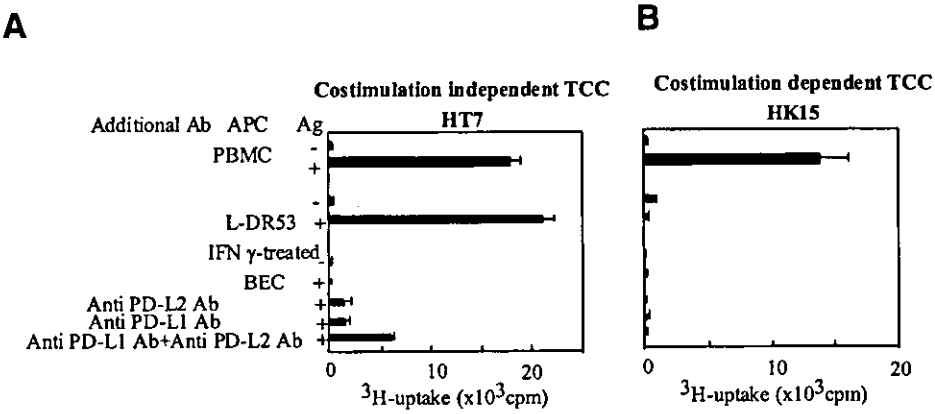

Fig. 2. Peripheral blood mononuclear cells (PBMCs), L-DR53, and biliary epithelial cells (BECs) as antigen-presenting cells (APCs). (A) Human leukocyte antigen (HLA)-DR53-restricted costimulation-independent HT7 and (B) costimulation-dependent HK15 clones were cocultured with antigen (Ag)-pulsed or control PBMCs, L-DR53, interferon (IFN)γ-treated BECs as APCs. Both HT7 and HK15 proliferated when cocultured with Ag-pulsed HLA-DR53-positive PBMCs. HT7, but not HK15, proliferated when cocultured with Ag-pulsed L-DR53. Neither HT7 nor HK15 proliferated when cocultured with Ag-pulsed IFNγ-treated BECs. Next, HT7 and HK15 were cocultured with Ag-pulsed BECs with blockade of programmed death (PD 1) ligands through neutralizing anti-programmed death 1 ligand 1 (PD-L1) Ab or neutralizing anti-programmed death 1 ligand 2(PD-L2) Ab. HT7 proliferated partially when cocultured with Ag-pulsed BECs in conjunction with blocking agents for both PD-L1 and PD-L2, and HK15 did not proliferate with Ag-pulsed BECs in conjunction with blocking agents for both PD-L1 and PD-L2. TCC, T-cell clone.

for 72 hours. Thymidine-uptake or IFNγ production was then measured. BECs strongly regulated the proliferation and IFNγ production of TCCs (17,060 ± 1,464 cpm vs. 616 ± 276 cpm for ^{3}H-TdR, $P < .0001$, 87,400 ± 6,953 pg/mL vs. 80 ± 10 pg/mL for IFNγ, $P < .0001$) (Fig. 3A). We next investigated the dependence of this regulation on cell–cell contact between BECs and T cells using a transwell compartment culture system. TCCs and Ag-pulsed irradiated PBMCs were cocultured in the lower compartment, while BECs were added to the lower or upper compartment as AC. BECs in either the lower or upper compartment similarly suppressed the proliferation of TCCs (21,264 ± 744 cpm vs. 5,246 ± 458 cpm or 6,266 ± 844 cpm) (Fig. 3B), indicating that BECs regulate T-cell activation not only via the cell-contact–dependent manner but also via the cell-independent manner.

BECs Regulate T-Cell Activation by Producing PG-E2. To further determine the mechanism of T-cell regulation by BECs as ACs, TCCs were cocultured with Ag-pulsed PBMCs as APCs (18,173 ± 331cpm) and BECs as ACs (8,155 ± 1,358 cpm) ($P = .0002$, compared with Ag-pulsed PBMCs only) in the presence of various agents that block the putative suppressive molecules. The TCCs were not activated in the presence of anti-TGFβ Ab (8,296 ± 250 cpm), anti–IL-10 Ab (7,250 ± 258 cpm), or NG-monomethyl-L-arginine (7,595 ± 550 cpm),

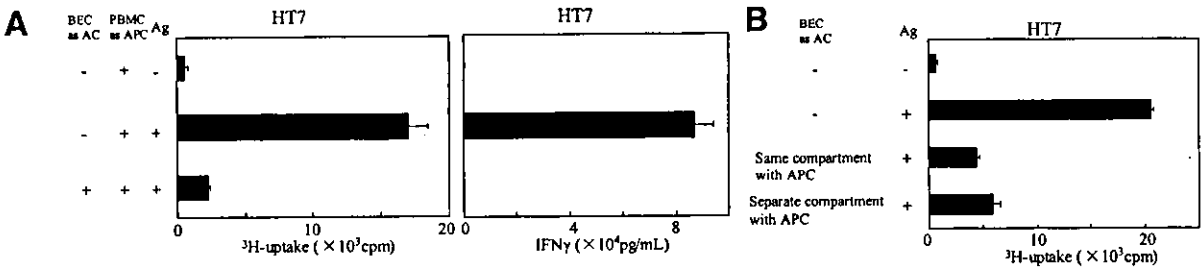

Fig. 3. Biliary epithelial cells (BECs) regulate the activation of TCCs in a cell-contact-independent manner. (A) The effect of BECs on the proliferation of and interferon (IFN)γ production by HT7 was assessed by culturing with antigen (Ag)-pulsed irradiated peripheral blood mononuclear cells (PBMCs) as antigen-presenting cells (APCs) in the presence or absence of BECs as accessory cells (ACs) for 72 hours. ^{3}H-thymidine uptake and the concentration of IFNγ in culture supernatants were then measured. BECs strongly regulated both the proliferation of and IFNγ production by HT7. (B) To determine whether the regulation of T-cell activation by BECs required cell-to-cell contact, a transwell compartment culture system was utilized. Ag-pulsed irradiated PBMCs and HT7 were cocultured in the lower compartment of a 24-well plate, BECs were added to either the lower or upper compartment. ^{3}H-thymidine uptake was measured after 72 hours. BECs in the upper compartment suppressed the proliferation of HT7 at levels similar to suppression seen following culture of the BECs in the lower compartment.

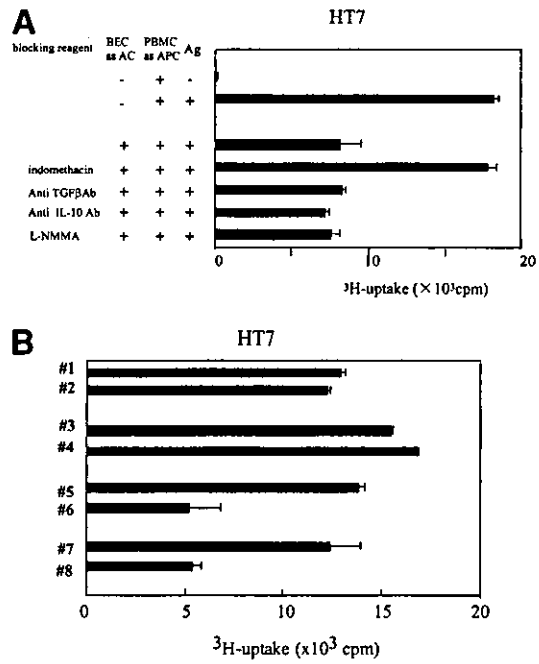

Fig. 4. Biliary epithelial cells (BECs) regulate T-cell activation via prostaglandin E2 (PG-E2). (A) To determine the mechanism by which BECs regulate T-cell activation in a cell-contact-independent manner, HT7 cells were cocultured with antigen (Ag)-pulsed BECs with blockade of PG-E, transforming growth factor (TGF)β, interleukin (IL-10, or nitric oxide using indomethacin, neutralizing anti-TGF antibody, neutralizing anti-IL-10 Antibody, or NG-monomethyl-L-arginine. (B) ^{3}H-thymidine uptake of T-cell clones (TCCs) was then measured using culture supernatants obtained from coculture of BECs, TCCs, and irradiated peripheral blood mononuclear cells.

which block the production of nitric oxide, and was activated in the presence of indomethacin (17,778 ± 572 cpm), which block the production of PG-E2, respectively (Fig. 4A). The supernatant derived from the various culture groups described in Table 1 was added to the T-cell proliferation assay. Supernatants of #6 and #8 inhibited the proliferation of TCCs while others did not (Fig. 4B and Table 1).

