mouse model. Furthermore, an association between susceptibility to PBC and MHC class II molecules, such as HLA DR8, has been demonstrated [31]. A high prevalence of PBC is observed within some families [32]. However, the prevalence of PBC never reaches 100 % among monozygotic twins, suggesting that both genetic and environmental factors are involved in the etiology of autoimmune diseases ([32] and references therein).

In the present study, we demonstrated that the pathogenetic mechanism underlying these PBC-like CNSDC is autoimmunity through the transmission of 'self'-reactive lymphocytes to naïve mice. In addition, we assessed the role of autophagy in the pathological alterations observed in our PBC mouse model, as well as the influence of the strain differences.

Methods

Bacterial Strain and Culture Conditions

Live *S. intermedius* NCDO 2227^T, the type strain, were used in the present series of experiments. The bacteria were cultured in Brain Heart Infusion broth (BD Biosciences) anaerobically using the AnaeroPak system (Mitsubishi Gas Chemical).

Mice

C57BL/6 and BALB/c mice were purchased from SLC (Hamamatsu, Japan), and C3H/HeJ mice were purchased from Crea Japan Ltd. RAG2$^{-/-}$ mice [33, 34] were kindly provided by Professor Abe (Research Institute for Biological Sciences, Science University of Tokyo, Japan). C57BL/6 mice were bred in the animal facility of the Department of Microbiology and Immunology, Tokyo Women's Medical University. Approximately 6-week-old female mice were used in all the experiments; the study design and the use of the mice were approved by the ethics review committee for animal experiments at Tokyo Women's Medical University.

Treatment of Mice with Bacteria

Mice were intraperitoneally (i.p.) injected with *S. intermedius*, (2×10^8 CFU/mouse in 200 μL of phosphate buffered saline [PBS]) once a week for a total of 8 weeks. Simultaneously, a group of control mice were inoculated weekly with 200 μL of PBS for 8 weeks. All the mice were sacrificed under deep anesthesia using diethyl ether at the indicated time after the final inoculation. Tissues and sera samples were then obtained for use in the following examinations.

Histopathology

Multiple 4-μm-thick sections were deparaffinized and stained with hematoxylin-eosin (H&E) and Masson's trichrome staining. Serial sections were subjected to immunohistochemical staining by incubation with mAbs against CD3 (abcam), CD45R/B220 (BD PharMingen), F4/80 antigen (BMA Biochemicals AG), or polyclonal anti-LC3B Ab (abcam), then stained according to a peroxidase technique using a Vectastain Elite ABC Kit (Vector Laboratories).

Spleen Cell Transfer to RAG2$^{-/-}$ Mice

Spleen cells (5×10^7 cells in 200 μL of PBS/mouse) obtained 1 week after the completion of the series of *S. intermedius* inoculations were intravenously (i.v.) injected into RAG2$^{-/-}$ mice. Ten days after the spleen cell transfer, the mice were sacrificed to examine the tissues for pathological damage. Simultaneously, spleen cells (5×10^7 cells in 200 μL of PBS/mouse) obtained from PBS-inoculated C57BL/6 mice inoculated weekly with PBS for 8 weeks were i.v. injected into a control group of RAG2$^{-/-}$ mice.

Antibody Production in S Intermedius-inoculated Mice

Gp-210 C-terminal peptides were synthesized using a peptide synthesizer (Model 432A Synergy; Applied Biosystems) and F-moc chemistry, as previously described ([35] and references therein). The peptide was purified using reverse-phase high-performance liquid chromatography (HPLC), and a purity of greater than 90 % was attained. Using mouse gp210 as an antigen, an ELISA was performed as described elsewhere [35].

To study anti-nuclear antibody (ANA) production, HEp-2 cells were used [36]. Hep2 cells were seeded onto glass-bottom dishes (MATSUNAMI), fixed with 95 % ethanol, and blocked with 3 % non-fat milk containing 2 % TritonX-100 in PBS. The cells were then stained using a 1:100 dilution of each mouse serum in PBS followed by incubation with FITC-conjugated anti-mouse IgG (Jackson ImmunoResearch). The reactions were visualized using the LSM 510 laser-scanning microscope (Carl Zeiss MicroImaging, LSM 510; Carl Zeiss Co., Ltd., Jena, Germany).

Electron Microscopic Examination

Liver tissues obtained from BALB/c mice at 1 week after the final *S. intermedius*-inoculation were used. The tissues were fixed using 2 % glutaraldehyde in PBS, followed by osmification, dehydration, and direct embedding in EPON 812 RESIN (TAAB Laboratory Equipment Ltd., Berks, England). Ultrathin sections were stained using uranyl acetate and lead citrate, mounted on copper grids, and observed

using a transmission electron microscope (H-7650; Hitachi Inc., Tokyo, Japan).

Flow Cytometry

Single-cell suspensions from spleens and liver containing infiltrating cells from *S. intermedius*-inoculated and PBS-inoculated mice were obtained at 1 week after the final inoculation. Hepatic mononuclear cells were isolated using 35 % Percoll solution containing 100 U/mL of heparin [37]. Cells were washed twice in staining buffer containing 1 % BSA in PBS, resuspended, and then incubated with labeled antibodies to CD3 (BD Biosciences), TCR$\gamma\delta$ (BD Biosciences), CD4 (BD Biosciences), CD8 (BD Biosciences), CD69 (BD Biosciences), and NK1.1 (eBioscience), which were diluted in staining buffer, for 30 min at 4°C. Cells were then washed in staining buffer and analyzed immediately using an EpicsXL (Beckman Coulter Inc., CA, USA).

Statistical Analyis

Data were analyzed by Mann–Whitney *U*-test and analysis of variance (ANOVA) for comparison between groups where applicable. Statistical significance was defined as $p<0.05$.

Results

Pathology of *S. intermedius*-inoculated Mice

In our previous study, PBC-like CNSDC was observed in the livers of *S. intermedius*-inoculated BALB/c (H-2^d) mice [28]. To study strain differences in *S. intermedius*-inoculated mice livers, C57BL/6 (H-2^b) and C3H/HeJ (H-2^k) mice were examined. In a representative liver from a C57BL/6 mouse obtained 1 week after the last of the 8 weekly inoculations with *S. intermedius*, inflammation in the portal area, over the hepatic parenchyma, was observed, as suggested by enlarged portal tracts accompanied by moderate to marked inflammatory cellular infiltrates of lymphocytes, plasma cells, and granulocytes. In addition biliary epithelial cell damage with focal disruption of the basement membrane was observed (Fig. 1a, b). In a representative liver from a C57BL/6 mouse at 1 week after the final inoculation with PBS, the inflammatory cellular infiltrates and biliary epithelial cell damage around the portal tracts and the inflammation in the hepatic parenchyma were none to mild (Fig. 1c). In the livers of C57BL/6 mice at 1 week after the final *S. intermedius*-inoculation, CD3-positive cells were predominantly observed in the cellular infiltrates around the bile ducts (Fig. 1d, e), whereas relatively few B220-immunoreactive or F4/80 pan-macrophage antigen-immunoreactive cells were observed in the infiltrating cells (Fig. 1f, g, respectively). *S. intermedius*-inoculation might upregulate CD3-positive cell-mediated immune responses, as observed in *S. intermedius*-inoculated BALB/c mice [28]. Thus, the pathological findings observed in the C57BL/6 mouse livers were quite similar to those observed in BALB/c mice, as described in our previous report [28].

In a representative liver from a *S. intermedius*-inoculated C3H/HeJ mouse, marked inflammation was observed in the hepatic parenchyma close to the portal tracts (Fig. 1h, i), the findings of which were not compatible with CNSDC and more closely resembled those of septic liver. These findings suggested that depending on the mouse strain, the genetic background might influence the PBC-like hepatic alterations that are induced by long-term *S. intermedius* inoculation.

Other types of tissue damage that are occasionally associated with PBC were simultaneously examined. In the salivary glands of *S. intermedius*-inoculated C57BL/6 mice, mild periductular cellular infiltrates were observed (Fig. 1j), but not in the salivary glands of PBS-inoculated C57BL/6 mice (Fig. 1k). In contrast, inflammatory cellular infiltrates were not observed in the salivary glands of *S. intermedius*-inoculated C3H/HeJ mice (0 of 3 mice) (Fig. 1l). Thus, the difference in the response to repeated *S. intermedius* inoculations between the mouse strains was observed not only in the liver, but also in the salivary glands.

Production of Autoantibodies

We studied the serum levels of anti-gp210, since anti-gp210 antibody has been detected in patients with PBC [4], and in our previous study, *S. intermedius*-inoculated BALB/c mice produced anti-gp210 antibodies [28]. *S. intermedius*-inoculated C57BL/6 mice sera showed significantly higher titers of anti-gp210, compared with sera obtained from PBS-inoculated C57BL/6 mice (Fig. 2a). Generally, ANA is clinically detected using HEp2 immunofluorescence [38]. *S. intermedius*-inoculated C57BL/6 mice also produced ANAs, as shown by staining using HEp2 cells (Fig. 2b).

Although anti-gp210 antibodies reportedly produce a characteristic perinuclear rim-like membranous pattern (RL/M) [39], the ANA pattern appeared as multiple nuclear dots (MND), similar to an RL/M pattern. The MND pattern has also been observed in PBC [39]. As ANA was detected using diluted whole mouse sera, not only anti-gp210 but also other ANAs may have been present in the tested sera (Fig. 2b).

