and BDCA-2, respectively, and mDCs are identified as BDCA-1$^+$ and CD19$^-$ cells (10, 11). Langerin is a C-type lectin expressed in LCs from the skin and the small intestine, and Langerin$^+$ LCs are involved in immunological surveillance at the surface (12).

In the periphery, immature DCs such as LCs capture antigens and, under the influence of inflammatory stimuli, subsequently migrate. LCs represents a population of DCs only found in epithelia. Although several chemokines have been reported to attract immature DCs *in vitro*, macrophage inflammatory protein-3α (MIP-3α, also known as CCL20) is the main chemokine expressed in epithelium and recruiting LCs to mucosal surfaces (13). Indeed, it has been demonstrated recently that the receptor for MIP-3α, namely CCR6, is expressed in LCs (13, 14).

Although DCs surely play an important role in the pathogenesis of the cholangiopathy in PBC, more studies are needed to explain the bile duct-specific autoimmunity. The aim of this study is to characterize periductal DCs in PBC and to clarify the interaction between BECs and DCs in terms of the periductal cytokine milieu and biliary innate immunity. This study may open avenues for understanding and eventually the control of the specific functions of biliary DCs.

Materials and methods

Patients and tissue preparation

A total of 72 needle or wedge liver biopsy specimens were obtained from the patients with PBC and the controls including chronic viral hepatitis (CVH) associated with hepatitis C virus (HCV), hepatic sarcoidosis, drug-induced liver injury and no significant histopathological changes (so-called histologically 'normal liver') (Table 1). Liver tissue specimens were fixed in 10% neutral-buffer formalin. The histopathological diagnosis was established by a histological review by at least two pathologists with consideration of clinical and laboratory data. All PBC patients were examined before ursodeoxycholic acid therapy, and AMA was detected by immunofluorescence microscopy in all PBC cases. The diagnosis of drug-induced liver injury was clinicopathologically performed.

Table 1. Hepatobiliary diseases examined in this study

Patients	Case number	Average age (years)	Male/ female	Histological stage
Primary biliary cirrhosis	26	59	2/24	I/II/III = 14/9/3
HCV-related chronic hepatitis	18	62	6/12	F1/F2/F3 = 6/8/4
Hepatic sarcoidosis	6	52	3/3	
Drug-induced liver injury	14	49	5/9	
Normal liver	8	60	3/5	

HCV, hepatitis C virus.

Benzbromarone, tiquizium bromide, analgesic agent, Chinese medicines (two cases), protein and health food were speculated as a causative drug by lymphocyte stimulation test in seven patients. According to Councils for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method (15) scoring system, 'unlikely', 'probable' and 'possible' were two, five and seven cases respectively. Liver histology also confirmed specific findings such perivenular zonal necrosis, cholestasis, granulomatous changes and eosinophil/neutrophil infiltration, suggesting drug-induced liver injury.

More than 10 sections, 3 μm thick, were prepared from each paraffin block; three of them were stained with haematoxylin and eosin, Gomori's reticulum and orcein stain for histological diagnosis and evaluation. The remaining sections were processed for immunohistochemistry. In addition to fixed specimens, fresh surgical specimens were available in seven cases each of PBC and HCV-related CVH, and used for immunohistochemistry. Informed consent to conduct research was obtained from these patients. This study was approved by the Kanazawa University Ethics Committee.

Immunohistochemistry and immunocytochemistry

After deparaffinization, the sections were incubated in Target Retrieval Solution (Dako Japan, Tokyo, Japan) at 95 °C for 20 min for the pretreatment of tissue before immunostaining. Following endogenous peroxidase blocking in methanolic-hydrogen peroxide for 20 min and incubation in normal goat serum (diluted 1:10; Vector Lab, Burlingame, CA, USA) for 20 min, the sections were incubated at 4 °C overnight with a primary mouse monoclonal antibody against human Langerin (clone 12D6; 2.5 μg/ml; Abcam Japan, Tokyo, Japan), human BDCA-2 (clone 104C12.08; 5 μg/ml; AbCys S.A., Paris, France) or human MIP-3α (clone 308B7.06; 5 μg/ml; Dendritics S.A., Lyon, France) and then Dako Envision-HRP or the CSA system (Dako Japan) was used. After the benzidine reaction, sections were weakly counterstained with haematoxylin. No positive staining was obtained when the primary monoclonal antibody was replaced with an isotype-matched, non-immunized immunoglobulin as a negative control of the staining procedures.