Next, we measured the concentration of PG-E2 in culture supernatants obtained from cocultures of BECs, TCCs, and irradiated PBMCs in the designated combinations (Fig. 5A). BECs (#1) or TCCs (#2) produced low levels of PG-E2 (Fig. 5A and Table 1). While BECs produced low amounts of PG-E2 upon coculture with Ag nonpulsed irradiated PBMCs (#3), Ag-pulsed irradiated PBMCs (#4), or Ag nonpulsed irradiated PBMCs and TCCs (#5), BECs produced large amounts of PG-E2 upon coculture with Ag-pulsed irradiated PBMCs and TCCs (#6) (Fig. 5A and Table 1). While the supernatants obtained from culturing BECs in the presence of the supernatants from cultures of TCCs and Ag nonpulsed irradiated PBMCs (#7) contained only very low levels of PG-E2, supernatants obtained by culturing BECs in the presence of the supernatants obtained from cultures of TCCs and Ag-pulsed irradiated PBMCs (#8) contained high levels of PG-E2 (Fig. 5A and Table 1). All supernatants obtained from BEC cultures contained high levels of TGFβ (7,013-13,606 pg/mL) but did not contain detectable levels of either IL-10 (<8 pg/mL) or nitrite (<2 mmol/L) (data not shown).

Some parenchymal cells or tumor cell lines produce PG-E2 following stimulation with proinflammatory cytokines, including TNFα and IL-1β, or LPS.[19–21] Since the supernatants obtained by culturing TCCs with Ag-

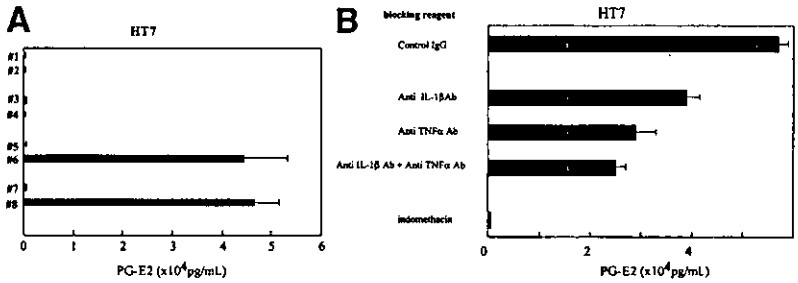

Fig. 5. Biliary epithelial cells (BECs) produced prostaglandin E2 (PG-E2) when stimulated by direct cellular interaction or by indirect produced cytokine. (A) PG-E2 production was assessed by enzyme-linked immunosorbent assays using culture supernatants obtained from coculture of BECs, T-cell clones (TCCs), and irradiated peripheral blood mononuclear cells (PBMCs). BECs, activated HT7, BECs with inactivated TCCs, or BECs with supernatant of inactivated TCCs produced low levels of PG-E2. High levels of PG-E2 were detected when BECs were cocultured with activated TCCs or when BECs were cultured with supernatant of activated TCCs. (B) BECs were cultured in the presence of supernatant of activated TCCs with neutralizing anti-tumor necrosis factor (TNF)α antibody, anti-interleukin 1 Ab, control immunoglobulin G, or indomethacin. The concentration of PG-E2 in the culture supernatant was then measured. The production of PG-E2 was partially blocked by anti-TNFα and anti-interleukin-1β and was completely blocked by indomethacin. IgG, immunoglobulin G; IL, Interleukin.

pulsed irradiated PBMCs contained TNFα (2,080 ± 120 pg/mL) and IL-1β (2,480 ±190 pg/mL), we examined the effect of these proinflammatory cytokines on BECs to produce PG-E2. BECs produced high levels of PG-E2 after stimulation with recombinant TNFα, recombinant IL-1β, or LPS (93,333 ± 11,614, 180,000 ± 24,944, and 61,000 ± 2,055 pg/mL, respectively). Furthermore, the production of PG-E2 by BECs in the presence of the supernatants obtained from cultures of TCCs and Ag-pulsed irradiated PBMCs (56,333 ± 3,091), which was blocked by indomethacin (570 ± 83) ($P < .0001$ compared with cultures of TCCs and Ag-pulsed irradiated PBMCs), could be partially blocked by neutralizing anti-TNFα Ab (29,333 ± 4,028) ($P < .001$ compared with cultures of TCCs and Ag-pulsed irradiated PBMCs), anti-IL-1β Ab (39,333 ± 1,886) ($P < .01$, compared with cultures of TCCs and Ag-pulsed irradiated PBMCs) or combination of anti-TNFα Ab and anti-IL-1β Ab (24,667 ± 2,357) ($P < .001$ compared with cultures of TCCs and Ag-pulsed irradiated PBMCs) (Fig. 5B). These observations indicate that TNFα and IL-1β from TCCs with Ag-pulsed irradiated PBMCs induced the production of PG-E2 by BECs.

Discussion

The activation of T cells requires 2 signals: the first mediated by interaction of the T-cell receptor with peptide-MHC complexes on the APC, and the second a costimulatory signal, mediated by the interaction of a set of ligands and cognate receptors. The interaction of B7 molecules (CD80 or CD86) on APC with CD28 on T cells is required for full T-cell activation and effector function. As BECs do not express B7 molecules, as determined by flow cytometry, the lack of a costimulatory signal is speculated to be the mechanism preventing BECs from functioning as APCs.[22] However, while the TCCs used in the present study, HT7, could proliferate in response to the T-cell receptor–peptide/MHC signal alone in a costimulation–independent manner, Ag-pulsed HLA-DR–expressing BECs still could not induce proliferation. This observation suggests that Ag expressed BECs on their HLA-DR may actively conduct a regulatory signal to T cells, which recognize the Ag that overcame the initial T-cell receptor–peptide/MHC stimulation.

It was therefore of interest to investigate the role of the BEC as either an APC or an AC in the activation of Ag-specific $CD4^+$ T cells. Interestingly, in the experimental context herein, BECs did not function as APCs. This lack of activation results in part from the expression of inhibitory molecules, such as PD-L1 and PD-L2. BECs as ACs regulated T-cell activation through production of PG-E2. Also, TNFα and IL-1β, produced during the coculture of Ag-specific T cells with Ag-pulsed professional APCs induced PG-E2 production by BECs.

These data highlight several key mechanisms. First, the recently identified costimulatory molecule PD-1, a member of CD28 families, belongs to the immunoglobulin superfamily, which also contains an immunoreceptor tyrosine-based inhibitory motif within its cytoplasmic tail.[23] PD-L1 and PD-L2 are the binding partners for PD-1; both ligands inhibit T-cell activation *in vitro*.[24] *In vivo*, deficiency in or blockade of PD-1, PD-L1, or PD-L2 induces hypersensitivity leading to organ-specific autoimmune diseases.[25–28] PD-L1 is broadly expressed on APCs, including dendritic cells, macrophages, and B cells, while PD-L2 is selectively expressed on DCs and a subset of activated macrophages.[29,30] Recently, the expression of PD-L1 on nonhematopoietic cells has been reported, along with a possible functional role for these cells. Mouse keratinocytes activate IL-10-secreting T cells through a PD-L1-dependent pathway.[31] Human endothelial cells express PD-L1, suppressing cytokine synthesis by T cells.[32] Mouse sinusoidal endothelial cells in the liver expressing PD-L1 inhibit the proliferation and cell division of activated T cells expressing PD 1.[33] Thus, the inhibitory signal through PD-L1 appears to be involved in maintaining peripheral immune tolerance.

Prostaglandins, produced as metabolites of arachidonic acid by cyclooxygenase enzymes, play a pivotal role in the regulation of inflammatory and immune responses. PG-E2, a major product of arachidonic acid metabolism, exerts both pro- and anti-inflammatory actions *in vivo*.[34] PG-E2 is primarily produced by professional APCs, such as macrophages and dendritic cells, but can also be produced by tumor cells.[34] PG-E2 has pleiotropic effects on the immune system, including inhibition of T-cell–mediated IFNγ and IL-2 production, T-cell proliferation, natural killer cell activity, and the production of IL-1, TNF, and IL-12 by macrophages.[35–37] Despite reports that PG-E2 enhances human CD40-activated B-cell growth and IL-2–induced production of GM-CSF by T cells, PG-E2 generally exerts immunosuppressive effects.[38,39] Some epithelial cells from normal tissues, including human keratinocytes, rat intestinal epithelial cells, and rat retinal pigment epithelial cells, produce PG-E2, suppressing CD3-mediated T-cell activation.[40–42]

Recently, the expression of PD-L1 by nonhematopoietic cells has also been reported. Mouse keratinocytes, mouse liver sinusoidal epithelial cells, and human endothelial cells regulate the activation and synthesis of cytokines by T cells via PD-L1.[31–33] Thus, this inhibitory signal mediated by PD-L1 is speculated to function in peripheral immune tolerance. In this study, BECs expressed both PD-L1 and PD-L2, both of which were up-

regulated by IFNγ treatment. While the expression of PD-L1 by nonhematopoietic cells has been reported, the expression of PD-L2 by nonhematopoietic cells other than human endothelial cells has not yet been documented.[43]

The TCCs used in this study expressed PD 1, the receptor for PD-L1 and PD-L2. TCCs became partially activated upon coculture with Ag-pulsed BECs as APCs when the action of PD-L1 and PD-L2 was blocked using specific antibodies. Thus, BECs inhibit T-cell activation via PD-L1 and PD-L2. The activation of TCCs, even in the presence of agents blocking PD-L1 and PD-L2, however, was much weaker than that observed upon coculture of TCCs with Ag-pulsed PBMCs or L-DR53. These results indicate that additional regulatory molecules other than these PD-1 ligands must exist on BECs.