In addition, we presently do not know why all the *S. intermedius*-inoculated mice did not produce anti-gp210. In our previous study using BALB/c mice, anti-gp210 production was not observed in all the *S. intermedius*-inoculated BALB/c mice even after a 20-month observation period [28]. Further study examining whether strain differences in

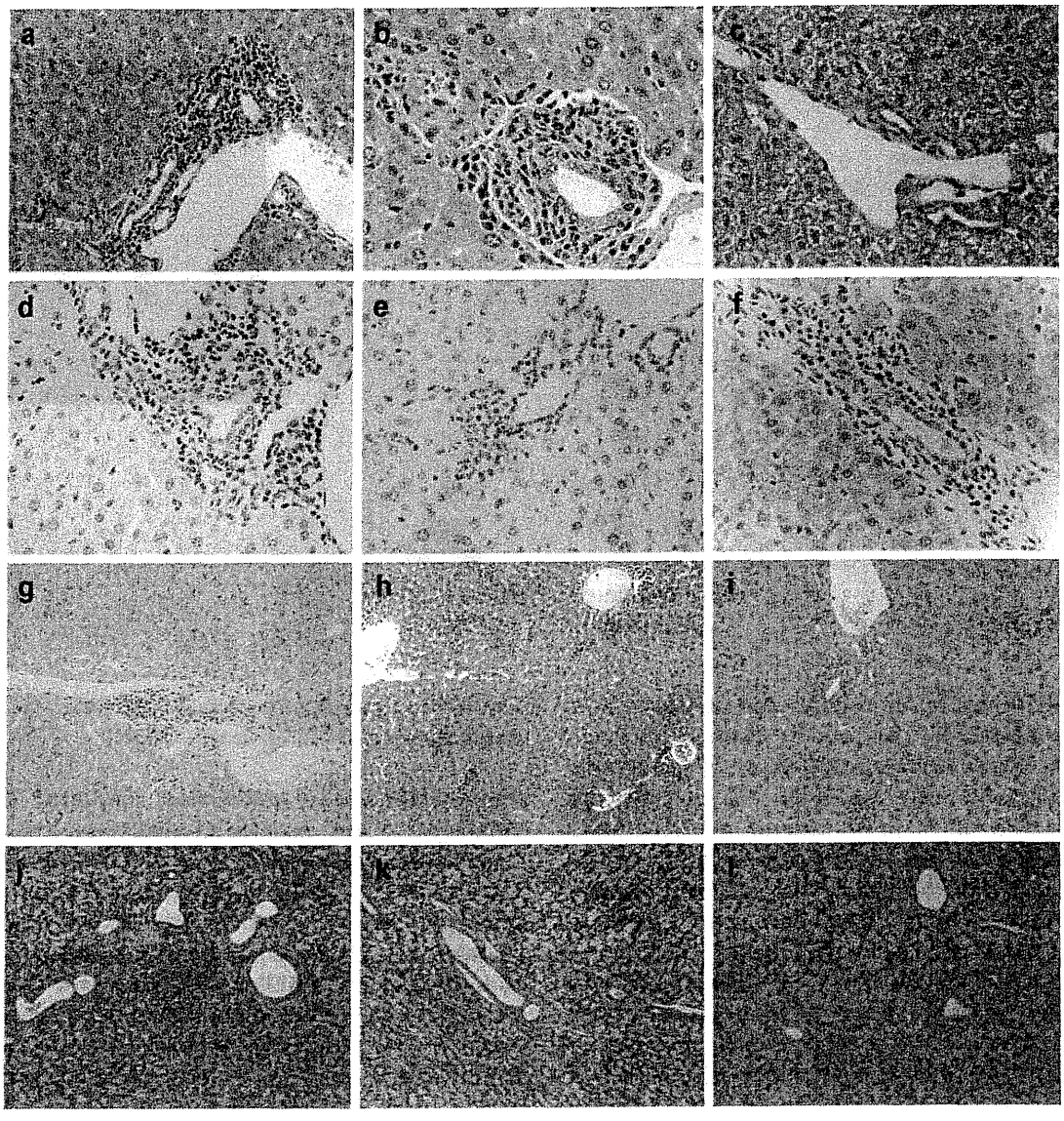

Fig. 1 Representative pathological findings in mouse livers. C57BL/6 mouse livers obtained 1 week after the final inoculation with *S. intermedius* (**a**, **b**) and 1 week after the final inoculation with PBS (**c**). Immunoreactivities for CD3 (**d**, **e**), B220 (**f**), and F4/80 pan-macrophage antigen (**g**). Liver from a *S. intermedius*-inoculated C3H/HeJ mouse obtained 1 week after the final inoculation with *S. intermedius* (**h**, **i**). C57BL/6 mouse salivary glands obtained 1 week after the final inoculation with *S. intermedius* (**j**) and 1 week after the final inoculation with PBS (**k**). C3H/HeJ mouse salivary glands were obtained 1 week after the final inoculation with *S. intermedius* (**l**)

anti-gp210 production exist after a longer observation period may be required.

Spleen Cell Transfer to RAG2$^{-/-}$ Mice

Next, we performed a transfer experiment using spleen cells obtained from *S. intermedius*-inoculated C57BL/6 mice; these cells were transferred to RAG2$^{-/-}$ mice. Before the transfer experiment, we performed the same series of *S. intermedius* inoculations to examine the reactivities of RAG2$^{-/-}$ mice without spleen cell transfer. In a representative liver obtained 1 week after the final inoculation with *S. intermedius* in a RAG2$^{-/-}$ mouse, the degree of inflammation in both the portal tract and the hepatic parenchyma was almost none to quite mild (Fig. 3a). In contrast, 2 weeks after the i.v. transfer of the spleen cells from *S. intermedius*-inoculated C57BL/6 mice, the livers in the recipient RAG2$^{-/-}$ mice showed cellular infiltration in the portal tract, especially around the bile ducts (Fig. 3b). On the other hand, in the livers of the recipient RAG2$^{-/-}$ control mice, which were i.v. transferred with spleen cells obtained from PBS-inoculated C57BL/6 mice, the degree of cellular infiltration was none

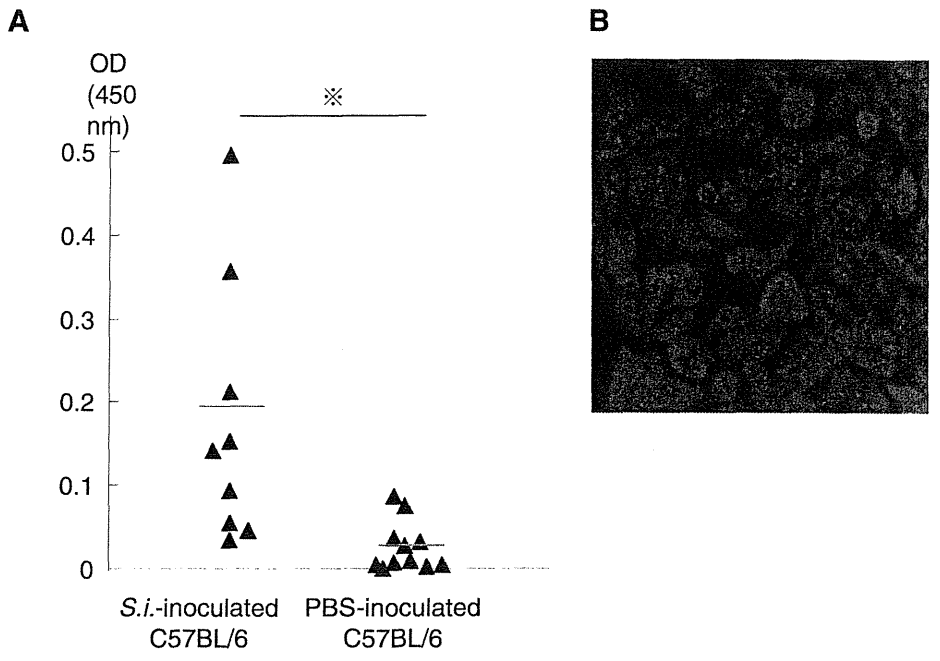

Fig. 2 Autoantibodies. Sera obtained from C57BL/6 mice 1 week after the last of 8 weekly inoculations with *S. intermedius* had significantly higher titers of anti-gp210 (**a**) than sera from PBS-inoculated C57BL/6 mice ($p<0.05$). *S. intermedius*-inoculated C57BL/6 mice also produced ANAs, as shown by staining using HEp2 cells (**b**)

to quite mild (Fig. 3c). In representative sections stained immunohistochemically, the majority of the cellular infiltrates in the portal tracts were CD3-positive (Fig. 3d), with a few B220-positive cells also visible (Fig. 3e). Although immunoreactivity to F4/80 was detected in Kupffer cells, immunoreactivity to F4/80 was not detected in the infiltrating cells around the bile ducts (Fig. 3f). These findings indicated that the majority of the infiltrating cells originated from the donor *S. intermedius*-inoculated C57BL/6 mice, since no mature CD3-positive cells are present in $RAG2^{-/-}$ mice because of the "knockout" of the RAG2 gene [40]. Thus, the CD3-positive cells were associated with the occurrence of *S. intermedius*-triggered PBC-like CNSDC in C57BL/6 mouse liver. Moreover, a few of the sera obtained from $RAG2^{-/-}$ mice that had received spleen cells from *S. intermedius*-inoculated mice exhibited higher antibody titers against gp210 than sera obtained from $RAG2^{-/-}$ mice that had received spleen cells from either PBS-inoculated mice or *S. intermedius*-inoculated $RAG2^{-/-}$ mice (Fig. 3g). Further study over a longer observation period is needed to determine whether homeostatic proliferation would be induced or if not bulk spleen cells, but purified T cells obtained from *S. intermedius*-inoculated mice transferred to $RAG2^{-/-}$ mice would produce larger numbers of gp-210-positive mice or higher anti-gp210 titers. However although the anti-gp210 titers were estimated at only one time point, *i.e.*, 2 weeks after the transfer of spleen cells, one of the $RAG2^{-/-}$ recipient mice definitely developed a higher anti-gp210 antibody titer. These results suggest that the transfer of pathogenic lymphocytes can induce PBC-like alterations in naïve $RAG2^{-/-}$ mice.

