, creating the first peak in the age distribution, and have similar characteristics compared to patients with PSC in foreign countries. By contrast, those with AIP, who were more than 50 years old, responded well to corticosteroid therapy. In addition, even after the exclusion of cases of sclerosing cholangitis complicated with AIP, the second peak in the age distribution was clearly evident. Therefore, we conclude that PSC patients without apparent involvement of the pancreas are present in the older patients and seem to be specific in Japan.

Keywords: Primary sclerosing cholangitis; Ulcerative colitis; Autoimmune pancreatitis

1. Introduction

Primary sclerosing cholangitis (PSC) has been thought to be a rare disease in Japan. In our previous study, the characteristics of PSC in Japan have been identified [1]. PSC patients in Japan were shown to have two peaks in the age distribution, which has never been observed in other countries [2–7]. The younger patients were more frequently complicated with inflammatory bowel diseases (IBD), whereas pancreatitis was often observed in the older patients [1]. Therefore, the younger patients in Japan had comparable characteristics to PSC patients in other countries [2–7]. However, our previous study was performed in 1995 and it remains unclear whether clinical characteristics of PSC have been changed since then.

In Japan, the diagnosis of PSC used to be done according to the diagnostic criteria from Mayo Clinic published in 1984 [8], and nowadays according to the new criteria from the same institute [9]. The recent criteria include the presence of cholestatic symptoms and the two to threefold increase in alkaline phosphatase level [9], but we notice that such findings are sometimes absent in PSC patients in Japan.

According to the workshops at annual meetings, IBD complicated with PSC has been shown to be atypical in various aspects [10]; first, mild colitis may sometimes be observed by a routine colonoscopy. Second, ulcerative colitis (UC) complicated with PSC tends to be atypical; mild in activity and sometimes right-sided colon predominant. On the other hand, accumulating evidences suggest that PSC cases complicated with pancreatitis are considered as a different entity of disease from PSC, especially after the proposal of the concept of autoimmune pancreatitis (AIP) [11–14].

At the workshop on PSC during Digestive Disease Week, Japan, 2003, we had a chance to perform a nation-wide survey of PSC by a questionnaire sent to gastroenterologists in Japan. In the present report, we analyzed the results of the survey, and compared the present results with our previous national survey performed in 1995 [1]. We also assessed the

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diagnostic criteria from Mayo Clinic of 2003 [9], to elucidate whether it is applicable to Japanese patients. Furthermore, we paid particular attentions to the aspect of complication of IBD and AIP.

2. Materials and methods

A questionnaire was sent to active members of the Japan Society of Hepatology, the Japanese Society of Gastroenterology, and the Japan Gastroenterological Endoscopy Society, and answers describing 388 cases were obtained from 90 physicians and surgeons.

These cases were analyzed from various aspects including diagnosis, complications, therapies and prognosis. Analyses were also performed for the difference in clinical features among total PSC patients, patients with IBD, and patients with AIP. Differences of the characteristics of PSC cases diagnosed before and after 1995 were also studied.

Differences in the incidence were examined by χ^2-test or Student's t-test, when applicable, and those with a $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Background of patients

Table 1 summarizes the major profiles of patients in the present study. Among 388 cases, 40 cases were diagnosed in 1975–1989, 79 cases in 1990–1994, 132 cases in 1995–2000, and 137 cases in 2000–2004. As has been reported before [1], male predominance (59%) was noted and there were two peaks in the age distribution at diagnosis (Fig. 1). Mean age was 47 years old, which is the same as our previous report [1]. Jaundice and itching, major symptoms in PSC patients included in the diagnostic criteria [9], were observed only 28 and 16%, respectively.

Table 1
Profiles of patients in the present survey

	Number of cases/total number	%
Male	222/379	59
Symptoms		
Jaundice	107/376	28
Itching	60/374	16
Eosinophilia (≧5%)	109/278	39
Positive anti-nuclear antibody	115/232	36
Bile duct damage		
Intra + extrahepatic	252/370	68
Intrahepatic	100/370	27
Extrahepatic	18/370	4.8
Ludwig's stage		
1	82/221	37
2	83/221	38
3	33/221	33
4	23/221	23
Complications		
Inflammatory bowel diseases	125/388	37
Autoimmune pancreatitis	28/388	7.2
Biliary stone	63/388	16
Biliary cancer	15/388	4.3