In previous studies, the interaction of HLA-DR–expressing or nonexpressing BECs and T cells induced low but significant proliferation of allogeneic primed T cells, in comparison with spleen cells or arterial endothelial cells.[44] BECs also inhibited CD25 expression on T cells after CD3-mediated stimulation.[45] These observations may result from a similar regulatory effect of BECs as that shown in our study. In addition, other epithelial cell lines from normal tissues, including human keratinocytes, human and rat intestinal epithelial cells, and rat retinal pigment epithelial cells, regulate CD3-mediated T-cell activation.[40–42, 46] The regulatory effect of these epithelial cells functions, in part, through PG-E2 or TGFβ. In the data herein, BECs regulated the T-cell proliferation in a cell-contact–independent manner, primarily via PG-E2. Interestingly, BECs produced low levels of PG-E2 when cultured alone, but they produced high levels of PG-E2 after stimulation with soluble factors produced by cocultures of activated T cells and Ag-pulsed professional APCs. Some of the soluble factors were subsequently shown to include TNFα and IL-1β.

In summary, the establishment of autoreactive T-cell clones and their influence by biliary epithelial cells are difficult. However, we believe that such studies are important and should include other biliary-specific diseases. There are, however, several key issues that are under study, including dissection of the role of PG-E2 and, in particular, further observations on the interrelationships of BECs following pretreatment with indomethacin. Similarly, based on our data suggesting cytokine regulation of PD-L1 and PD-L2 expression in BECs, further studies should include the use of specific cytokine antibodies. Finally, such studies should be done not only in PBC, but also in PSC to determine whether the biliary epithelium in PBC is unique to cholestatic diseases. However, our data are important, particularly because in the liver, Kupfer cells and sinusoidal endothelial cells are involved in the regulation of immunity through PD-L1 or nitric oxide synthesis.[33,47] We speculate that BECs may also regulate the immune system around bile ducts, where BECs are the main targets in PBC. When Ag-specific T cells are stimulated by professional APCs in the portal areas, BECs may regulate T-cell activation by producing PG-E2 after stimulation with inflammatory cytokines. BECs would also directly regulate T-cell activation through membrane-bound suppressive molecules, including PD-L1 and PD-L2. Decreased or abnormal expression and/or production of these inhibitory molecules may be one of the causes of autoimmune cholangitis. Therefore, the modification of these immune suppressive molecules, including PD-L1, PD-L2, and PG-E2, may mitigate PBC.

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Antibody titer to gp210-C terminal peptide as a clinical parameter for monitoring primary biliary cirrhosis

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***Background/Aims*: The presence of antibodies to the 210-kDa glycoprotein of the nuclear pore complex (gp210) is highly indicative of primary biliary cirrhosis (PBC). However, the significance of anti-gp210 antibody titers for monitoring PBC remains unresolved.**

***Methods*: We used an ELISA with a gp210 C-terminal peptide as an antigen to assess serum antibody titers in 71 patients with PBC.**

***Results*: Patients were classified into three groups: Group A in whom anti-gp210 titers were sustained at a high level, Group B in whom anti-gp210 status changed from positive to negative under ursodeoxycholic acid (UDCA) therapy, Group C in whom anti-gp210 antibodies were negative at the time of diagnosis. The rate of progression to end-stage hepatic failure was significantly higher in group A (60%) as compared to groups B (0%) and C (4.2%). The sustained antibody response to gp210 was closely associated with the severity of interface hepatitis. The significance of anti-gp210 antibody was confirmed by National Hospital Organization Study Group for Liver Disease in Japan.**

***Conclusions*: The serial quantitation of serum anti-gp210-C-terminal peptide antibodies is useful for monitoring the effect of UDCA and for the early identification of patients at high risk for end-stage hepatic failure.**

***Keywords*: Primary biliary cirrhosis; Anti-gp210 antibody; Clinical parameter; Ursodeoxycholic acid; End-stage hepatic failure; Liver transplantation; Interface hepatitis**

1. Introduction

Primary biliary cirrhosis (PBC) is an autoimmune disorder characterized by nonsuppurative inflammatory destruction of small bile ducts that can ultimately lead to biliary cirrhosis [1]. In addition to anti-mitochondrial antibodies (AMAs), a number of nuclear structures have been recognized as targets of the antinuclear antibodies (ANA) in PBC patients [2–4]. These include several components of the nuclear pore complex, such as the 210-kDa glycoprotein (gp210) and p62 proteins [3–5]. In this category of ANA, the autoantibody to gp210 is particularly significant as they are specifically detected in approximately 10–30% of PBC patients [6–13]. In addition, the presence of anti-gp210 antibody has been reported to be associated with the disease activity of PBC, indicating that the anti-gp210 antibody may potentially be useful as a prognostic marker of

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PBC [14–16]. However, these studies are largely cross-sectional and the significance of anti-gp210 antibody titers in a clinical setting remains unsettled.

In the present study, in order to elucidate the significance of anti-gp210 antibody titers in a clinical setting, we established an ELISA that quantitates the serum anti-gp210 level using a gp210 C-terminal peptide as an antigen. We then studied the antibody titers of PBC patients in serial serum samples that had been obtained over the course of treatment and stocked in our institution from August 1982 to March 2004. The significance of anti-gp210 antibody titers is discussed in terms of monitoring for the disease activity and the prediction of the long-term outcome of PBC.

2. Materials and methods

2.1. Patients and sera

Sera were obtained every 3–12 months from patients with PBC who were treated at our institution during the 22 years between August 1982 and March 2004 and were stored at −20 °C until use. All PBC patients were histologically diagnosed based on internationally accepted criteria [17,18] and classified by Scheuer's classification [19] using specimens obtained by needle liver biopsy. Of the 73 patients that were definitively diagnosed as PBC, two PBC patients who had episodes of moderate to severe autoimmune hepatitis (AST>500 IU/l, ALT>500 IU/l) and required initial administration of a moderate dose of prednisolone (PSL; 1 mg/Kg body weight) and have been given maintenance dose(20–25 mg/body) of PSL were excluded from the present study, leaving 71 PBC patients for the analysis. This group of eligible patients comprised 9 males and 62 females, ranging in age from 30 to 83 years (mean±SD 58.5±11.0, median 61). Twenty-nine, 16, 15 and 11 patients were in Scheuer's stage 1, 2, 3 and 4, respectively. While three patients were followed up without medication, 53 patients were treated with UDCA (600–900 mg/day) alone, one patient with bezafibrate (400 mg/day) alone and six patients with both UDCA and bezafibrate. PSL was transiently administered to eight patients in whom both histological and biochemical markers indicated the concomitant presence of mild autoimmune hepatitis. The maximum length of corticosteroid treatment in these patients was 6 months. D-penicillamin was administered to two patients who were resistant to the treatment including UDCA, bezafibrate, and PSL.

Sera were also obtained from 30 healthy blood donors, 36 patients with AIH who were negative for AMAs, 40 chronic hepatitis C(CHC) patients, 50 patients with liver cirrhosis (LC) caused by hepatitis B or hepatitis C virus infection, and 36 patients with collagen diseases including 10 cases of rheumatoid arthritis, nine cases of systemic lupus erythematodes and five cases of primary Sjogren's syndrome. The diagnosis of AIH was based on the criteria as described elsewhere [20].

The present study has been approved by Ethics Board at NHO Nagasaki Medical Center and NHO Study Group for Liver Disease in Japan (NHOSLJ) and conducted after obtaining informed consent from each subject.