Possible Involvement of Autophagic Mechanism in S Intermedius-inoculated Cholangitis Model

We simultaneously studied the possibility that an autophagy-mediated mechanism could be involved in this *S. intermedius*-triggered PBC model. To elucidate this, immunoreactivity to LC3B, an autophagosome marker, was studied. LC3B immunoreactivity was detected in the cytoplasm of some of the infiltrating cells around the portal tract of the livers in both BALB/c and C57BL/6 mice (Fig. 4a, b and 4c, d, respectively). On the other hand, LC3 immunoreactivity was detected in the cytoplasm of only a few of the infiltrating cells in the hepatic parenchyma, but not in the portal tract in C3H/HeJ mice (Fig. 4e). LC3B-immunoreactive cells were not detected in the livers from PBS-inoculated mice (Fig. 4f). We further performed electron microscopic examinations using BALB/c mouse livers obtained 1 week after the last of 8 weekly inoculations with *S. intermedius*. Autophagosome-like structures were observed in the infiltrating cells around the bile duct epithelial cells (Fig. 4g). These results indicated that autophagy might be one of the possible mechanisms involved in the pathogenesis in our cholangitis-harboring mouse model.

Profiles of the Cellular Infiltration

According to the reports by other groups, autoreactive $CD4^{+}$T cells that specifically target the PDC-E2-self-antigen are present in the peripheral blood and liver [41]. Autoreactive $CD8^{+}$T cells likewise have been characterized in PBC and are considered to be major effectors of tissue injury in

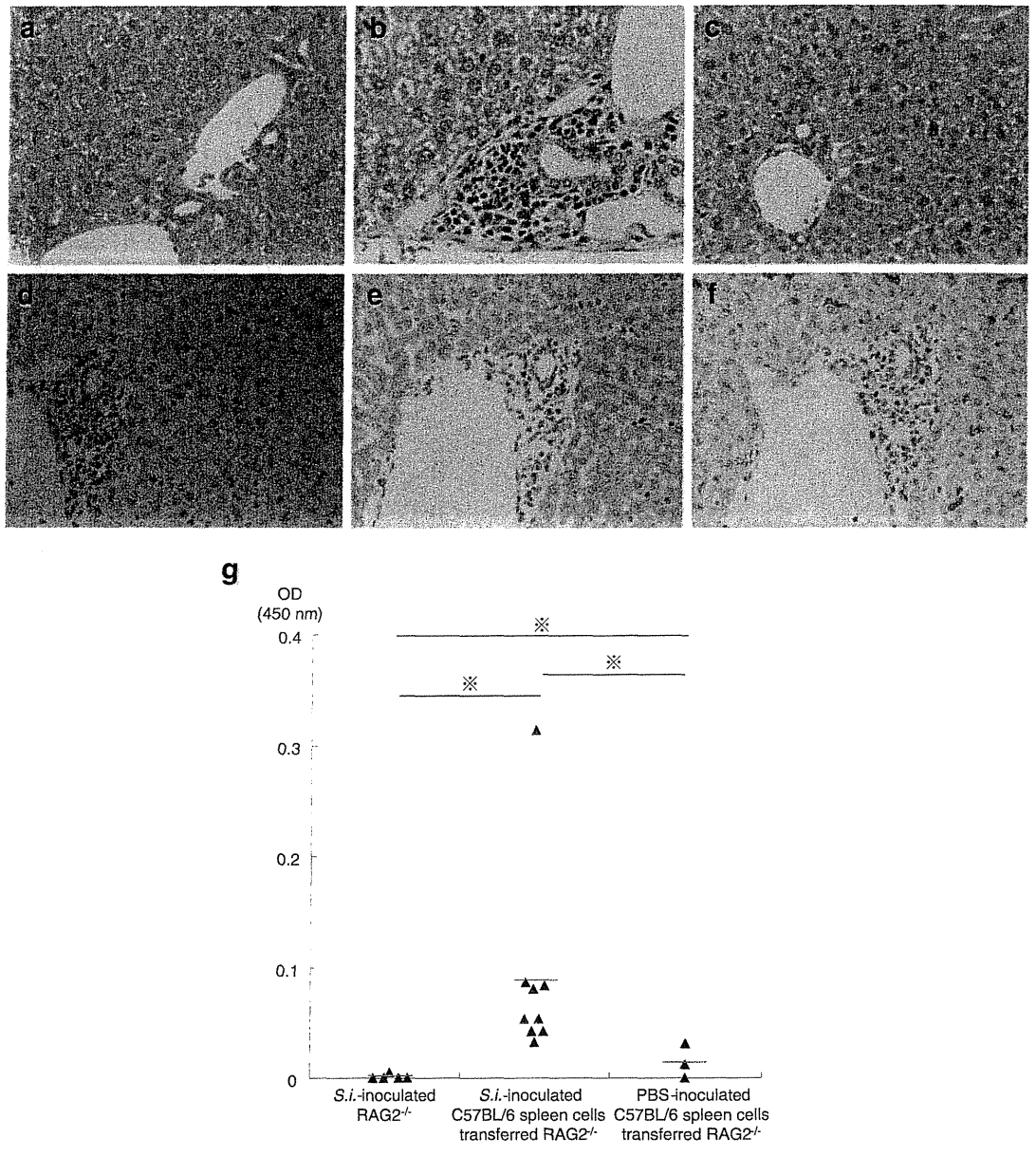

Fig. 3 Spleen cell-transfer to $RAG2^{-/-}$ mice. $RAG2^{-/-}$ mouse liver obtained 1 week after the last of the 8 weekly *S. intermedius* inoculations (**a**). $RAG2^{-/-}$ mouse liver obtained after the adaptive transfer of spleen cells obtained from *S. intermedius*-inoculated (**b**, **d**, **e**, **f**) and PBS-inoculated (**c**) C57/BL/6 mice (hematoxylin and eosin staining (**a**, **b**, and **c**) and immunoreactivities to CD3, B220, and F4/80 (in **d**, **e**, and **f**, respectively). The anti-gp210 titer was higher in sera obtained from $RAG2^{-/-}$ mice adaptively transferred with spleen cells obtained from *S. intermedius*-inoculated C57/BL/6 mice than in sera obtained from $RAG2^{-/-}$ mice after the adaptive transfer of spleen cells obtained from PBS-inoculated C57/BL/6 mice or spleen cells obtained from *S. intermedius*-inoculated $RAG2^{-/-}$ mice 1 week after the last of 8 weekly inoculations with *S. intermedius* (**g**) (※ $p<0.05$)

PBC ([41] and references therin). To identify the infiltrating cell subsets involved in this repeated microbial-inoculated CNSDC-harboring mouse model, we studied the cell profiles of the infiltrating cells. Single cell suspensions were obtained at the time of $RAG2^{-/-}$ transfer experiment, from donner C57BL/6 mice. Liver and spleen cells were each pooled from both *S. intermedius*-inoculated and PBS-inoculated C57BL/6 mice ($n=6$ each) and their surface molecules were studied using flow cytometry. Among the infiltrated cells in both the liver and the spleen, $TCR\gamma\delta^{+}$, $NK1.1^{+}$, and $CD3^{+}NK1.1^{+}$ cells were more abundant in the *S. intermedius*-inoculated mouse group than in the PBS-inoculated mouse group (Fig. 5). Furthermore, in the *S. intermedius*-inoculated mouse group, $CD3^{+}NK1.1^{+}$ cells

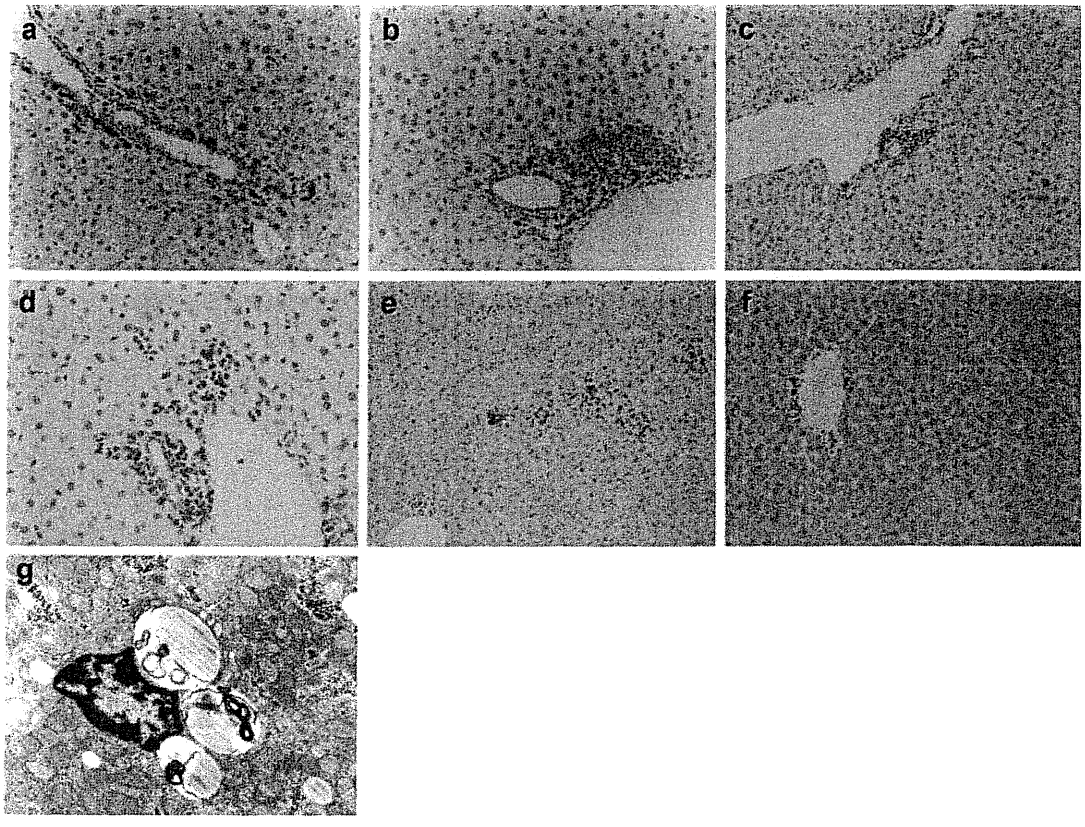

Fig. 4 Possible involvement of autophagic mechanism in *S intermedius*-inoculated cholangitis model. LC3B immunoreactivity was detected in the cytoplasm of some infiltrating cells around the portal tract in the livers of BALB/c mice (1 week and 20 months after the final inoculation; **a**, **b** respectively), 1 week after the final inoculation with *S. intermedius* in C57BL/6 mice (**c**, **d**), 1 week after the final inoculation with *S. intermedius* in C3H/HeJ mice (**e**), and 1 week after the last of 8 weekly inoculations with PBS in C57BL/6 mice (**f**). Electron microscopy of mouse livers obtained 1 week after the final inoculation with *S. intermedius* in BALB/c mice (**g**)