The simultaneous detection of Langerin and CCR6 was evaluated by double immunohistochemical staining. After staining for Langerin using a benzidine reaction, CCR6 staining was performed using human CCR6 antibody (rabbit polyclonal, 5 μg/ml, MBL, Nagoya, Japan) and HistoGreen (AbCys S.A.). Moreover, the simultaneous detection of BDCA-1 and CD19 was assessed using frozen sections. After fixation of frozen 5 μm sections in cold acetone for 10 min and the blocking of endogenous peroxidase, human CD19 antibody (clone HD37, 2 μg/ml, Dako Japan) was added and then Dako Envision-HRP was used. After the benzidine reaction, BDCA-1 staining

Liver International (2010)
© 2010 John Wiley & Sons A/S

was performed using human BDCA-1 antibody (clone L161; 5 μg/ml, GeneTex, Irvine, CA, USA), Histofine simple stain-AP (Nichirei Bioscience, Tokyo, Japan) and Vector Blue (Vector Lab).

Histological examination

The BDCA-2$^+$ cells, BDCA-1$^+$ and CD19$^-$ cells, and Langerin$^+$ cells were evaluated as pDCs, mDCs and LCs respectively. In a primary survey, we examined all portal tracts in each specimen and, for counting DCs, selected one or two representative portal tracts containing interlobular bile ducts (16) and expressed the results as the number of DCs per bile duct. For MIP-3α, the immunoreactivity in bile ducts was semiquantitatively graded as follows: 0, absence of expression; 1, weak constitutive expression; 2, moderate expression; and 3, strong expression. The final indices of PBC and the controls were defined as the mean of individual cases. In PBC, some interlobular bile ducts appeared normal, while others showed variable biliary epithelial damage including CNSDC. Therefore, the former and the latter were evaluated separately as normal and damaged bile ducts, respectively, in this study.

Cultured human biliary epithelial cells

Two cultured human BEC lines that were isolated from the explanted livers of two PBC patients were established in this study. These lines were incubated with a culture medium composed of D-MEM/F-12, Nu-Serum (Becton Dickinson, Bedford, MA, USA), ITS+ (Becton Dickinson), 5 μM forskolin (Wako, Osaka, Japan), 12.5 mg/ml of bovine pituitary extract (Gibco, Rockville, MD, USA), 1 μM dexamethasone (Sigma, St Louis, MO, USA), 5 μM triiodo-thyronine (Sigma), 5 mg/ml of glucose (Sigma), 25 mM sodium bicarbonate (Sigma), 1% antibiotics antimycotic, 20 ng/ml of human epidermal growth factor (Gibco) and 10 ng/ml of human hepatocyte growth factor (Gibco) (17). These cell lines had been confirmed to be BECs by the expression of a biliary-type cytokeratin, CK7. BECs were used between passages 6 and 10 for this study.

Treatment with cytokines and pathogen-associated molecular patterns

Cultured BECs were stimulated with recombinant human cytokines including interleukin (IL)-1β, IL-4, IL-6, IL-17, tumour necrosis factor (TNF)-α and interferon (IFN)-γ (1000 U/ml, PeproTech, London, UK) and TLR-related pathogen-associated molecular patterns (PAMPs) including Pam3CSK4 (TLR1/2 ligand, 100 ng/ml, Invivogen, San Diego, CA, USA), polyinosinic–polycytidylic acid [poly(I:C), a synthetic analogue of viral dsRNA, TLR3 ligand, 25 μg/ml, Invivogen] or lipopolysaccharide (LPS) (ultrapure grade, TLR4 ligand, 1 μg/ml, Invivogen) for 3 and 24 h and used for the preparation of samples for polymerase chain reaction (PCR) analysis and immunocytochemistry respectively.