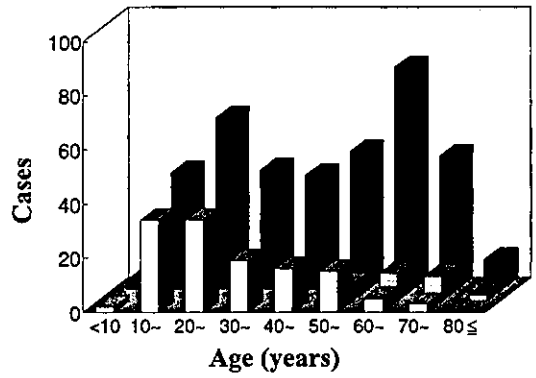

Fig. 1. Age distribution of patients with PSC in Japan. Black columns represent total cases ($n = 388$), white columns represent cases with IBD ($n = 125$), and dotted columns represent cases with AIP ($n = 28$).

3.2. Blood analyses

Alkaline phosphatase levels were normal (≤N, where N is the upper limit of normal value) in 12%, 1N–2N, in 23%, 2N–5N in 46%, and >5N in 18%. Thus, 35% of cases did not meet the diagnostic criteria that alkaline phosphatase levels increase to two- to three-fold [9]. Total bilirubin levels were normal (≤1.2 mg/dl) in 61% cases, 1.3–3.0 mg/dl in 16% cases, 3.1–10 mg/dl in 16% cases, and >10 mg/dl in 7%. Alanine aminotransferase levels were 40 IU/l or less in 27%, 41–100 IU/l in 37%, 101–200 IU/l in 22%, and >200 IU/l in 14%.

Eosinophilia (≥5%) was observed in 39%. Anti-nuclear antibody and perinuclear antineutrophil cytoplasmic antibodies were positive in 36 and 13% (22/172), respectively.

3.3. Diagnosis

Eighty percent (335/378) of patients were diagnosed by endoscopic retrograde cholangiopancreatography, 32% (121/378) by magnetic resonance cholangiopancreatograpy, 12% (45/378) by percutaneous transhepatic cholangiography, and 1.6% (6/378) by intraductal ultrasonography. Bile duct damage was observed mainly intra + extrahepatic (68%) (Table 1). Small duct PSC was observed only in six cases (1.6%).

Among 370 cases, liver biopsy was performed in 284 cases (78%); 239 cases (65%) by needle biopsy, 34 cases (9.2%) by surgical resection, nine cases (2.4%) by liver transplantation, and four cases (1.1%) by autopsy. Bile duct

damage of PSC was observed in 69% (193/279), and the findings of cholestasis were observed in 46% (1453/266). Ludwig's stage [15] was 1 in 37%, 2 in 38%, 3 in 33% and 4 in 23% (Table 1).

3.4. Complications in terms of age distribution

The incidence of complications of IBD was 37% (Table 1), which was increased to 61% in 206 cases in whom colonoscopy was performed. Among 125 cases with IBD, UC was 99 cases (79%), non-specific colitis was 12 cases (9.6%), Crohn's disease was eight cases (6.4%), and others such as eosinophilic colitis and unclassified colitis were six cases (4.8%). Among 99 cases with UC, 26 cases (26%) were atypical as UC, with 11 cases of right-sided colon predominant colitis.

Complication of AIP or sclerosing pancreatitis was observed only in 28/388 (7.2%). Gallstones and biliary cancers were observed in 16 and 4.3%, respectively. Among 63 cases of gallstones, 43 cases was gallbladder stones, 21 cases was common bile duct stones, and 17 cases were intrahepatic stones. There was no case in whom both IBD and AIP were present.

In terms of age distribution, there is a quite difference between PSC with IBD and with AIP. The peak of PSC patients complicated with IBD were mainly in teen to twenties, while PSC cases with AIP were only observed more than fifties (Fig. 1). Of note, the second peak of whole PSC patients in 50–60 years old was much larger than the peak of PSC wit AIP cases, and thus the second peak was clearly evident even after elimination of PSC cases complicated with AIP.

3.5. Therapies

As drug therapies, ursodeoxycholic acid (UDCA) was administered in 78% (304/388), and was answered to be effective in almost all cases (175 cases). Corticosteroid was administered in 31% (102/388), and was answered to be effective in 63 cases. Endoscopic therapy was performed in 14% (53/388); 30 cases with drainage, 21 cases with stenting, and 13 cases with balloon dilatation. Percutaneous transhepatic therapy was performed in 14% (53/388); 49 cases with drainage, seven cases with stenting, and seven cases with balloon dilatation.