were more abundant in the *S. intermedius*-inoculated mouse group than in the PBS-inoculated mouse group in the liver (28.1 % and 11.2 %, respectively) and in the spleen (19.6 % and 4.4 %, respectively) (Fig. 5). $CD4^{+}CD69^{+}$ spleen cells were upregulated in the *S. intermedius*-inoculated mouse group more strongly than in the PBS-inoculated mouse group (14.3 % and 7.9 %, respectively), and $CD8^{+}CD69^{+}$ liver-infiltrating cells from *S. intermedius*-inoculated C57BL/6 mice were also upregulated more strongly in infiltrating cells obtained from the *S. intermedius*-inoculated mouse group than those obtained from the PBS-inoculated mouse group (13.3 % and 5.1 %, respectively) (Fig. 5).

Discussion

PBC is thought to be a "complex disease" that can be attributed to the combined effects of multiple environmental and behavioral influences, genetic elements, and perhaps chance [42]. In the present study, we first demonstrated that mouse strains differed in their responses to repeated inoculation with *S. intermedius*. Repeated *S. intermedius*-inoculation induced CNSDC in the livers of C57BL/6 mice (Fig. 1) and BALB/c mice [28], whereas septic inflammation in the hepatic parenchyma, but not CNSDC, was induced in C3H/HeJ mice (Fig. 1). In addition, repeated *S. intermedius*-inoculated C57BL/6 mice exhibited not only a histological alteration in their liver, but also higher level of serum anti-gp210 (Fig. 2). These results indicated that the strain difference involved in the pathogenesis of repeated *S. intermedius*-inoculated CNSDC formation. Furthermore, CNSDC-like inflammatory region in the livers of recipient $RAG2^{-/-}$ mice with spleen cells transferred from *S. intermedius*-inoculated C57BL/6 mice, showed that most of the cellular infiltrations in the target livers were CD3 positive, indicating that these cells originated from the donor mice (Fig. 3). The finding observed indicated that our animal model of PBC-like CNSDC is of autoimmune etiology.

Next, we studied whether autophagy-mediated mechanisms might be involved in this mouse model. Autophagy takes part in many critical biological processes [43–45]. Autophagy is also a component of innate immunity [46]

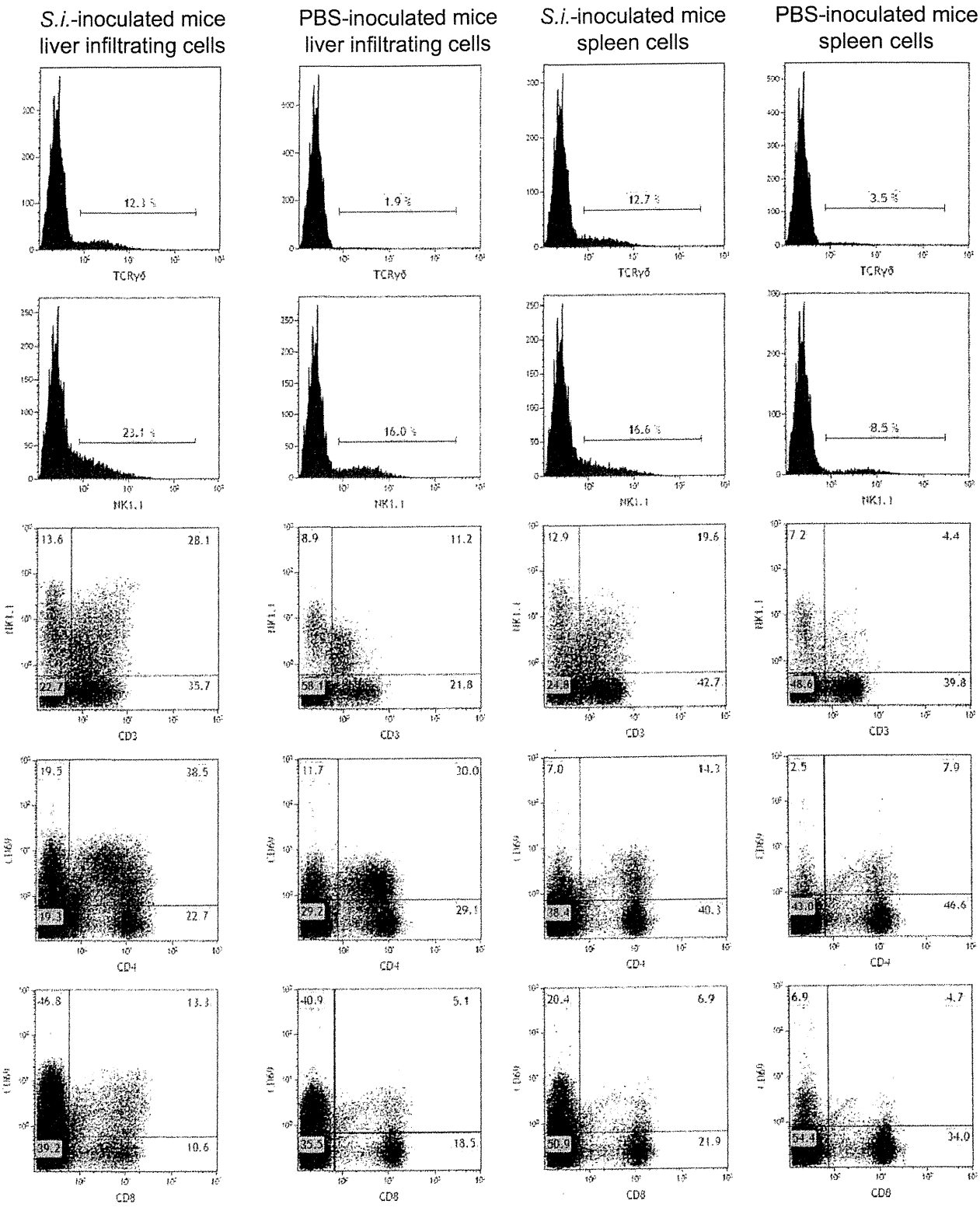

Fig. 5 Cell profiles of liver-infiltrating cells and spleen cells. Single cell suspensions were obtained from C57BL/6 mice at 1 week after the final inoculation with either *S. intermedius* or PBS. Liver-infiltrating cells and spleen cells were each pooled from 6 mice and their surface molecules were examined using flow cytometry

and is involved in the host defense elimination of bacterial pathogens, including *Streptococcus* species [47]. We observed that immunoreactivity for the autophagosome marker LC3 was markedly detected in the cytoplasm of infiltrating cells around the bile duct in *S. intermedius*-inoculated C57BL/6 and BALB/c mice, but not C3H/HeJ mice (Fig. 4). An electron microscope examination confirmed the presence of autophagosome-like structures in BALB/c mouse liver (Fig. 4). Based on these findings, we speculate that an autophagy-mediated mechanism, possibly induced by long-term inoculation with *S. intermedius*, may induce the breakdown of tolerance-targeting self/auto-antigens or commensal flora through molecular mimicry, possibly explaining the pathogenesis of *S. intermedius*-triggered PBC-like CNSDC. Obviously, further study to confirm this hypothesis is needed.

Autoantibody such as anti-gp210 has been detected in patients with PBC [4, 35]. Sera from subgroup of PBC patients are positive for ANAs [48]. These ANAs include two components of the nuclear pore complex specifically associated with a perinuclear pattern, that is gp210, p62, Sp 100 and promyelocytic leukemia proteins [4]. *S. intermedius*-inoculated C57BL/6 mice also produced both anti-gp210 antibodies and ANA as reported in our previous BALB/c mice [28]. In this report, we semi-quantitatively measured the serum levels of anti-gp210 as an anti-nuclear antibody. The ANA pattern resembled not only an RL/M pattern, but also an MND pattern, indicating a mixed pattern with other kind(s) of ANAs, such as anti-Sp100. A more detailed ANA analysis is required. However, our results support the usefulness of our mouse model as a PBC-like autoimmune cholangitis model.

Based on the flow cytometry analysis, the numbers of both liver and spleen $NK1.1^+$, $TCR\gamma\delta^+$, and $CD3^+NK1.1^+$ cells were higher in *S. intermedius*-inoculated C57BL/6 mice. $TCR\gamma\delta^+$ T cells play a role in protection during the early stage of infection. The protective mechanism of $TCR\gamma\delta^+$ T cells acts against bacterial infection in the liver [49]. $TCR\gamma\delta^+$ T cells are known to bridge innate and adaptive immune responses and are an important part of the immune surveillance system [50]. NKT cells have also been implicated as innate effector cells [41]. The involvement of NKT cells in the pathogenesis of PBC has been suggested [41, 51]. Mattener et al. reported that in a murine model of PBC, *N aromaticivorans* induced autoreactive AMAs and T-cell-mediated autoimmunity against small bile ducts via an NKT-dependent mechanism [52]. In addition, lymphocytic synergism between $TCR\gamma\delta^+$T cells and iNKT cells has been suggested in an airway hyperresponsiveness model [53]. Synergism between $TCR\gamma\delta^+$T cells and NKT cells might also exist in our *S. intermedius*-triggered CNDSC-harboring mouse model. Additional study regarding this point is needed. Furthermore, $CD4^+CD69^+$ spleen cells and $CD8^+CD69^+$ liver infiltrating cells were also upregulated. Both $CD4^+$ and $CD8^+$ T lymphocytes can be purified from liver biopsy samples obtained from PBC patients [54]. Therefore, the increase in activated $CD4^+$ and $CD8^+$ T cells might indicate a closer immunological response in human PBC.