2.2. Histological examination of liver biopsy samples

A total of 109 biopsies from the 71 patients were included in the histological review. The sections were stained with hematoxylin and eosin, Azan Mallory, reticulin silver impregnation, Victoria blue, orcein, and rodanin. Each biopsy was analyzed by two independent observers (M.I. and Y.T.). In case of initial disagreement in the assessment, consensus was reached on further review. The histological variables examined included fibrosis (0–4), portal inflammation (0–3), interface hepatitis (0–3), lobular inflammation (0–3), copper-associated protein deposition (0–1), chronic non-suppurative destructive cholangitis (CNSDC; 0–2), granuloma (0–2), ductal paucity (0–3), and ductal proliferation (0–3). Upon completion of the evaluation of each of these variables, a numerical necroinflammatory grade (A0–A3) and a histological staging using Scheuer's classification (stage 1–4) were determined.

2.3. Peptide synthesis

The human gp210 C-terminal peptide, a 25-mer with the following sequence, SPNALPPARKASPPSGLWSPAYASH, was synthesized with a peptide synthesizer (Model 432A Synergy; Applied Biosystems, Fostercity, CA) using F-moc chemistry as previously described [21]. The peptide was purified with reverse phase HPLC, and the resulting purity was greater than 90% as determined by HPLC analysis.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The wells of flat-bottom polystyrene microtiter plates (Maxisorp, Nalge Nunc International, Denmark) were coated with 100 μl of a 5 μg/ml solution of the purified gp210 C-terminal peptide, which was dissolved in 0.1 M carbonate buffer, pH 9.6. The plates were incubated at 4 °C for 24 h, followed by further 2-hour incubation with 100 μl of phosphate buffered saline (PBS) containing 1% BSA. The plates were dried at room temperature and stored at 4 °C until use. Next, 100 μl of each serum sample, diluted 1:100 in PBS containing 0.05% v/v Tween 20, was added to a well, incubated for 1 h at room temperature, then washed four times with PBS-Tween 20. The optimal dilution of peroxidase-conjugated anti-human IgG (Medical and Biological Laboratories, Nagoya, Japan) was added to each well and allowed to incubate for 1 h. After washing four times with PBS-Tween 20, 100 μl of substrate solution containing 3,3′,5,5′-tetramethylbenzidine and H_2O_2 was added to each well. After a 30-minute incubation at room temperature, the reaction was stopped by adding 100 μl of 0.5 M H_2SO_4 and the optical density at 450 nm was read using an automatic plate reader (MPRA4i, TOSO, Japan). The antibody titers were calculated with reference to the standard serum, which had been diluted to four different concentrations (200, 100, 50 and 0 units/ml).

2.4.1. Sera from primary biliary cirrhosis (PBC) patients in NHOSLJ

To validate the results in our institution, we obtained sera from 16 Hospitals, which are the members of NHOSLJ. These include 219 serum samples derived from 83 PBC patient (16 males and 67 females, age range 34–84 years, mean±SD 63.5±10.6 years, median 63.5 years) in whom the serum samples were available for anti-gp210 antibody determination at the time of diagnosis by liver biopsy and at least once before the conclusion of observation period.

2.5. Statistical analysis

Results are presented as mean±SD or as percentages. Comparison between values was accomplished using the Student *t*-test and Fisher's exact test. The rate of progression to end-stage hepatic failure was estimated by the Kaplan–Meier method, and compared by log-rank test. A two-tailed *P*-value of <0.05 was considered significant. Statistical analyses were performed using Statcel 2 and Excel 2003 software.

3. Results

3.1. Clinical course of PBC patients during the observation period

From August 1982 to March 2004, 71 patients were followed up at our institution for varying periods of 6–216 months (72.8±54.8 months, median 64 months) after the initial definitive diagnosis of PBC using histological criteria. Among the 71 patients, five died of hepatic failure

and three patients underwent OLT because of progression to hepatic failure. Three patients, who were negative for anti-gp210 antibodies, developed hepatocellular carcinoma (HCC) and two of them died of HCC. The death of three other patients was not attributable to PBC but attributable to myelodysplastic syndrome, adult T cell leukemia and arrhythmia. Twelve patients were followed up by nearby hospitals and the patients' information was available for this study. The remaining 50 patients are alive and are followed up at our institution.

3.2. Quantitation of antibody titers to the gp210 C-terminal peptide by ELISA

The antibody titers to the gp210 C-terminal peptide in the 71 PBC patients, 30 healthy subjects and 162 patients with various diseases including AIH, CHC, LC and collagen diseases are shown in Fig. 1. When serum antibody titers that were greater than 6.0 unit/ml (mean value +5 SD of the titers of 30 healthy subjects) were arbitrary determined as positive for antibodies, 23 PBC patients (32.4%) were positive at the time of diagnosis. In contrast, none in AIH, CHC, LC and collagen diseases were positive for anti-gp210 antibodies. These results indicate that the present ELISA system to detect anti-gp210 C-terminal peptide antibodies is highly specific for PBC (specificity 100.0%).

3.3. Comparison of various clinical and histological parameters and the long-term outcome between anti-gp210-positive and -negative PBC patients

The histological stage of the patients positive for anti-gp210 antibodies was significantly more advanced when compared to that of patients negative for anti-gp210 antibodies at the time of diagnosis (Table 1). There was no significant difference in serum biochemical parameters between the two

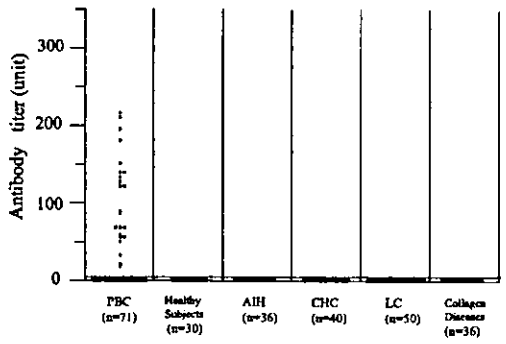

Fig. 1. Serum antibody titers to the gp210 C-terminal peptide in patients with PBC. Each closed circle represents the antibody titer of one patient at the time of diagnosis. Antibody titers more than 6.0 unit/ml are determined to be positive. Sera from various diseases including AIH, CHC, LC, and collagen diseases are all less than 6.0 unit/ml, while 32.4% of sera from patients with PBC were positive.

Table 1
Comparison of clinical parameters between anti-gp210-positive and anti-gp210-negative PBC patients at the time of diagnosis

	Anti-gp210 antibody		*P* value
	Positive	Negative	
Patients number (%)	23 (32.4)	48 (67.6)	
Male/female (%)	5 (21.7)/18 (78.3)	4 (8.3)/44 (91.7)	0.174
Age	57.6+12.6	58.8±10.3	0.654
Observation (months)	73.4±54.3	72.5±55.6	0.950
Cases of hepatic failure (%)	6 (26.1)	2(4.2)	*0.012*
gp210 antibody titer (unit)	104.7±58.3	1.6±1.5	<0.0001
AMA (Index value)	99.8±68.6	120.1±71.1	0.228
Stage			
1+2 (%)	10 (43.5%)	35 (72.9%)	
3+4 (%)	13 (56.5%)	13 (27.1%)	0.020
Mayo risk score	4.3±0.7	4.1±0.9	0.482
Albumin (g/dl)	3.9±0.6	4.1±0.4	0.066
T. bilirubin (mg/dl)	1.1±0.8	0.8±0.5	0.252
AST (IU/l)	77.9± ±61.2	55.2±41.1	0.068
ALT (IU/l)	94.1±97.0	63.5±45.1	0.073
ALP (IU/l)	646.8±355.3	694.6±516.5	0.695
T. Chol. (mg/dl)	229.2±64.5	230.1±54.5	0.952
IgG (mg/dl)	1953.7±779.8	1963.8±521.7	0.959
IgA (mg/dl)	466.6±283.2	378.0±173.0	0.113
IgM (mg/dl)	662.7±545.4	424.6±261.0	0.015

Normal range: albumin 3.7–5.2 g/l, T. bilirubin 0.3–1.5 mg/dl, AST 12–37 IU/l, ALT 6–39 IU/l, ALP 115–359 IU/l, T. cholesterol 128–256 mg/dl, IgG 870–1700 mg/dl, IgA 110–410 mg/dl, IgM 35–220 mg/dl.

groups except IgM level ($P=0.015$, Table 1). Mayo risk score at the time of diagnosis was not different between the two groups (Table 1).

During the observation period, six out of the 23 patients positive for anti-gp210 antibodies progressed to hepatic failure resulting in OLT ($n=2$) or death ($n=4$), while only two out of the 48 patients negative for anti-gp210 antibodies progressed to hepatic failure resulting in OLT ($n=1$) or death ($n=1$) (Table 1 and Fig. 2(a)). The incidence of the progression to hepatic failure was significantly higher in anti-gp210 positive patients as compared to that in the anti-gp210 negative patients ($P=0.012$; Table 1).