Operations were performed in 10% (40/388). Liver transplantation was performed in 10% (40/388). Mean age of liver transplanted patients was 27 years old (3–66), and 22 cases (55%) was complicated with IBD.

3.6. Prognosis

Seventeen percent of the patients (66/388) died during the follow up period; 32 cases by liver failure, seven cases by biliary cancers, and seven cases after the surgery, and five cases by colon-related diseases.

4. Discussion

4.1. Differences of the characteristics of PSC cases diagnosed before and after 1995

We demonstrated in the previous report that there were two peaks in age distribution, one in the twenties and the other in the fifties and sixties [1]. In the current study, the age distribution of PSC cases diagnosed before and after 1995 was similar with two peaks (Fig. 2). The age distribution of cases with IBD and pancreatitis was also identical before and after 1995. These results suggest that the situation of PSC patients is similar before and after PSC patients in Japan, in which heterogeneous patients are included (Table 2).

Table 3 shows the differences of the characteristics of PSC cases before and after 1995. The prevalence of eosinophilia was higher after 1995. The distribution of Ludwig's stage was different, with more stage 4 after 1995. The distribution of bile duct damage was also different with less cases of extrahepatic bile duct damage after 1995. Since the cases only with extrahepatic bile duct damage seem different from typical PSC cases, more extensive diagnostic approach may have reduced the prevalence of these patients.

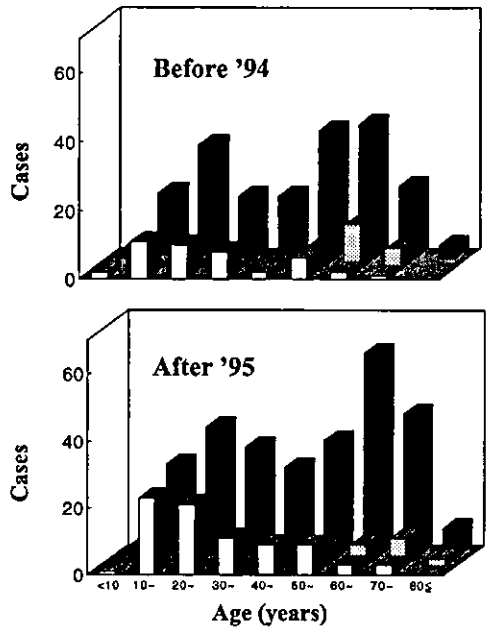

Fig. 2. Age distribution of PSC cases diagnosed before and after 1995. The upper figure shows the patients before 1994 in the previous survey [1], and black, white and dotted columns represent total cases ($n = 191$), those with IBD ($n = 42$) and those with pancreatitis ($n = 29$), respectively. The lower figure shows the patients after 1995 in the present survey, and black, white and dotted columns represent total cases ($n = 269$), those with IBD ($n = 80$) and those with AIP ($n = 21$), respectively.

Table 2
Differences in characteristics among total PSC patients, patients with IBD, and patients with AIP

	Total cases (388 cases)	Cases with IBD (125 cases)	Cases with AIP (28 cases)
Sex (male cases) (%)	59	63	64
Rate of death (%)	7	13%	18
Cases with jaundice (%)	28	19	41#
Total bilirubin level (mg/dl)	3.1 ± 5.6	2.4 ± 4.8	3.2 ± 3.2
Alkaline phosphatase level (×*N*)	3.4*N* ± 2.8*N*	3.6*N* ± 2.3*N*	4.5*N* ± 3.0*N*
Alanine aminotransferase level (IU/l)	113 ± 124	115 ± 118	186 ± 172#
Elevated alkaline phosphatase (≥2*N*) (%)	64	66	79*,#
Eosinophilia (%)	39	41	32
Positive for anti-nuclear antibody (%)	36	37	35
Bile duct damage compatible with PSC by liver histololgy (%)	69	79	29*,#
Ludwig's stage (1 (%)/2 (%)/3 (%)/4 (%))	37/38/15/10	31/35/26/8	54/46/0/0
Bile duct damage (intra + extrahepatic (%)/intrahepatic (%)/extrahepatic (%))	68/25/5	74/22/4	64/18/18*,#
Complication of bile duct cancer (%)	4	2	0
Cases with steroid therapy (%)/effective cases (%)	31/16	36%/15%	61/61*,#
Cases with UDCA therapy (%)/effective cases (%)	78/45	94%/50%	39*,#/29
Endoscopic therapies (%)	14	6*	46*,#
Transhepatic therapies (%)	14	8	14
Operation (%)	10	6	18#
Liver transplantation (%)	10	17	0*,#

* $P < 0.05$ vs. total cases.
# $P < 0.05$ vs. cases with IBD (χ^2 test).