Collating our results with the criteria for determining whether a condition may be considered as being autoimmune, as outlined by Witebsky's postulates and the modern revision by Rose and Bona [55], the following critical parameters were met: (i) repeated inoculation with *S. intermedius* successfully reproduced PBC-like CNSDC in both BALB/c and C57BL/6 mice in a strain-favorable manner, (ii) direct proof was obtained indicating that the transfer of pathogenic T cells induced CNSDC in naïve $RAG2^{-/-}$ mice (Fig. 4), and (iii) the serum levels of anti-gp210 antibodies were elevated in repeated *S. intermedius*-inoculated mice. These multiple lines of evidence indicate that the mechanism involved in our established animal model has an autoimmune origin.

Mattener et al. reported a PBC mouse model induced by intravenous or oral inoculation with *N aromaticivorans* [52]. We agree with their proposal that neither *N aromaticivorans* nor *S. intermedius* is likely to be the only cause of PBC. These bacteria may contribute to the breakdown in tolerance that in turn leads to the development of PBC. Interestingly, in our previous observation [56], an initial breakdown in tolerance was induced by repeated inoculation with *S. intermedius* but not *E. coli* in the liver of BALB/c mice. However, repeated inoculation with *E. coli* in BALB/c mice resulted in an autoimmune pancreatitis (AIP)-like alteration in the pancreas [56]. Depending on the bacterial species and the presence or absence of other factor(s), such as genetic elements, different bacteria may have different likelihoods of causing a breakdown in tolerance or triggering autoimmune disease in different organs.

In conclusion, our *S. intermedius*-inoculated model clearly resembled human PBC. Clarification of the pathogenetic mechanism involved in this model may provide new insights regarding therapeutic approaches for autoimmune epithelial inflammation, such as PBC, prior to the commencement of a terminal disease stage.

Acknowledgments The authors thank Professor Takehiko Uchiyama (Tokiwa University) for profound discussion, Mr. Hideyuki Takeiri, Mrs. Noriko Sakayori, Mr. Mizuho Karita and Mr. Yasuhide Shigematsu for their skillful technical assistance, Dr. Yutaka Arimura and Mr. Hidehiro Ueshiba for their help with the series of bacterial inoculations in the mice, and Mr. Masamichi Yoshikawa for the intensive animal care. This work was financially supported in part by Grants-in-Aid for Scientific Research (C23590522 to I.H. and C21590496 to J.Y) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Disclosure The authors declare that we have no conflicts of interest.

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総　説

PBCの疾患感受性遺伝子による病態の解明

中村　稔[*1,*2]

Analysis of disease-pathway by identifying susceptibility genes to primary biliary cirrhosis

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(Received October 9, 2012)

summary

High concordance rate in monozygotic twins and familial clustering of patients with primary biliary cirrhosis (PBC) indicate the involvement of strong genetic factors in the development of PBC. Recent genome-wide association studies (GWASs) and subsequent meta-analyses in European descent have identified *HLA* and 21 non-*HLA* susceptibility loci which are involved in IL12/IL12R signaling, TNF/TLR-NFKB signaling and B cell differentiation in the development of PBC. To identify susceptibility loci for PBC in Japanese population, a GWAS and subsequent replication study was performed in a total of 1327 PBC cases and 1120 healthy controls. In addition to the most significant susceptibility region at HLA, two significant ($p<5\times10^{-8}$) susceptibility loci (*TNFSF15* and *POU2AF1*) were identified. Although these susceptibility loci are different from those identified in European descent (IL12A, IL12RB2, SPIB), these loci are involved in the same signaling pathways, differentiation of T lymphocyte to Th1 cells and differentiation of B lymphocyte to plasma cells. Among 21 non-HLA susceptibility loci for PBC identified in GWASs of European descent, 10 loci (*CD80*, *IKZF3*, *IL7R*, *NFKB1*, *STAT4*, *TNFAIP2*, *CXCR5*, *MAP3K7IP1*, *rs6974491*, *DENND1B*) showed significant associations in the Japanese population. The comparative analysis of disease-susceptibility genes in multiple ethnicities may provide an important clue for the dissection of disease-pathogenesis.

Key words——disease-susceptibility gene; single nucleotide polymorphism (SNP); genome-wide association study (GWAS); primary biliary cirrhosis (PBC)

抄　録

原発性胆汁性肝硬変（PBC）の発症には，家族集積性や双生児による研究から強い遺伝的素因の関与が示唆されていたが，近年，欧米人を対象としたゲノムワイド関連解析（GWAS）により，PBC疾患感受性遺伝子としてHLA領域の遺伝子多型の他に，IL12/IL12Rシグナル伝達，TLR/TNFα-NFkBシグナル伝達，B細胞の成熟・分化，上皮細胞の分化・アポトーシスなどに関連する計21の遺伝子多型が報告された．本邦においても，国立病院機構肝ネットワーク研究班，厚生労働省難治性疾患克服研究事業“難治性の肝・胆道疾患に関する調査研究班に登録されたPBC 1,327名と健常者1,120名のDNA検体を用いて全国規模のGWAS共同研究を実施し，日本人PBCの発症に関わる新規疾患感受性遺伝子を2個（*TNFSF15*，*POU2AF1*）同定した．これらの遺伝子は欧米人で報告されたPBCの疾患感受性遺伝子（*IL12A*, *IL12RB2*, *SPIB*）とは異なっていたが，免疫応答においては同一のシグナル伝達系やリンパ球の分化・成熟の経路に位置しており，集団間で疾患感受性遺伝子が異なっていてもPBCの疾患発症経路は共通であることが示唆された．また，欧米で同定された21個の疾患感受性遺伝子の内10遺伝子（*CD80*, *IKZF3*, *IL7R*, *NFKB1*, *STAT4*, *TNFAIP2*, *CXCR5*, *MAP3K7IP1*, *rs6974491*, *DENND1B*）が日本人でもPBCの疾患感受性遺伝子であることが確認された．複数の集団での疾患感受性遺伝子の比較検討は疾患発症機構の解明のための重要な手がかりとなることが期待される．

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はじめに

原発性胆汁性肝硬変（PBC）は中年女性に好発する比較的まれな（患者総数は全国で約5-6万人と推定）胆汁うっ滞性肝疾患で，初期には無症状のことが多いが，進行すると全身の掻痒感，食道静脈瘤，腹水，黄疸，脳症が出現，肝不全に至り肝移植以外には救命方法がない難病である．ミトコンドリアや核成分に対する自己抗体（抗ミトコンドリア抗体，抗gp210抗体，抗セントロメア抗体，抗sp100抗体など）が出現すること，他の自己免疫疾患の合併率が高いことや門脈域の小葉間胆管周囲にリンパ球の浸潤を認めることなどから肝内小葉間胆管を標的とする自己免疫疾患と考えられている[1,2]．一方，PBCには家族集積性（患者同胞の発症危険率10.5）が高いこと，一卵性双生児におけるconcordance rate（60%）が高いことからその発症には強い遺伝的素因の関与が示唆されていた[3]．

近年，ヒトゲノム全域の膨大な多様性情報の集積（HapMap計画）とゲノムワイド関連解析（genome-wide association study：GWAS）技術の進歩により，様々な疾患において遺伝子多型と疾患感受性との関連の解析が可能となった．PBCにおいてもこの手法（GWAS）を用いて2009年にカナダからヨーロッパ系集団のPBC疾患感受性遺伝子座が2か所（*IL12A*, *IL12RB2*）初めて同定された[4]．その後，イタリア，カナダ，イギリスからメタアナリシスを含めたGWASの報告が相次ぎ，現在までにヨーロッパ系集団の疾患感受性遺伝子が計21か所（*STAT4*, *IRF5*, *IKZF3*, *MMEL1*, *SPIB*, *DENND1B*, *CD80*, *IL7R*, *CXCR5*, *TNFRSF1A*, *CLEC16A*, *NFKB*, *RAD51L1*, *MAP3K7IP1*, *PLCL2*, *RPS6KA4*, *TNFAIP2*, 7p14 and 16q24）同定された[5~7]．これらの疾患感受性遺伝子が集団差を超えてPBC発症に共通であるか否かは疾患の発症機構の解明だけでなく人類遺伝学上の興味ある課題であり，特に日本人のような比較的遺伝的均質性の高い集団における疾患感受性遺伝子の同定が待たれていた．

本総説では，まずヨーロッパ系集団で実施されたPBC-GWASのこれまでの成果を紹介した後[4~7]，日本人PBC-GWAS共同研究により最近明らかとなった日本人のPBC疾患感受性遺伝子について紹介するとともに，これらの結果から推定されるPBC疾患発症経路について我々の考え方を紹介する[8]．

欧米人でのPBC-GWAS研究

2009年にPBCを対象とした初めてのGWASがカナダのグループからNew England Journal of Medicineに発表された[4]．PBC患者536人とコントロール1536人を対象として300,000以上のSNPsを解析した結果，最もPBC発症と強い関連の認められたのはHLA class II領域（染色体6p21.3）の中の*HLA-DQB1*（OR：1.75, $P = 1.78 \times 10^{-19}$）であった．HLA領域には，*HLA-DQB1*以外にも*C6orf10*, *HLA-DPB1*, *BTNL2*などPBCの発症と有意に関連している遺伝子多型（OR：1.4-2.8, $P < 3 \times 10^{-7}$）が13 locus同定された．非HLA領域では，発症と関連する疾患感受性遺伝子12遺伝子座が同定されたが，その中で*IL12A*（OR：1.54, $P = 2.42 \times 10^{-14}$）と*IL12RB2*（OR：1.51, $P = 2.76 \times 10^{-11}$）が最も有意性が高く，*STAT4*（OR：1.65, $P = 4.67 \times 10^{-5}$），*IRF5-TNPO3*（OR：1.52, $P = 1.52 \times 10^{-7}$）はGWASの有意レベル（$p < 5 \times 10^{-8}$）には達しなかった．