Isolation of RNA and real-time polymerase chain reaction

For the evaluation of MIP-3α mRNA, total RNA was extracted from cultured BECs using the RNeasy Total RNA System (Qiagen, Hilden, Germany) following the manufacturer's instructions. Then, 1 μg of total RNA was reverse-transcribed with an oligo-(dT) primer and ReverTra Ace (Toyobo, Osaka, Japan) to synthesize a cDNA template for PCR. To carry out relative quantification, real-time quantitative PCR was performed for measurements according to a standard protocol using the Brilliant II SYBR Green QPCR Reagents and Mx300P QPCR system (Stratagene Japan, Tokyo, Japan) and the relative gene expression was calculated using the comparative cycle threshold method. Specific primers were as follows: MIP-3α forward, 5′-GCGCAAATCCAAAACAGACT-3′, and reverse, 5′-CAAGTCCAGTGAGGCACAAA-3′, and glyceraldehyde 3 phosphate dehydrogenase (GAPDH, internal positive control), forward, 5′-GGCCTCCAAGG AGTAAGACC-3′, and reverse, 5′-AGGGGTCTACATGG CAACTG-3′.

In vitro chemotaxis assay

The MIP-3α-induced chemoattractant activity of human LCs was determined by an invasive assay. Human LCs were prepared according to the previous report (18). Briefly, monocytes obtained from peripheral blood mononuclear cells using Monocyte Isolation Kit II (Miltenyi Biotec K.K., Tokyo, Japan) were cultured in a complete medium supplemented with granulocyte macrophage-colony stimulating factor (GM-CSF) (100 ng/ml, PeproTech), IL-4 (10 ng/ml, PeproTech) and transforming growth factor (TGF)-β1 (10 ng/ml, PeproTech). At days 2 and 4, a fresh medium supplemented with GM-CSF and TGF-β1 was added. At days 5–7 of culture, cells were resuspended in 96-well plates assembled with the Collagen I Cell Invasion Assay (Trevigen, Gaithersburg, MD, USA). Induction to LCs was confirmed by a Langerin positivity. The supernatants of IL-1β- or Pam3CSK4-stimulated BECs for 2 days were added to the medium of LCs at a concentration of 5% and the degree of LC migration was evaluated according to the manufacturer's instruction. To confirm the MIP-3α-mediated process, LCs were incubated with additional human recombinant MIP-3α (1000 U/ml, PeproTech) or sufficient neutralizing anti-MIP-3α antibody (5 μg/ml, R&D systems, Minneapolis, MN, USA) in the culture. Normal rabbit IgG (5 μg/ml, R&D systems) was used as a negative control.

Statistical analysis

Data were analysed using Welch's *t*-test. $P < 0.05$ was considered statistically significant.

© 2010 John Wiley & Sons A/S

Results

Detection of plasmacytoid dendritic cells, myeloid dendritic cells and Langerhans cells in liver sections

Immunohistochemistry revealed that BDCA-2^+ pDCs were mainly scattered in the portal tracts and, in particular, prominent in inflammed portal tracts in the cases of PBC, CVH and drug-induced liver injury (Fig. 1A and B). In contrast, in normal livers and hepatic sarcoidosis, there were few BDCA-2^+ cells in the portal tracts. The distribution of BDCA-2^+ cells was not anatomically correlated with bile ducts, irrespective of diseased livers and those attaching bile ducts were hardly found (Fig. 1A and B). Double immunolabelling of BDCA-1 and CD19 revealed that BDCA-1^+ and $CD19^-$ mDCs were also present in the portal tracts (Fig. 1C and D). In contrast to pDCs, mDCs were mostly distributed around and within bile ducts and especially prominent in PBC (Fig. 1C and D). $Langerin^+$ LCs as well as mDCs were found around and within bile ducts and several $Langerin^+$ LCs were rather accentuated around the damaged interlobular bile ducts in PBC (Fig. 2A). In CVH and drug-induced liver injury, several $Langerin^+$ LCs were scattered within portal tracts, but no peribiliary accentuation was seen in the controls including drug-induced liver injury (Fig. 2B and C). In hepatic sarcoidosis and normal liver, $Langerin^+$ LCs were almost unrecognizable (2D). Double staining of Langerin and PAS revealed that several LCs were located within PAS-positive biliary basement membranes, demonstrating that LCs had infiltrated the biliary epithelial layer (Fig. 2E). Moreover, double immunostaining of Langerin and CCR6 revealed that these LCs express CCR6 (Fig. 2F).