The complication of IBD was increased after 1995, and that of pancreatitis was decreased after 1995. The increase in the percentage of the complication of IBD may be due to the recognition that IBD complicated with PSC is relatively mild and that total colonoscopy should be performed even without the symptoms due to the colon [10]. In contrast, the decrease in the percentage of the complication of pancreatitis may be due to the fact that many doctors do not diagnose the cases with sclerosing cholangitis complicated with AIP as PSC.

UDCA was administered in 78% of PSC patients in the current study, while 49% of the patients were treated by UDCA before 1995 [1]. The increase and preference of UDCA may be due to the reports published after 1995, demonstrating effectiveness of UDCA for PSC [16–18]. However, the effect of UDCA on the long-term prognosis of this disease is to be elucidated [19].

4.2. Applicability of the diagnostic criteria proposed by Mayo Clinic to PSC patients in Japan

As known, the diagnostic criteria in 2003 from Mayo Clinic includes three items; (1) typical characteristic abnormalities involving the biliary tree, (2) compatible clinical and biochemical findings, and (3) exclusion of secondary sclerosing cholangitis. In particular, radiographic findings of biliary tree are regarded as a diagnostic standard. In Japanese cases, cholangiographic studies are also employed in most

Table 3
Differences of the characteristics of PSC cases diagnosed before and after 1995

	Diagnosed in 1994 and before (192 cases)	Diagnosed after 1995 (269 cases)
Eosinophilia (≥5%) (%)	27 (39/142)	41 (82/199)
Ludwig's stage (%)		
1	26 (26/100)	39 (59/152)
2	49 (49/100)	39 (59/152)
3	20 (20/100)	11 (17/152)
4	5.0 (5/100)	11 (17/152)
Bile duct damage (%)		
Intra + extrahepatic	69 (129/160)	68 (176/260)
Intrahepatic	17 (32/187)	26 (68/260)
Extrahepatic	14 (26/187)	6.2 (16/260)
Complication of IBD (%)	24 (43/176)	33 (80/242)
Complication of pancreatitis (%)	16 (29/150)	8.5 (21/246)

Cases in 1994 and before are those included in the previous study [1], and cases after 1995 are those diagnosed after 1995 in the present study. All data presented in this table are significantly different between two groups (χ^2-test).

cases for diagnosis of PSC. Endoscopic retrograde cholangiography was performed in 335/378 (80%) of patients with PSC, suggesting that this modality is considered to be the most reliable approach for diagnosis by gastroenterogists in Japan.

However, Japanese PSC patients fail to meet the Mayo's diagnostic criteria in two aspects. First, in the criteria, clinical symptoms such as history of IBD and cholestatic symptoms are required for the diagnosis. However, in this survey, jaundice and itching were noted only in 28 and 16% of PSC patients, respectively. The history of IBD was observed only in 37%. Therefore, obviously clinical symptoms should not be rigorously involved in the criteria. Second, "compatible biochemical findings" are described as "two to three-fold increases in serum alkaline phosphatase for longer than 6 months." By contrast, our national survey demonstrates that alkaline phosphatase levels were normal in 12% and 1*N*–2*N* in 23%. Thus, 35% of PSC patients are not diagnosed as having PSC if the Mayo's criterion is utilized. Taken together, we propose that the criteria of clinical and biochemical findings should not be strictly applied for the diagnosis of Japanese PSC patients.

4.3. Differences in characteristics among total PSC patients, patients with IBD, and patients with AIP

Complication of AIP or sclerosing pancreatitis was observed in 7.2% (Table 1). It has been demonstrated that extrahepatic bile ducts as well as pancreatic duct are frequently involved in patients with AIP, resulting in stricture of distal bile ducts mimicking PSC [12,13,20,21]. Recently, Hyodo et al. reported that strictured common bile ducts were noted in all five patients with AIP investigated [21]. Nakazawa et al. proposed in 2001 that PSC cases associated with chronic pancreatitis and with better prognosis could be categorized as atypical PSC, and should not treated as prototype PSC. [13]. Therefore, it is very likely that PSC patients with AIP reported in this survey include atypical PSC, proposed by Nakazawa. However, it is noteworthy that the second peak of the whole PSC patients in the age distribution was still evident even after the exclusion of the cases with AIP, demonstrating that PSC cases without apparent involvement of the pancreas, corresponding to at least some of the second peak, are present in Japan. This subgroup seems to be specific to Japan, which have different characteristics compared to those in foreign countries [2–7].