その後カナダの同じグループから，前回のGWASで$P < 1 \times 10^{-4}$であった遺伝子を対象としてヨーロッパと北米の症例を加えて解析した結果，新たに*IRF5-TNPO3*（OR：1.57, $P = 8.66 \times 10^{-13}$），17q12-21（*ZPBP2*）（OR：0.72, $P = 3.50 \times 10^{-13}$），*MMEL1*（OR：1.33, $P = 3.15 \times 10^{-8}$）がPBCの疾患感受性遺伝子として同定され，2010年にNature Geneticsに報告された[5]．また，同誌に発表されたイタリアのグループの解析結果は，カナダから発表された*IL12A*と*IL12RB2*のPBC発症への関連をreplicationすると共に，新たに*SPIB*（OR：1.46, $P = 7.9 \times 10^{-11}$），*IRF5-TNPO3*（OR：1.63, $P = 2.8 \times 10^{-10}$），17q12-21（*IKZF3*）（OR：1.38, $P = 1.7 \times 10^{-10}$）をPBCの疾患感受性遺伝子として同定したものであった[6]．

2011年には，イギリスのグループから1840人のPBC患者と5163人のWellcome Trust Case Control Consortiumのコントロールを対象とした507,467 SNPsのGWASの結果がNature Geneticsに発表された[7]．それまでに報告されていた欧米人で有意な遺伝子多型の多くがreplicationされると共に，新たにPBC発症に関連する12の遺伝子多型（*STAT4*, *DENND1B*, *CD80*, *IL7R*, *CXCR5*, *TNFRSF1A*, *CLEC16A*, *NFKB*, *RAD51L1*, *MAP3K7IP1*）が同定された．

以上，ヨーロッパ系集団の GWAS で有意水準に達した PBC 疾患感受性遺伝子として，現在までに HLA 以外に 21 の遺伝子座が報告されているが，そのほとんどは T 細胞の活性化，T 細胞・B 細胞の分化・成熟，TNF/TLR-NFkB シグナル伝達などの免疫関連遺伝子であることから，PBC は自己免疫疾患に属することが GWAS の結果からも裏付けられた（表 2）.

日本人での PBC-GWAS

本邦においては，2010 年 10 月から国立病院機構肝ネットワーク研究班（参加 31 施設）と厚生労働省難治性疾患克服研究事業“難治性の肝・胆道疾患に関する調査研究班（参加 26 施設）を中心とした全国規模の PBC-GWAS 共同研究が開始され，現在までに PBC 1274 症例（表 1），健常人コントロール 1091 例の DNA 検体を用いて Axiom をプラットフォームとして約 60 万 SNPs に対する解析が行われ，日本人 PBC の発症に関わる新規疾患感受性遺

表 1　解析した PBC 症例の臨床プロフィル

	ゲノムワイド関連解析 (n＝487)	再現性の確認 (n＝808)
症例数：男/女	57/430	120/688
年齢：範囲	33–90	24–85
中央値	66	61
平均±標準偏差	64.7±11.3	61.1±11.4
*臨床病期：ステージ 1	320	646
2	110	121
3	57	39
抗ミトコンドリア抗体陽性率（%）	87.3%	86.4%
他の自己免疫疾患の合併（%）		
シェーグレン症候群	12.5%	14.5%
自己免疫性甲状腺炎	5.5%	10.8%
関節リウマチ	3.7%	4.0%
全身性硬化症	3.3%	3.3%
CREST 症候群	1.8%	1.7%

（文献 8 より改変）

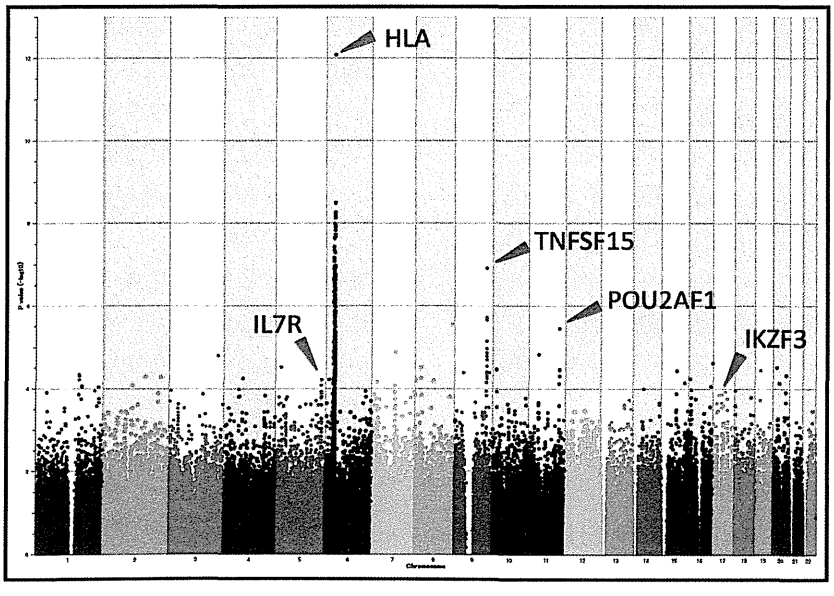

図 1　日本人おける PBC 関連遺伝子のマンハッタンプロット
日本人の PBC 487 症例と健常人コントロール 476 例の GWAS の結果を示す．PBC 発症と最も強い関連の認められたのは HLA class II 領域（染色体 6p21.3）であった．HLA 以外の領域では，TNFSF15, POU2AF1 に強い関連を認めた．

伝子が2箇所（*TNFSF15*, *POU2AF1*）同定された（図1）[8]．また，欧米で報告されていたHLA以外の疾患感受性遺伝子21遺伝子座の日本人でのreplication study（PBC 1327症例，健常人コントロール1120例）の結果，10遺伝子（*CD80*, *IKZF3*, *IL7R*, *NFKB1*, *STAT4*, *TNFAIP2*, *CXCR5*, *MAP3K71P1*, *rs6974491*, *DENND1B*）が日本人でも疾患感受性遺伝子であることが確認された（表2）[8]．一方．欧米人で最も有意性の高い疾患感受性遺伝子であるIL12A, IL12RB2は日本人では疾患感受性遺伝子ではないことも明らかとなった(表2)[8]．

TNFSF15は腫瘍壊死因子スーパーファミリーに属するサイトカインで，Th1細胞上のレセプター（DR3）に結合することにより，Th1細胞の増殖を促進し炎症局所をTh1環境に維持するのに関わっている（図2）．また，Th17細胞上のDR3に結合

表2 GWASにより同定されたPBCの疾患感受性遺伝子

遺伝子	染色体	機能	報告				OR	p-value	疾患感受性遺伝子として報告されているPBC以外の自己免疫性疾患[5]
			カナダ[1]	イタリア・カナダ[2]	イギリス[3]	日本[4]			
HLA-DQB1	6p21.3	抗原提示	○	○	○	○			many autoimmune diseases
TNFSF15	9P32	Th細胞の共刺激，Th1, Th17細胞の増殖				○	1.56	2.84×10^{-14}	CD, UC, AS
POU2AF1	11q23.1	B細胞の分化				○	1.39	2.38×10^{-8}	none
IL12A	3q25.33-q26	IL12シグナル伝達，Th1細胞への分化	○	○	○				celiac disease, MS
IL12RB2/SCHIP1	1p31.2	IL12シグナル伝達，Th1細胞への分化	○	○	○				psoriasis, CD, UC, AS, SSc, BD
STAT4	2q32	IL12シグナル伝達，Th1細胞への分化			○	○	1.35	1.11×10^{-6}	RA, SLE, SjS, SSc, psoriasis
IRF5/TNPO3	7q32.1	TLR-IFNシグナル伝達		○	○				SLE, RA, SSc, SjS, UC
IKZF3-ZPBP2-GSDMB-ORMDL3	17q12-21	B細胞の分化・アポトーシス，上皮細胞の分化・アポトーシス，小胞体ストレスの制御 など		○	○	○	1.44	3.66×10^{-9}	asthma, CD, T1D, UC
MMEL1	1p36	membrane metallo-endopeptidase-like 1，ペプチド結合を切断		○	○				RA, celiac disease, MS
SPIB	19q13	B細胞の分化		○	○				none
DENND1B	1q31	guanine exchange factors (GEFs) for RAB35，貪食に関連			○	○	1.14	4.05×10^{-2}	childhood asthma, CD
CD80	3q13	T細胞の共刺激			○	○	1.48	3.04×10^{-9}	celiac disease, JIA, AD
IL7R	5p13	B細胞・T細胞への分化・成熟			○	○	1.47	3.66×10^{-8}	MS, UC
CXCR5	11q23	BLCの受容体，リンパ球の遊走，接着			○	○	1.42	4.11×10^{-4}	none
TNFRSF1A	12p13	TNFα受容体，TNFα-NFκBシグナル伝達，アポトーシス			○				MS
CLEC16A	16p13	C type lectin containing family，機能の詳細は不明			○				MS, RA, CD, T1D, celiac disease
NFKB1	4q24	ストレス，サイトカインなどの様々な刺激に迅速に応答して様々な遺伝子の転写を制御			○	○	1.35	1.42×10^{-7}	none
RAD51L1	14q24	DNA修復			○				none
MAP3K7IP1 (TAB1)	22q13	IL1/TLR-NFκBシグナル伝達，TGFβシグナル伝達			○	○	1.29	8.59×10^{-4}	none
rs6974491	7p14	intergenic			○	○	1.33	4.98×10^{-3}	none
rs11117432	16q24	intergenic			○				none
PLCL2	3p24	B細胞受容体からのシグナル伝達の負の制御			○				none
RPS6KA4	11q13	TLR刺激によるサイトカイン産生の抑制			○				none
TNFAIP2	14q32	TNFα induced protein 2，機能の詳細は不明			○	○	1.22	6.34×10^{-4}	none