3.4. Comparison of various clinical and histological parameters and the long-term outcome among the anti-gp210 antibody positive patients

The 23 patients positive for anti-gp210 C-terminal peptide antibodies at the time of diagnosis were classified into two groups depending on the change in antibody titers upon UDCA treatment. As depicted in Fig. 2(a), patients in group A consisted of 10 patients in whom the anti-gp210 antibody titers were sustained at a high level (more than 50 units/ml) during the observation period (median 63 months). In contrast, patients in group B consisted of 13 patients in

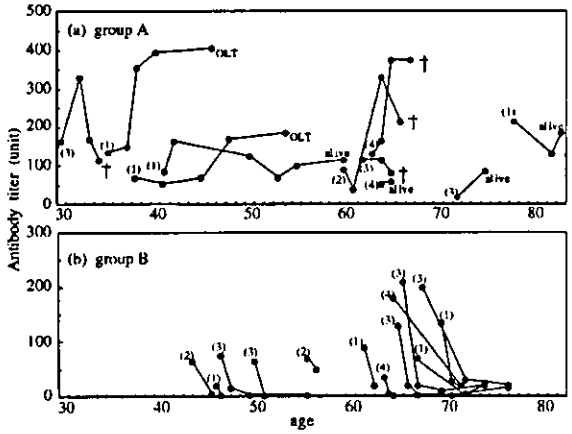

Fig. 2. The change of serum antibody titers to the gp210 C-terminal peptide in patients with PBC. The anti-gp210 antibody titers were plotted against the age of each patient at the time of measurement. The number in parenthesis indicates the histological stage (Scheuer's classification) of each patient at the time of diagnosis by first liver biopsy. (a) The profile of antibody titers in patients who were classified into group A. Anti-gp210 antibody titers were sustained at a high level in these 10 patients, of whom four patients died of end-stage hepatic failure, two patients undertook OLT, and four patients are alive without any sign of hepatic failure. (b) The profile of antibody titers in patients who were classified into group B. Anti-gp210 antibody status changed from positive to negative or weakly positive as early as 6 months after the initiation of UDCA therapy, and the titer was sustained at a normal or low level during the observation period. All 13 patients in group B are alive without any sign of hepatic failure.

whom the anti-gp210 antibody titers decreased to normal, or to a low level (less than 50 units/ml), as early as 6 months after the initiation of UDCA treatment and were sustained at this level during the entire observation period (median 60 months) (Fig. 2(b)).

The distribution of the histological stages of the patients in group A was similar to that in group B ($P=1.00$) at the time of diagnosis (Table 2). The levels of total serum bilirubin and AST were significantly higher in group A as compared to group B ($P=0.018$ and 0.037, respectively; Table 2). Mayo risk score at the time of diagnosis was similar between the two groups ($P=0.534$) (Table 2). There was no significant difference in the serum level of IgM between the two groups at the time of diagnosis ($P=0.786$) (Table 2). Furthermore, the serum level of IgM was similar between the two groups (group A: 493.4±327.7 mg/dl, group B: 421.4±337.1 mg/dl, $P=0.643$) at the end of observation period.

Finally, six out of 10 patients in group A progressed to hepatic failure resulting in OLT ($n=2$) or death ($n=4$) (Fig. 2(a) and Table 2). In contrast, none of the patients in group B progressed to hepatic failure and all 13 patients were alive at the conclusion of the observation period (Fig. 2(b) and Table 2). The incidence of end-stage hepatic failure was significantly higher in group A as compared to group B ($P=0.002$; Table 2).

Table 2
Comparison of clinical parameters between groups A and B at the time of diagnosis

	Group A	Group B	*P* value
Patients number	10	13	
Male/female (%)	2 (20)/8 (80)	3 (23.1)/10 (76.9)	1.000
Age	55.2±16.2	59.5±9.2	0.754
Observation (months)	83.7±60.5	65.5±50.1	0.439
Cases of hepatic failure (%)	6 (60)	0 (0)	*0.002*
gp210 antibody titer (unit)	109.3±54.8	101.4±62.9	0.485
AMA (Index value)	85.4±70.4	109.6±68.3	0.429
Stage			
1+2 (%)	4 (40)	6 (46.2)	
3+4 (%)	6 (60)	7 (53.8)	1.000
Mayo risk score	4.4±0.9	4.2±0.6	0.534
Albumin (g/dl)	3.9±0.7	3.9±0.5	0.931
T. bilirubin (mg/dl)	1.6±1.1	0.7±0.3	0.018
AST (IU/l)	91.2±53.9	67.7±66.6	0.037
ALT (IU/l)	100.8±59.7	89.0±120.5	0.780
ALP (IU/l)	821.8±442.6	525.7±226.8	0.052
T. Chol. (mg/dl)	257.1±84.3	207.8±33.8	0.067
IgG (mg/dl)	1779.1±450.8	2074.6±943.4	0.395
IgA (mg/dl)	434.4±252.3	488.9±310.8	0.668
IgM (mg/dl)	699.1±610.7	634.6±513.4	0.786

Group A: patients in whom anti-gp210 titers were sustained at a high level. Group B: patients in whom anti-gp210 status changed from positive to negative or weakly positive after initiation of UDCA therapy. See Table 1 for normal range.

3.5. Kaplan–Meier assessment of transplantation free survival

The 48 PBC patients who were negative for anti-gp210 C-terminal peptide antibodies at the time of diagnosis were classified as group C and the prognosis was compared with patients positive for anti-gp210 antibodies (groups A and B). As shown in Fig. 3, the Kaplan–Meier assessment revealed a significant difference in transplantation free survival between groups A and B ($P=0.0427$) and groups A and C ($P=0.0046$), but revealed no significant difference

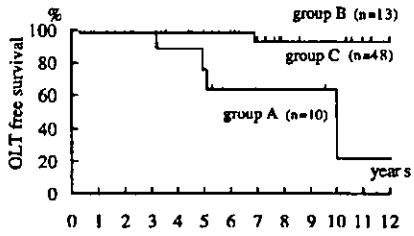

Fig. 3. Probability of survival free of liver transplantation calculated by Kaplan–Meier method in patients with PBC who were classified into groups A, B and C based on the anti-gp210 antibody profile. The rate of end-stage hepatic failure (transplantation or death without transplantation due to hepatic failure) is significantly higher in group A as compared to groups B and C (group A vs. B, $P=0.0427$; group B vs. C, $P=0.4056$; group C vs. A; $P=0.0046$) by log-rank test.

between groups B and C ($P=0.4056$). The rate of transplantation or death due to progression to hepatic failure was calculated as 23.8, 0 and 2.1% in groups A, B and C, respectively, at 5 years, and 78.9, 0 and 6.8% in groups A, B and C, respectively, at 10 years.

3.6. Comparison of histological scores among groups A, B and C at the time of diagnosis

The score of interface hepatitis was significantly higher in group A (1.9 ± 0.9) as compared to groups B (0.9 ± 0.7) ($P=0.012$) and C (1.1 ± 0.9) ($P=0.015$). The score of lobular inflammation was higher in group A (1.3 ± 0.5) as compared to groups B (0.8 ± 0.6) and C (1.0 ± 0.7), although the difference was not statistically significant. The activity score, which corresponds to both interface hepatitis and lobular inflammation, was also significantly higher in group A (2.0 ± 0.7) as compared to groups B (1.3 ± 0.5) ($P=0.015$) and C (1.3 ± 0.8) (0.009). In contrast, the score of portal inflammation was very similar among three groups; group A (1.7 ± 0.5), group B (1.7 ± 0.8,), group C (1.6 ± 0.8).