Percentage of sex distribution was similar among three subgroups; PSC with IBD, PSC with AIP and PSC without AIP. Unexpectedly, rate of death was similar among three groups, since younger cases with IBD are considered to progress more rapidly and the cases with AIP may have better prognosis due to a good response to corticosteroid therapy [11–14]. This may be due to the fact that many younger patients with IBD were relieved by liver transplantation.

Jaundice and elevated liver tests were more frequent in patients complicated with AIP. These data suggest that these patients are more likely to be subjected to obstructive jaundice due to the swelling of the pancreas head. The less frequent prevalence of bile duct damage and lower Ludwig's stage detected by liver histology, more frequent extrahepatic bile duct damage, and no complication of bile duct cancer in patients complicated with AIP suggest that these patients have entirely different characteristics to the cases without AIP. The more cases with effective steroid therapy, less cases treated with UDCA, more cases with endoscopic therapy, and no case subjected to liver transplantation in the cases with AIP also lead to the same conclusion. In contrast, more cases with operation in the cases with AIP suggest that many patients were operated under the diagnosis of pancreatic cancers.

Taken together, the current survey suggested that PSC cases without apparent pancreatic involvement are present in the second peak in the age distribution of the whole patients, and different from younger PSC patients frequently complicated with IBD. At the workshop on PSC during Digestive Disease Week, Japan 2003, the presence of IgG4-related autoimmune cholangiopathy without pancreatitis was advocated as a subtype of PSC. Kamisawa et al. recently reported that the infiltration of IgG4-positive plasma cells was observed not only in the pancreas, but in the bile duct of AIP cases [22]. Therefore, the patients with IgG4-realted autoimmune disease, which clinically manifest only the sclerosing cholangitis lesion, may overlap the PSC patients without pancreatic lesion we reported herein. Further work is needed to clarify this possibility.

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ミトコンドリア抗体：最近の展開

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索引用語：抗ミトコンドリア抗体，PDC-E2，分子相同性，xenobiotics

1 はじめに

抗ミトコンドリア抗体（anti-mitochondrial antibody; AMA）は原発性胆汁性肝硬変（primary biliary cirrhosis; PBC）患者血清のおよそ90％以上に検出され，PBCの診断に繁用されている．日常臨床において胆道系酵素の上昇を認めた場合，悪性腫瘍や総胆管結石などの精査を行うと同時にAMAを検索し，陽性であればPBCを考えて入院・肝生検を勧める，というのが通常の流れであろう．

ここ20年にわたり，PBCの研究はこのAMA—正確に述べればミトコンドリアに存在する自己抗原に対する免疫反応の解析—を中心に進められ，かなりの進歩がみられている．宿主の免疫反応のうち，T細胞による自己抗原に対する免疫反応は，本特集号では下田・喜多によって述べられており，本稿では自己抗体（B細胞による自己抗原に対する免疫反応）に焦点を絞り，PBCの診断，および病因におけるAMAの位置づけにつき何がわかっており，何がわかっていないのか，について概説する．

2 AMAによって認識される自己抗原

はじめに，AMAの認識するミトコンドリア抗原について整理しておく．AMAはミトコンドリア内膜に存在する2-オキソ酸脱水素酵素複合体（2-oxo acid dehydrogenase complex; 2-OADC）と呼ばれる酵素群，ことにその中のいくつかの酵素を認識することがわかっている．2-OADCはさらにピルビン酸脱水素酵素（pyruvate dehydrogenase complex; PDC），分岐鎖オキソ酸脱水素酵素（branched chain 2-oxo acid dehydrogenase complex; BCOADC），オキソグルタル酸脱水素酵素（2-oxoglutarate dehydrogenase complex; OGDC）の3種に分類され，それぞれの酵素はE1，E2，E3のサブユニットから構成されている．これらのうち，AMAが特異的に認識するものはそれぞれのE2コンポーネント（PDC-E2，BCOADC-E2，OGDC-E2），および以前はprotein Xと呼ばれていたE3 binding protein（E3BP）が主である．加えて，AMAは頻度は低いもののPDC-E1αをも認識することが知られている（表1）．

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