1）文献4, 2）文献5, 6, 3）文献7 4）文献8 5）Jan. 2007からJuly 2012に報告されている疾患
○それぞれの論文で報告された疾患感受性遺伝子
CD : Chron's disease, UC : ulcerative colitis, AS : ankylosing spondylitis, SSc : systemic sclerosis, RA : rheumatoid arthritis, SLE : systemic lupus erythematodes, T1D : type 1 diabetes, AT : autoimmune thyroiditis, SjS : Sjogren syndrome, MS : multiple sclerosis, BD : Behcet's disease, JIA : juvenile idiopathic arthritis, AD : atopic dermatitis

することにより TH17 細胞の増殖も促進することが報告されている（図 2）[9~11]．IL12, IL12R, STAT4 は T リンパ球を Th1 細胞へ分化させるための鍵となる一連の分子であることが知られているが，IL23 による Th17 細胞の誘導の制御に関与している事も知られている[12]．即ち日本人の疾患感受性遺伝子である *TNFSF15*，欧米人の疾患感受性遺伝子である *IL12A*, *IL12RB2*，日本人と欧米人に共通の疾患感受性遺伝子である *STAT4* は何れも T 細胞の Th1 細胞への分化・増殖，IFNγ 産生経路，さらには Th17 細胞の分化経路に位置する遺伝子と考えられる（図 2）．このことから日本人と欧米人では疾患感受性遺伝子は異なっても同じ疾患発症経路（Th1, Th17 環境へのシフト）があることが示唆された

また，POU2AF1 は，B リンパ球の形質細胞への分化・成熟過程の様々なステップで重要な役割を果たしている転写因子であるが，欧米の疾患感受性遺伝子である *SPIB* の転写を調節する転写因子でもある[13~15]．IKZF3 は B 細胞の形質細胞への分化，長期 B 細胞記憶に必須の転写因子であり[16]，CXCR5 はリンパ節での T–B interaction に必要であることが最近明らかになった T follicular helper cell（Tfh）の表面に発現し，Tfh の胚中心へのホーミングに重要な役割を果たしていると考えられている[17]．従って，これら *POU2AF1*, *SPIB*, *IKZF3*, CXCR5 の遺伝子多型が PBC 発症に関係していることは，リンパ節の胚中心での T–B interaction，B 細胞の形質細胞への分化，長期 B 細胞記憶が PBC の発症に関連していることを示唆しており極めて興味深い（図 3）．

CD80 は抗原提示細胞に発現する共刺激分子であり，T 細胞の CD28 や CTLA4 と結合することにより T 細胞の活性化や非活性化などの調節に関連している[18]．IL7R は骨髄のストローマ細胞から分泌される IL7 の受容体であり，骨髄幹細胞から T 細胞，B 細胞への分化・成熟に重要なサイトカインで

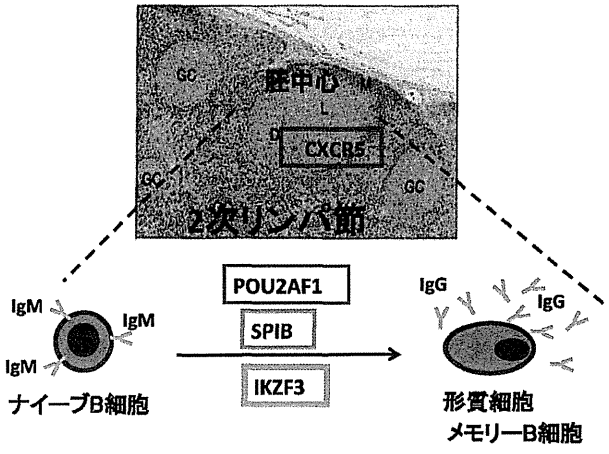

図 3　B リンパ球の分化と POU2AF1

B リンパ球の分化との関連を認めた疾患感受性遺伝子を示す．

CXCR5, POU2AF1, SPIB, IKZF3 は何れも B 細胞の形質細胞，長期生存メモリー B 細胞への分化・増殖に関与している．

POU2AF1 rs4938534 A allele は POU2AF1 の発現を増加させることも報告されている．

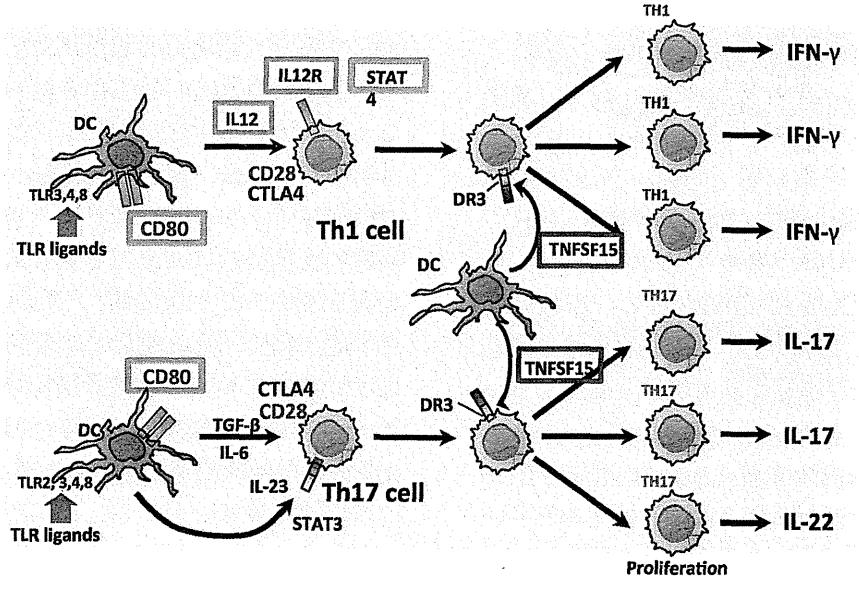

図 2　T リンパ球の分化と TNFSF15

T リンパ球の分化との関連を認めた疾患感受性遺伝子を示す．CD80, IL12A, IL12RB2, STAT4, TNFSF15 は何れも T リンパ球の Th1, Th2 細胞への分化・増殖に関与している．今回同定された TNFSF15 rs4979462 T allele は TNFSF15 の発現を増加させることも報告されている．

ある[19]．これらはリンパ球への分化・成熟やT細胞の活性化がPBC発症に関連していることを示唆している．

以前よりTNFやTLRがPBCの発症に関与していることが推測されていたが[20]，欧米人のGWASにおいてTNF/TLR-NFkBシグナル伝達に関連した6個の疾患感受性遺伝子が同定された（*IRF5*/*TNPO3*，*TNFRSF1A*，*NFkB1*，*MAP3K7IP1*，*RPS6KA4*，*TNFAIP2*）[4~7]（表2）．この内，*NFkB1*, *MAP3K7IP1*, *TNFAIP2*の3遺伝子は日本人で疾患感受性遺伝子であることが確認された．また，上皮細胞の分化やアポトーシス，小胞体ストレスに関連した遺伝子領域（chromosome 17q12-21）も日本人でPBCの疾患感受性遺伝子であることが確認された．今後は日本人のGWASの解析症例を増やすことにより，さらに多くの疾患感受性遺伝子を同定し，日本人の疾患感受性遺伝子座の遺伝子構築の全貌を明らかにする必要がある．

PBC発症に関連するshared autoimmune susceptibility loci

以上，現在までにGWASで報告されたHLA以外のPBC疾患感受性遺伝子は，今回日本人で初めて同定された*TNFSF15*と*POU2AF*を含めると23遺伝子座となる．その内12遺伝子座（13/23＝52.2％）は他の自己免疫疾患でも報告されている疾患感受性遺伝子座（shared autoimmune susceptibility loci）に相当する（表2）．これらの自己免疫疾患には，クローン病（CD），潰瘍性大腸炎（UC），セリアック病（celiac disease），多発性硬化症（MS），関節リウマチ（RA），全身性硬化症（SSc），全身性ループスエリトマトーデス（SLE），シェーグレン症候群（SjS），Ⅰ型糖尿病（T1D），気管支喘息（asthma），乾癬（psoriasis），強直性脊椎炎（AS），ベーチェット病（BD），若年性特発性リウマチ（JIA），アトピー性皮膚炎（AD）などの多数の疾患が含まれている．PBC発症にも他の自己免疫疾患と共通した複数の疾患感受性遺伝子が関与していることが示唆されるが，shared autoimmune susceptibility lociの12遺伝子座の内，10遺伝子座が消化管の粘膜免疫機構の異常が原因と考えられているCD, UC, Celiac diseaseの疾患感受性遺伝子に一致することは，PBCの発症に粘膜免疫機構の異常が関与している可能性を示唆しておりきわめて興味深い．特に，今回の日本人PBC-GWASで同定された*TNFSF15*はクローン病や潰瘍性大腸炎などの炎症性腸疾患の疾患感受性遺伝子であることが欧米人や日本人でよく知られており，原発性胆汁性肝硬変発症と炎症性腸疾患の発症に共通した遺伝的素因として注目される[21]．また，PBCマウスモデルであるdnTGFβIIマウスからIL12p40を欠損させると胆管炎が発症しなくなることも，胆管炎発症にIL12シグナル伝達経路が重要であることを示唆していると考えられる[22]．

おわりに

欧米人と日本人のPBC疾患感受性遺伝子の比較検討から，PBCの疾患感受性遺伝子には日本人と欧米人との間に集団差を認めても共通の疾患発症経路が存在することが明らかになった．特にTh1環境へのシフトに関わる疾患経路（日本人：*TNFSF15*，*STAT4*，欧米人：*IL12A*，*IL12RB2*，*STAT4*），2次リンパ節胚中心でのBリンパ球の形質細胞への分化，長期B細胞記憶の成立に関わる疾患経路（日本人：*POU2AF1*，*IKZF3*，*CXCR5*，欧米人：*SPIB*，*IKZF3*，*CXCR5*）の重要性が示唆された．