3.7. Long-term outcome of PBC patients in NHOSLJ

To confirm the significance of anti-gp210 antibody titers for the long-term outcome of PBC patients, we measured anti-gp210 antibody titers of serum samples from NHOSLJ. Sixty-three patients (male/female = 8/55, age 34–82, 63.5 ± 10.5, median 62 years) were in early stage (Scheuer's stage 1 ± 2) and 20 patients (male/female = 8/12, age 47–84, 63.4 ± 10.9, median 65 years) were in late stage (Scheuer's stage 3 ± 4) at the time of diagnosis. The patients were classified into three groups (A, B or C) based on the anti-gp210 antibody profile as described above. As for 63 patients in early stage, four out of 10 in group A, zero out of three in group B, and one out of 50 in group C progressed to end-stage hepatic failure during the observation period (group A: range 11–156, 79.2 ± 53.5, median 85.5 months, group B: range 55–144, 99.3 ± 44.5, median 99 months, group C: range 6–275, 78.1 ± 64.4, median 59.5 months). As for 20 patients in late stage, nine out of 10 in group A and one out of 10 in group C progressed to end-stage hepatic failure during the observation period (group A: range 29–330, 98.5 ± 90.2, median 68 months, group C: range 6–167, 91.5 ± 53.6, median 87.5 months). Thus, the rate of progression to end-stage hepatic failure was significantly higher in group A as compared to group C in both early ($P=0.00196$) and late stage ($P=0.00035$) of PBC patients. As shown in Fig. 4, the Kaplan–Meier assessment revealed a significant difference in transplantation free survival between groups A and C in both early stage- and late stage-patients at the time of diagnosis(log-rank test $P=0.00078$ for early stage and $P=0.0167$ for late stage).

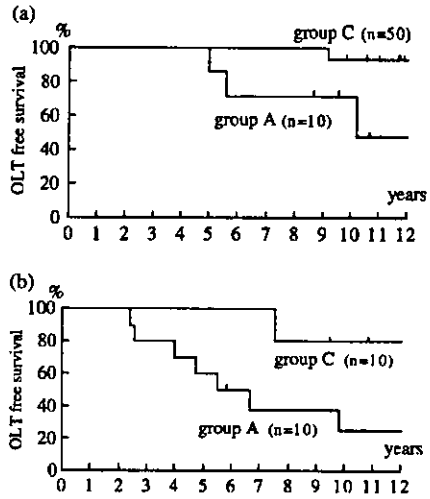

Fig. 4. Probability of survival free of liver transplantation calculated by Kaplan–Meier method in patients with PBC in NHOSLJ. The data from patients who could be classified into groups A or C based on their anti-gp210 antibody profile were used for analysis. (a) The rate of end-stage hepatic failure (transplantation or death without transplantation due to hepatic failure) is significantly higher in group A as compared to group C in early stage patients at the time of diagnosis ($P=0.000783$) by log-rank test. (b) The rate of end-stage hepatic failure is significantly higher in group A as compared to group C in late stage patients at the time of diagnosis ($P=0.0167$) by log-rank test.

4. Discussion

Our results indicated that there are at least three different categories of PBC patients based on the anti-gp210 antibody profile. Group A comprised patients in whom the anti-gp210 antibodies were sustained at a high level under UDCA therapy. Group B consisted of patients in whom anti-gp210 antibody status changed from an initial positive to negative or weakly positive after the initiation of UDCA therapy. Group C contained those patients that were negative for anti-gp210 antibodies at the time of diagnosis. Importantly, the incidence of progression to end-stage hepatic failure was significantly higher in group A as compared to groups B and C, indicating that a sustained antibody response to gp210 is closely associated with the progression to end-stage hepatic failure. Since the Mayo risk score calculated at the time of diagnosis did not significantly differ among these three groups, the profile of anti-gp210 antibody titers should be useful for early identification of the patients who will potentially progress to end-stage hepatic failure. In addition, these observation was confirmed by the study in NHOSLJ.

The association of the presence of anti-gp210 antibodies with the disease activity of PBC has been reported by three groups [14–16]. These studies, however, were largely cross-sectional and the antibody titers to gp210 were not quantitated [14–16]. Thus, the clinical significance of the presence of anti-gp210 antibodies has remained unsettled so far. Therefore, the present study, which is a long-term

follow-up study using serially stocked sera, provides for the first time a definitive evidence that the PBC patients who are at high risk for the progression to end-stage hepatic failure can be identified by the serial quantitation of anti-gp210 C-terminal peptide antibodies using ELISA. According to our Kaplan–Meier analysis, the risk for the progression to end-stage hepatic failure is 11.3 times and 11.6 times higher in group A than that in group C, at 5 and 10 years, respectively.

Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid that is widely used for the treatment of PBC. UDCA therapy significantly delayed the progression of histological stage and prolonged survival free of liver transplantation in many studies [17,18,22–27], whereas UDCA therapy did not demonstrate an improvement in the incidence of survival free of liver transplantation in some studies [28,29]. Thus, there still remains some controversy for the effect of UDCA on the long-term outcome of PBC. In this context, our method to monitor the effect of UDCA by measuring anti-gp210 C-terminal peptide antibodies should be useful for the identification of patients who are resistant to UDCA therapy.

Poupon et al. reported that the severity of lymphocytic piecemeal necrosis and lobular inflammation in the first biopsy specimen, but not the severity of the bile duct lesions, was highly correlated to the progression of fibrosis and the development of cirrhosis under UDCA treatment [18,25]. In our study, the activity score corresponding to interface hepatitis and lobular inflammation was significantly higher in patients in group A, in whom anti-gp210 antibody titers were sustained at high levels. In contrast, the activity score was significantly lower in patients in group B, in whom anti-gp210 antibody titers decreased to normal or low levels. The degree of portal inflammation was similar between the two groups. Thus, it is considered possible that the anti-gp210 C-terminal peptide antibody titer is a clinical parameter in serum, which indicates the histological activity rather than the portal inflammation in the majority of PBC patients who have been sensitized to anti-gp210 antibody-production. However, approximately 20–25% of PBC patients who progressed to end-stage hepatic failure were consistently negative for antibodies to gp210 C-terminal peptide. In order to early identify these patients with poor prognosis, the new prognostic markers other than anti-gp210 antibodies are now under investigation.

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Is primary biliary cirrhosis a model autoimmune disease?

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Abstract

Primary biliary cirrhosis (PBC) has been coined a model autoimmune disease. In fact, it does share many similarities with other autoimmune diseases, but there are striking differences that illustrate the uniqueness of the immunopathology. Firstly, similar to other autoimmune diseases, there is an intense humoral and cellular response to an intracytoplasmic antigen. There is also an overlap of the epitopes recognized by autoreactive $CD4^+$, $CD8^+$ T cells as well as B cells. Patients with PBC are also predominantly female, and there is a higher family history of other autoimmune diseases. In contrast, however, there are no specific HLA associations in PBC. Further, there are no spontaneous or induced animal models of PBC. In addition, early in the biliary lesions of PBC, there is an eosinophilic infiltration and, often, there are granulomas. Finally, unlike several other human autoimmune diseases, patients with PBC have recognition of but one major epitope, and there is no evidence for determinant spreading. Hence, although the immune response of PBC has been vigorously defined, there remain major gaps in understanding the most difficult issue of all, namely etiology.

Keywords: Primary biliary cirrhosis; Autoimmune diseases; Epitope; Female predominance; Molecular mimicry; Xenobiotics

1. Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease that predominantly affects middle-aged women and is histologically characterized by infiltration of lymphocytes in portal tracts and destruction of intrahepatic small bile ducts, causing liver fibrosis and eventually, liver failure [1,2]. The most characteristic feature in PBC is the presence of anti-mitochondrial antibodies (AMA), positive in 90–95% of patients with PBC long before clinical signs or symptoms appear [2]. Other autoimmune diseases, such as Sjögren's syndrome, Hashimoto's thyroiditis, scleroderma and rheumatoid arthritis often coexist. In this paper, we will discuss the immunological characteristics of PBC, and whether PBC is a model auto-

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Table 1
The features of PBC compared to other autoimmune diseases

Similar features to other autoimmune diseases	Different features from other autoimmune diseases
• humoral and cellular autoimmunity • overlapping epitopes of B and T cells • coexistence of other autoimmune diseases • female predominance • higher prevalence in patient's family • possible involvement of molecular mimicry and xenobiotics in the etiology	• only BEC are affected despite mitochondrial autoantigens are ubiquitous • no evidence for determinant spreading • no specific-HLA association • involvement of X chromosome monosomy in the female susceptibility • No evidence for fetal microchimerism • eosinophilic infiltration and granuloma formation in early biliary lesions • no animal model

immune disease, compared with other autoimmune diseases (Table 1).

2. Autoantigens and immune responses

Similar to other autoimmune diseases, there are intense humoral and cellular responses to intracytoplasmic antigens. The autoantigens in PBC are located on the inner mitochondrial membrane and identified as members of the 2-oxo-acid dehydrogenase enzyme complexes (2-OADC) [3,4]. Among them, the major autoantigen in PBC is PDC-E2, and anti-PDC-E2 is positive in approximately 90–95% of PBC sera [5].