Evaluation of Langerhans cells

Because the primary survey revealed that the distribution of LCs was closely associated with bile ducts, the number of $Langerin^+$ LCs attaching to or embedding in bile ducts was calculated in each bile duct. As shown in Figure 3, the number of $Langerin^+$ LCs in damaged bile ducts in PBC was relatively large in comparison with that in normal bile ducts in PBC or interlobular bile ducts in the controls ($P < 0.05$).

Expression of macrophage inflammatory protein-3α in liver sections

The expression of MIP-3α was basically found in infiltrating mononuclear cells, periportal hepatocytes and bile ducts and particularly prominent in the damaged bile ducts in PBC (Fig. 4A). Some MIP-3α-positive mononuclear cells and periportal hepatocytes were found in the control diseased livers as well as PBC, but were faint or lacking in normal livers (Fig. 4B). Moreover, the semiquantitative evaluation also showed the

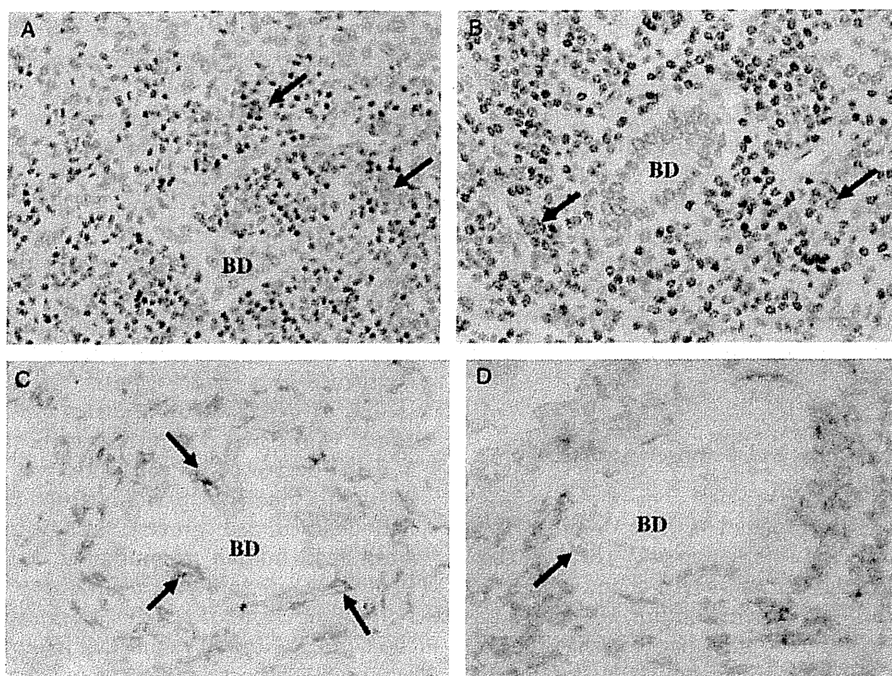

Fig. 1. Detection of plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs) in liver sections. Blood dendritic cell antigen (BDCA)-2^+ pDCs (arrows) are scattered in inflammed portal tracts of primary biliary cirrhosis (PBC) (A) and chronic viral hepatitis (CVH) (B) patients, but their distribution is not related to bile ducts (BD). BDCA-1 (blue)-positive and CD19 (brown)-negative mDCs are mostly distributed around and within BD in PBC (C), but are rare in CVH (D). (A) and (B) show the immunohistochemistry for BDCA-2 using formalin-fixed, paraffin-embedded sections. (C) and (D) show double immunohistochemistry for BDCA-1 (brown) and CD19 (blue) using frozen sections.