今回同定された日本人PBC疾患感受性遺伝子（12遺伝子）は，他の自己免疫疾患の中では，クローン病，潰瘍性大腸炎の疾患感受性遺伝子と最も多く重複しており，PBCと炎症性腸疾患との間に共通した疾患発症経路が存在することが示唆された．これらの疾患発症経路は，PBCの原因究明や根治的治療法の開発にきわめて重要な手がかりを提供しているものと思われ，特にTNFSF15を中和する生物学的製剤の開発は，PBCの根治的治療法となる可能性がある[9,10]．

今後は，欧米，東アジア諸国を含めた世界規模の共同研究により，PBCの疾患感受性遺伝子，疾患発症経路を網羅的に同定して疾患発症に関わる遺伝子構築を明らかにすると共に，PBCの治療反応性，病理学的活動性，自己抗体の産生プロフィール，転帰など，PBCの病態・病型分類に基づく層別化解析を行い，PBCの病態形成，進展に関する分子標的を同定し新しい治療法を開発する必要がある．

謝　辞：ここで紹介しました日本人PBC-GWASのデータは，東京大学人類遺伝学教室　西田奈央博士，川嶋実苗博士，徳永勝士教授との共同研究によるものです．また，DNA検体の収集は以下の御施

設の先生方との共同研究“日本人原発性胆汁性肝硬変の発症・進展に関わる遺伝因子の網羅的遺伝子解析（Genome-wide association study : GWAS）（研究代表者：中村　稔）”によるものです．ここに深謝申し上げます．

国立病院機構肝ネット共同研究班（31施設）

旭川医療センター，北海道医療センター，仙台医療センター，東京病院，横浜医療センター，まつもと医療センター松本病院，金沢医療センター，大阪医療センター，呉医療センター，岡山医療センター，小倉医療センター，九州医療センター，大分医療センター，災害医療センター，高崎総合医療センター，西群馬病院，西埼玉中央病院，相模原病院，名古屋医療センター，東名古屋病院，京都医療センター，嬉野医療センター，東京医療センター，南和歌山医療センター，米子医療センター，熊本医療センター，別府医療センター，東広島医療センター，信州上田医療センター，国立国際医療研究センター戸山病院，長崎医療センター

厚生労働省難治性疾患克服研究事業“難治性の肝・胆道疾患に関する調査研究班（27施設）

東北大学大学院消化器病態学，福島県立医科大学消化器・リウマチ膠原病内科学，東京女子医科大学消化器内科，東京慈恵会医科大学器官病態・治療学消化器内科，帝京大学医学部内科，帝京大学医学部溝口病院第4内科，慶応義塾大学消化器内科，新潟大学消化器内科，順天堂大学静岡病院消化器内科，金沢大学大学院形態機能病理学，金沢大学大学院恒常性制御学，金沢大学医薬保健研究域先端医療技術学，信州大学消化器内科，岡山大学大学院消化器・肝臓・感染症内科，愛媛大学大学院先端病態制御内科学，高知大学医学部消化器内科，九州大学大学院病態修復内科学，長崎大学消化器病態制御学，大分大学医学部総合内科第一，福岡大学医学部消化器肝臓病学内科，産業大学医学部第3内科，鹿児島大学大学院消化器疾患生活習慣病学，九州大学大学院形態機能病理学，九州大学大学院消化器総合外科，京都大学医学部肝胆膵移植外科，東京大学肝胆膵外科・人工臓器・移植外科，国立病院機構長崎医療センター臨床研究センター

遺伝子解析施設（2施設）

国立国際医療研究センター・肝炎免疫研究センター，東京大学人類遺伝学教室

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特集 ゲノムワイド関連解析からみえてきた消化器疾患

Gastrointestinal Research

PBCの疾患感受性遺伝子による病態の解明

中村 稔*,**

Summary
原発性胆汁性肝硬変（PBC）の発症には，家族集積性や双生児による研究から強い遺伝的素因の関与が示唆されていたが，近年，欧米人を対象としたゲノムワイド関連解析（GWAS）により，PBC疾患感受性遺伝子としてHLA領域の遺伝子多型のほかに，①インターロイキン12（IL12）/IL12Rシグナル伝達，②TLR/TNFα-NFκBシグナル伝達，③B細胞の成熟・分化，④上皮細胞の分化・アポトーシスなどに関連する遺伝子多型の重要性が明らかとなった．今後は，①欧米人以外の人種を対象としたGWASによるPBC疾患感受性遺伝子の同定，②PBCの病態・病型分類（治療反応性，病理学的活動性，自己抗体のプロフィール，転帰など）にもとづくGWAS層別化解析などにより，PBCの発症・病態形成に関与するdisease pathwayの理解が飛躍的に進むことが期待される．

Key words
疾患感受性遺伝子　一塩基多型（SNP）　ゲノムワイド関連解析（GWAS）　原発性胆汁性肝硬変（PBC）

はじめに

近年，ヒトゲノム全域にわたる膨大な多様性情報の集積と数十万～百万種の一塩基多型（single nucleotide polymorphism：SNP）を一度に多数の検体が解析できるプラットフォームの開発により，ゲノムワイド関連解析（genome-wide association study：GWAS）が可能となった．2007年にこのプラットフォームが市販化されて以来，さまざまな疾患や形質に関連した遺伝子が同定されてきたが，米国国立ヒトゲノム研究所（National Human Genome Research Institute：NHGRI）GWAS Catalogによると，2011年6月までのGWASの論文発表は1,449件で，対象となる形質あるいは疾患は237に，報告された遺伝子座は500に達している（http://www.genome.gov/gwastudies）．

自己免疫性肝胆道疾患においても，2009年に原発性胆汁性肝硬変（primary biliary cirrhosis：PBC）を対象としたはじめてのGWASの成果がカナダから報告され，PBC発症と関連する新たな疾患感受性遺伝子〔*interleukin-12α*, *IL12 re-*

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ceptor β2, signal transducer and activator of transcription 4 (*IL12A*, *IL12RB2*, *STAT4*)〕が同定された[1]．その後，イタリア，カナダ，イギリスからメタアナリシスを含めたGWASの報告があいつぎ，PBCの新たな疾患関連遺伝子多型の情報が集積されるとともに，遺伝子多型からみたPBCの病態の理解が進んでいる[2)~4)]．本稿では，GWASによるPBCの発症要因の解明についての現状と，今後の展望について概説する．

1 PBCの発症には遺伝的素因の関与が示唆されていた

PBCは中年女性に好発する肝内小葉間胆管を標的とした慢性の肝疾患で，初期には無症状のことが多いが，進行すると全身の瘙痒感，食道静脈瘤，腹水，黄疸，脳症が出現して肝不全に至り，最終的には肝臓移植以外に救命方法がない難治性疾患である．ミトコンドリアや核成分に対するさまざまな自己抗体（抗ミトコンドリア抗体，抗gp210抗体，抗セントロメア抗体，抗sp100抗体など）が出現することや，門脈域にリンパ球の浸潤を認めることから，小葉間胆管を標的とする自己免疫疾患と考えられている．PBCには家族集積性（患者同胞の発症危険率10.5）の高いこと，一卵性双生児における一致率（60%）が高いこと，またほかの自己免疫疾患の合併率が高いことなどから，その発症には強い遺伝的素因の関与が示唆されていたが，その詳細についてはヒトゲノム全域の膨大な多様性情報の集積（国際HapMap計画）とGWAS技術の開発までは不明であった．

2 候補遺伝子法による遺伝子多型解析

PBCの発症や進行，自己抗体産生に関連した遺伝的素因を明らかにするために，候補遺伝子法によるSNPの解析が1990年代からおこなわれてきた．しかし，初期の解析では対象とした症例数が不十分な報告が多く，またHapMapなどによるヒトゲノムの多様性情報がなかったために，多数のタグSNPを用いての系統的関連解析が不可能であった．したがって，再現性が確認された遺伝子多型はきわめて限られており，人種差を超えて再現性が確認されているのはhuman leukocyte antigen（HLA）とcytotoxic T lymphocyte antigen 4（CTLA4）のみにすぎない[5)~10)]．Vitamin D receptor（VDR），solute carrier family 4, anion exchanger, member 2（SLC4A2），IL1，IL10，apoprotein E，mannose-binding lectin（MBL），endothelial nitric oxide synthase（eNOS），chemokine receptor 5（CCR5），cytochrome P450 2E2（CYP2E2），programmed cell death 1（PDCD1），keratin，ataxin 2-binding protein 1（A2BP1），endocannabinoid receptorなど多くの遺伝子多型とPBC発症との関連が報告されていたが，GWASで$p<10^{-5}$の有意性で同定されたのは皆無であり，CTLA4もオッズ比（odds ratio：OR）：1.39，$p=1.41\times10^{-5}$と，GWASレベルの有意水準には達していない[1)~4)]．

3 GWASによる遺伝子多型解析からHLAのほかに多くの疾患感受性遺伝子座が同定された

2009年にPBCを対象としたはじめてのGWASが，カナダのグループから“*N Engl J Med*”誌に発表された[1]．PBC患者536例とコントロール1,536例を対象として300,000以上のSNPを解析した結果，最もPBC発症と強い関連の認められたのは，HLAクラスⅡ領域（染色体6p21.3）のなかのHLA-DQB1（OR：1.75，$p=1.78\times10^{-19}$）であった．HLA領域には，HLA-DQB1以外にもC6orf10，HLA-DPB1，butyrophilin-like 2（BTNL2）など，PBCの発症と有意に関連している遺伝子多型（OR：1.4-2.8，$p<3\times10^{-7}$）が13 locus同定された．非HLA領域では，発症と関連する疾患感受性遺伝子12 locusが同定されたが，そのなかでIL12A（OR：1.54，$p=2.42\times10^{-14}$）とIL12RB2（OR：1.51，$p=2.76\times10^{-11}$）が最も