The E2 subunits of the 2-OADC share structural similarities in terms of their lipoic acid binding domain, E3 binding domain, and catalytic and E2 binding domain. Several studies, using either oligopeptides or recombinant fusion proteins, have shown that the B cell epitope of PDC-E2 is located in the lipoyl domain, whereas the other domains are nonreactive. By expressing various recombinant peptides spanning the entire human cDNA, PBC sera recognize recombinant peptides corresponding to the outer lipoyl domain (residues 1–96) and the inner lipoyl domain (residues 128–227) of PDC-E2 [6]. Furthermore, the residues 167–186 of PDC-E2 are thought to be the minimal B cell epitope by inhibition assays [5]. Interestingly, antibody binding occurs when the antigen complexes with lipoic acid [7], and there are only five proteins that contain lipoic acid, and four of the five are autoantigens in PBC [8].

It has been hypothesized that the destruction of biliary epithelial cells (BEC) in PBC is mediated by autoreactive T cells infiltrating in the liver [2,9]. Proliferation assays demonstrate that the $CD4^+$ helper T cells derived from the patients with PBC respond positively to amino acid residues 163–176 within the inner lipoyl domain and amino acid residues 36–49 within the outer lipoyl domain of PDC-E2 ($CD4^+$ T cell epitopes) in the context of HLA-DR53. The frequencies of these autoreactive $CD4^+$ T cells are significantly higher in the liver than in the peripheral blood [10,11]. $CD8^+$ cytotoxic T cell (CTL) epitopes are identified as amino acid residues 159–167 and 165–174 of PDC-E2 in the context of HLA-A2.1, and the frequencies of these autoreactive CTL are also significantly higher in the liver than in the peripheral blood [12–14]. Interestingly, these T cell epitopes are overlapping not only each other, but also with the B cell epitopes. These findings suggest that the lipoyl domain of PDC-E2 constitutes the most significant immunodominant regions in PBC.

These features are also found in other autoimmune diseases. In type 1 diabetes (T1D), three out of five autoreactive B cell epitopes and three out of four HLA-DRB1*0401-restricted autoreactive T cell epitopes overlap on the glutamic acid decarboxylase-65 (GAD65), which is the major autoantigen [15]. In multiple sclerosis (MS), the epitopes recognized by the autoantibody and T cells against myelin basic protein (MBP) are shared [16,17]. In contrast, there is no evidence for determinant spreading in PBC, unlike several other autoimmune diseases. The observation that immune responses undergo determi-

nant spreading is considered to be a major finding, shaping current theories regarding autoimmunity and molecular mimicry [18]. We emphasise that autoreactive T and B cells recognize only one major epitope in PBC.

3. Genetic factors

Although there is a female predominance in PBC (ratio of 9:1) [19], the cause is still unknown. Most autoimmune diseases also show female predilection, perhaps related to estrogens [20], but there are many other genes that have estrogen responsive elements. We should also note that genes implicated in immunological tolerance are located on the X chromosome [21]. Recently, the involvement of an abnormal X chromosome in the female susceptibility to PBC has been suggested [22], and it was shown that the frequency of X monosomy in peripheral blood cells from female PBC patients was significantly higher than in chronic hepatitis C and healthy controls using fluorescence in situ hybridization. This is the first report about the association of X chromosome monosomy with autoimmunity. Unlike scleroderma, there is no evidence for fetal microchimerism in PBC [23].

As in other autoimmune diseases, PBC is also more frequent in people having a family history of the disease, found in 1 – 6.4% of families [24,25]. Limited studies once indicated the association of HLA-DR8 with PBC [26], but, in general, no particular HLA haplotype is considered to be associated with the occurrence of PBC, even in familial cases. This is different from other autoimmune diseases, i.e., MS and HLA-DR2, T1D and HLA-DR4 – DQB1*0302 [17,27].

4. Environmental factors

Both environmental and genetic factors play a role in the pathogenesis of autoimmune diseases. Interestingly, the onset of PBC in family members often occurs within a few years of each other [25]. This suggests that environmental factors may be important for the etiology of PBC (Fig. 1). It has been suggested that molecular mimicry between microbial agents and self-antigens might induce autoimmune diseases. For instance, amino acids 247–279 of GAD65 share sequence homology with the P2-C protein of coxsackie B virus, and this virus is considered to initiate T1D [28]. In the murine model, it is shown that the lymphocytic choriomeningitis virus (LCMV) infection induces T1D when LCMV glycoprotein is expressed on β cells [29].

In PBC, bacteria, especially *Escherichia coli*, have been considered to be involved in molecular mimicry, because their PDC-E2 has homology with human PDC-E2 [30] and also the observation that the patients with PBC have frequently had urinary tract infection. Indeed, the role of molecular mimicry has been evaluated in $CD4^+$ helper T cells and $CD8^+$ CTL in the pathogenesis of PBC, and it has been shown that these T cells react to the peptides derived from several kinds of bacteria [31,32]. Recently, a new bacterial candidate was identified as *Novosphingobium aromaticivorans*, because the amino acid sequences of two proteins of this bacterium have higher degree of homology with the immunodominant epitope of human PDC-E2 than any known microorganism. The sera from PBC patients but not controls react against these proteins of *N. aromaticivorans*, and the titer is at least 100-fold higher than against *E. coli*. Importantly, a bacterial strain from the *Sphingomonas* genus showing 96% DNA homology with *N. aromaticivorans* has cleavage activity on 17β-estradiol, transforming the inactive conjugated to the free active form [33]. These results suggest that this bacterium may initiate the autoimmune response in PBC.

5. Histopathological features

Eosinophilic infiltration and granuloma formation are often seen in the hepatic lesions of PBC. Grades of eosinophilic infiltration of the portal tracts are positively correlated with grades of lymphoplasmacytic infiltration, granulomas and florid duct lesions in the portal tracts, and negatively correlated with grades of bile duct disappearance [34]. These data imply that eosinophilic infiltration and granulomas are present in the early stage of PBC, but their roles in the pathogenesis of PBC are still unclear. These latter features are generally not found in other

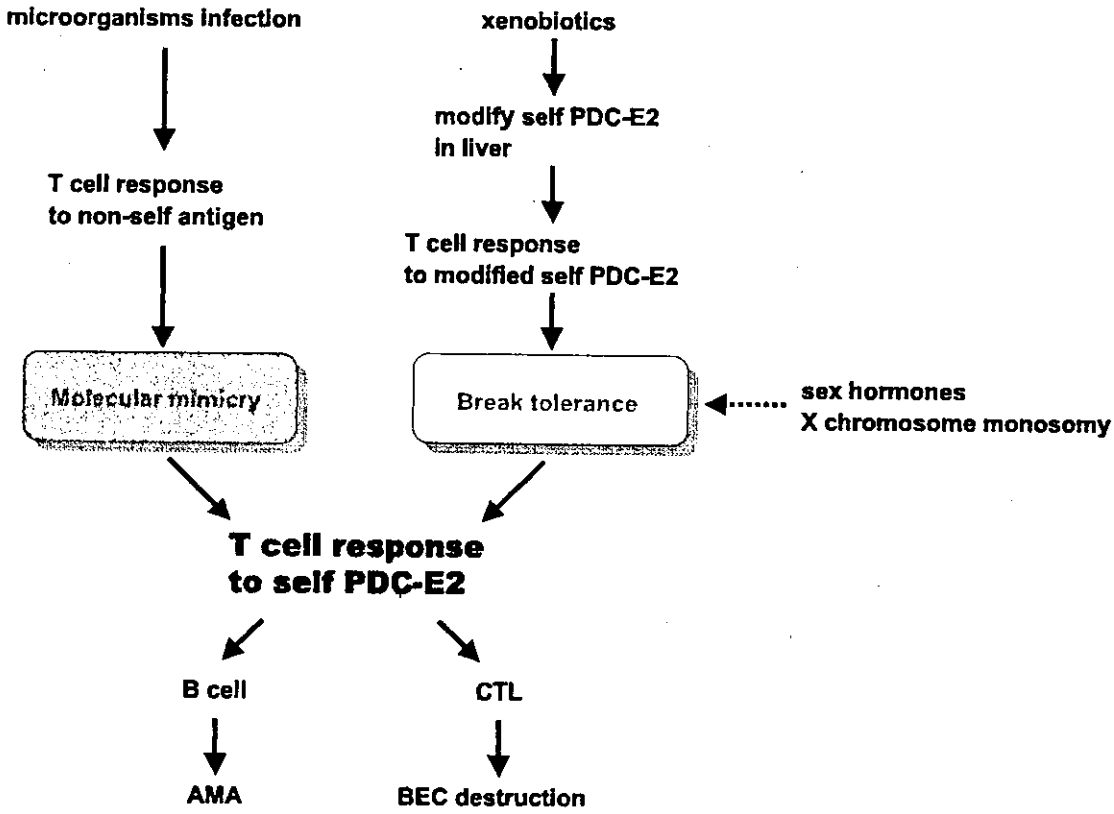

Fig. 1. Hypothetical immune mechanism in PBC.

autoimmune diseases, although they can be seen in several autoimmune animal models and in rheumatoid arthritis [35,36].