© 2010 John Wiley & Sons A/S

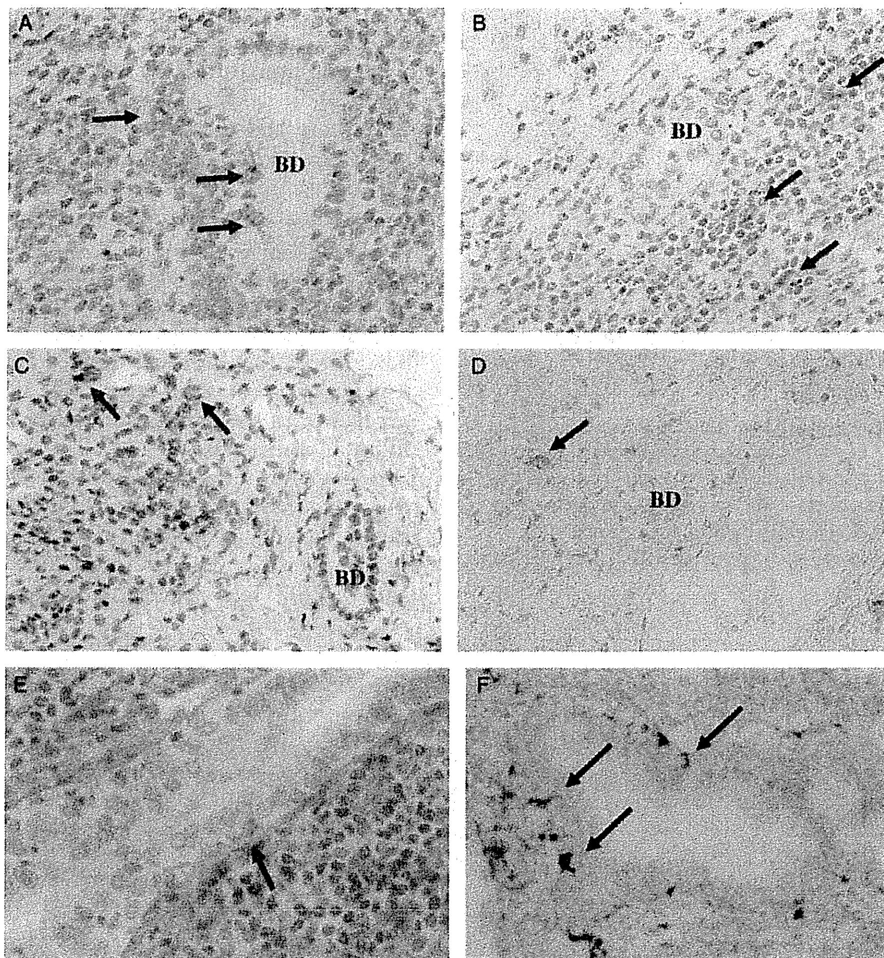

Fig. 2. Detection of Langerhans cells (LCs) in liver sections. (A) $Langerin^{+}$ LCs are found around and within bile ducts (BD) showing cholangitis in primary biliary cirrhosis (PBC). In chronic viral hepatitis (CVH) (B) and drug-induced liver injury (C), several $Langerin^{+}$ LCs are scattered in the portal tracts, but periductal accumulation is not found. (D) In normal liver, $Langerin^{+}$ LCs are very rare. (E) Some $Langerin^{+}$ LCs are located within PAS-positive biliary basement membranes in PBC. Arrowheads denote $Langerin^{+}$ LCs. (F) $Langerin^{+}$ LCs (brown) express CCR6 (green). Arrows and 'BD' denote representative positive cells and BD, respectively, in each figure. (A, B, C and D) Immunohistochemistry for Langerin using formalin-fixed, paraffin-embedded sections. (E) Double immunohistochemistry for Langerin and histochemistry of PAS staining. (F) Double immunohistochemistry for Langerin and CCR6.

expression of MIP-3α to be statistically significant in the damage bile ducts of PBC, compared with normal bile ducts of PBC and interlobular bile ducts of the controls ($P < 0.05$) (Fig. 4C).