6. Other issues

There are several other issues in PBC compared to other autoimmune diseases. Why are only bile ducts affected in PBC despite the fact that mitochondrial antigens are ubiquitous. In general, the autoantigens in systemic autoimmune diseases are ubiquitous, such as the components of the cell nucleus (dsDNA, histones, snRNP) in systemic lupus erythematosus (SLE). On the other hand, autoantigens in organ-specific autoimmune diseases are localized in a specific organ, such as the central nervous system antigens (MBP, proteolipid protein and myelin oligodendrocyte glycoprotein) in MS and the pancreatic islet cell antigens (GAD65, insulin, proinsulin and insulinoma-associated-2) in T1D. One of the reasons why only bile ducts are affected in PBC may be that liver is a unique organ for metabolism and degradation for xenobiotics and an altered immune response [37]. Many chemicals are detoxified through the liver and secreted in bile through biliary epithelial cells. During these processes, xenobiotics may modify autoantigens in liver, break tolerance and initiate an autoimmune response. Interestingly, immunization of rabbits with a xenobiotic organic compound, 6-bromohexanoate, coupled to bovine serum albumin induces the production of AMA [38]. Unfortunately, there is no animal model of PBC. It is clear that animal models of autoimmune diseases are powerful tools for immunopathogenesis and therapies, such as the non-obese diabetic mice of T1D, the experimental allergic encephalomyelitis mice of MS and New Zealand mice for SLE.

7. Conclusion

PBC is one of the most homogenous autoimmune diseases. The autoreactive B cells, $CD4^+$ T cells and $CD8^+$ CTL recognize overlapping epitopes on the mitochondrial autoantigens. These autoantigens are well characterized, and there are data that molecular mimicry and xenobiotics may be involved in the initiation of disease. Female predilection and familial high prevalence are similar to other autoimmune diseases, and the involvement of X chromosome monosomy in the female susceptibility of PBC has been suggested for the first time as an important factor for autoimmune disease. However, there is neither evidence for determinant spreading nor specific-HLA association. Histopathologically, PBC has the unusual features of eosinophilic infiltration and granuloma formation in early biliary lesions. Furthermore, there is no animal model of PBC.

Take-home messages

- The epitopes of autoreactive B cells, $CD4^+$ T cells and $CD8^+$ CTL overlap with each other.
- X chromosome monosomy may be involved in female susceptibility of PBC.
- Molecular mimicry may be involved in the initiation of PBC.
- Only bile ducts are affected in PBC despite the fact that mitochondrial autoantigens are ubiquitous. Xenobiotics may be involved in this mechanism.

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Mitochondria and autoimmunity in primary biliary cirrhosis

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Abstract

Primary biliary cirrhosis is an enigmatic autoimmune liver disease that predominantly affects women and is characterized by antimitochondrial antibodies and specific destruction of small bile ducts. Interestingly, patients with this disease not only have high titer antibodies to mitochondria, but also highly directed, liver-specific CD4 and CD8 cells directed at the same mitochondrial autoantigens. These mitochondrial autoantigens are all members of the 2-oxo dehydrogenase complex family and include the E2 component of pyruvate dehydrogenase as the major autoantigen. Moreover, the epitopes recognized by CD4, CD8 T cells and autoantibody, are all directed within the same region, namely the lipoyl domain of pyruvate dehydrogenase complex-E2. All cells in the body have mitochondria but there appear to be specific destruction of biliary cells. We believe that this specific destruction is secondary to a highly directed mucosal response that focuses on biliary cells because of the involvement of a polymeric immunoglobulin receptor, the presence of immunoglobulin A in mucosal secretions, and the unique apoptotic properties of biliary epithelium.

Keywords: Primary biliary cirrhosis; Antimitochondrial antibodies; 2-Oxo-acid dehydrogenase complexes; IgA; Biliary epithelial cells; Apoptosis

1. Introduction

Although mitochondria are found in all eukaryotic cells and are physiologically essential for aerobic respiration, patients with the autoimmune biliary disease, primary biliary cirrhosis (PBC), develop high titer of antimitochondrial antibodies (AMA) against the some of the enzyme complexes involved in the tricarboxylic acid cycle. PBC is a chronic autoimmune cholestatic liver disease that predominantly affects middle-aged women and histologically characterized by infiltration of lymphocytes in portal triad and destruction of intrahepatic small bile ducts followed by liver fibrosis and eventually, liver failure

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(Kaplan, 1996; Gershwin et al., 2000). PBC has a worldwide prevalence of approximately 5/100,000 and an annual incidence of approximately 6/1,000,000. About 90% of patients with PBC are women. Most commonly, the disease is diagnosed at middle age.

Although mitochondrial antigens are the targets of immunological responses in PBC, it is enigmatic why AMA are directed against selected mitochondrial proteins and the immunological attack is predominantly bile ducts-specific (especially, small bile ducts) despite the fact that mitochondrial antigens are ubiquitous. In this article, we will focus on the immunological responses to mitochondrial autoantigens, the molecular identities of the mitochondrial antigens, the physiology of BEC with regard to current concepts on the possible molecular pathological mechanism(s) in PBC, and the experimental evidence supporting a possible mechanism of mitochondrial antigen-specific immunoglobulin A (IgA)-mediated specific destruction of intrahepatic small bile ducts in patients with PBC.

2. Mitochondrial proteins as autoantigens in PBC

2.1. Discovery of AMA and the molecular identification of major mitochondrial autoantigens in PBC

Several reports in the late 1950s showed that sera of patients with PBC contained highly titers of elevated circulating complement-fixing autoantibodies to liver, kidney and other human tissue antigens, indicating that PBC may have an autoimmune pathogenesis (Gajdusek, 1957; Mackay, 1958). Later, Walker et al. (1965) showed that PBC sera, but none of the controls exhibited a characteristic cytoplasmic pattern of immunofluorescence on human tissue sections rich in mitochondria. In contrast, no such staining was found in tissue poor in mitochondria. In addition, they showed the cytoplasmic fluorescence was abolished by prior absorption with rat-liver mitochondria, but not by the other subcellular fractions. These studies represent the early identification of association of AMA in sera of patients with PBC. It was not until the late 1980s that the mitochondrial autoantigens recognized by AMA were identified as members of the 2-oxo-acid dehydrogenase enzyme complexes (2-OADC) (Coppel et al., 1988; Gershwin et al., 1987), including the E2 subunit of pyruvate dehydrogenase complex (PDC-E2), the E2 subunit of branched-chain 2-oxo-acid dehydrogenase complex (BCOADC-E2), the E2 subunit of 2-oxo glutarate dehydrogenase complex (OGDC-E2), dihydrolipoamide dehydrogenase-binding protein (E3BP), the E1α subunit of pyruvate dehydrogenase (PDC-E1α), and the E1α subunit of branched-chain 2-oxo-acid dehydrogenase complex (BCOADC-E1α) (Mackay and Gershwin, 1989; Mori et al., 2001).

The most common AMA is anti-PDC-E2 and approximately 90–95% of PBC sera have antibodies to PDC-E2 (Van de Water et al., 1988b). While some patients have only anti-PDC-E2, most patients developed autoantibodies to one or more other mitochondrial autoantigens. Patients who have only anti-OGDC-E2 or anti-BCOADC-E2 are rare (Table 1). While AMA occur before clinical signs appear (Metcalf et al., 1996), they are very rarely found in non-PBC individuals. Moreover, AMA has very potent inhibitory effects on the in vitro catalytic activity of the enzyme with which they react (Van de Water et al., 1988a; Fregeau et al., 1989). Serum containing AMA rapidly inhibits PDC enzyme function at high serum dilutions (1:500–1:5000) and affinity purified antibodies against each of the 2-OADC enzymes specifically inhibit its own enzyme activity but not other related 2-OADC enzymes (Van de Water et al., 1988a).

2.2. B cell epitopes of the 2-OADC E2s

The E2 subunits of the 2-OADC share structural similarities in terms of their lipoic acid binding domain, E3 binding domain, and catalytic and E2 binding domain (Fig. 1). Several studies, using either

Table 1
Frequencies of antimitochondrial antibodies in PBC

Antigen	Frequency (%)
PDC-E2	95
BCOADC-E2	53–55
OGDC-E2	39–88
PDC-E1α	41–66
E3BP	95