Detection of macrophage inflammatory protein-3α in cultured biliary epithelial cells

The mRNA of MIP-3α was constantly expressed in cultured BECs under normal conditions. Moreover, its expression was upregulated by several cytokines including IL-1β, TNF-α and IL-17 and all PAMPs examined in this study (Fig. 5A). In addition to this molecular analysis, immunocytochemistry also demonstrated that MIP-3α protein was strongly expressed in cytokine- and PAMP-stimulated BECs, compared with non-stimulated BECs (Fig. 5B and C).

Chemotactic effects of macrophage inflammatory protein-3α to Langerhans cells

The degree of LCs that transmigrated in an assay chamber containing the culture medium of IL-1β- or PamCSK4-treated BECs was larger than that of the non-treated supernatant and these increased migrations were inhibited by neutralizing MIP-3α antibody, suggesting that the MIP-3α secreted by BECs chemoattracted LCs (Fig. 6).

Discussion

Dentritic cells form a network comprising different populations that initiate and differentially regulate immune responses and regulate the generation of inflammatory cells including T cells and are known as a regulator of continuous chronic inflammation. Although

© 2010 John Wiley & Sons A/S

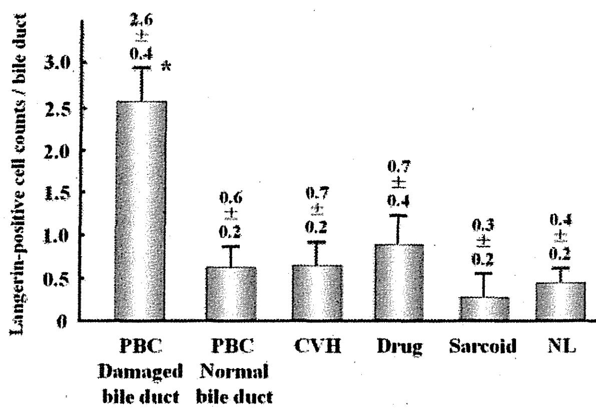

Fig. 3. Semiquantative evaluation of Langerhans cells (LCs). The number of Langerin$^+$ LCs attached to damaged bile ducts in primary biliary cirrhosis (PBC) was relatively large in comparison with that attached to normal bile ducts in PBC or interlobular bile ducts in chronic viral hepatitis (CVH), drug-induced liver injury (Drug), hepatic sarcoidosis (Sarcoid) and normal livers (NL). Bars indicate the mean ± SEM. $^*P < 0.05$.

studies have already demonstrated that extensively inflammed biliary mucosa of PBC contain several DCs including immature DCs (8), S-100$^+$ DCs (7) and LCs (19, 20), it is necessary to investigate the extent of infiltration and the mechanism of migration to understand the immune response forming cholangiopathy, especially chronic cholangitis, in PBC. The present study revealed that periductal DCs were mostly mDCs and that the major representative type of mDCs, Langerin$^+$ LCs, were attached to or embedded in the damaged bile ducts of PBC patients. These results suggest that LCs existing around and within bile ducts play an important role as APCs presenting bile duct-derived autoantigens. LCs are a population of immature DCs involved in antigen processing in the skin and intestinal mucosa and can be found in diseased tissues (13). The presence and function of LCs closely associated with bile ducts, therefore, are suggested to be of importance in the establishment of bile duct-specific autoimmune phenomena in PBC.

Because LCs possess chemokine receptors (CCR4 and CCR6), their ligands including CCL17, CCL22 and

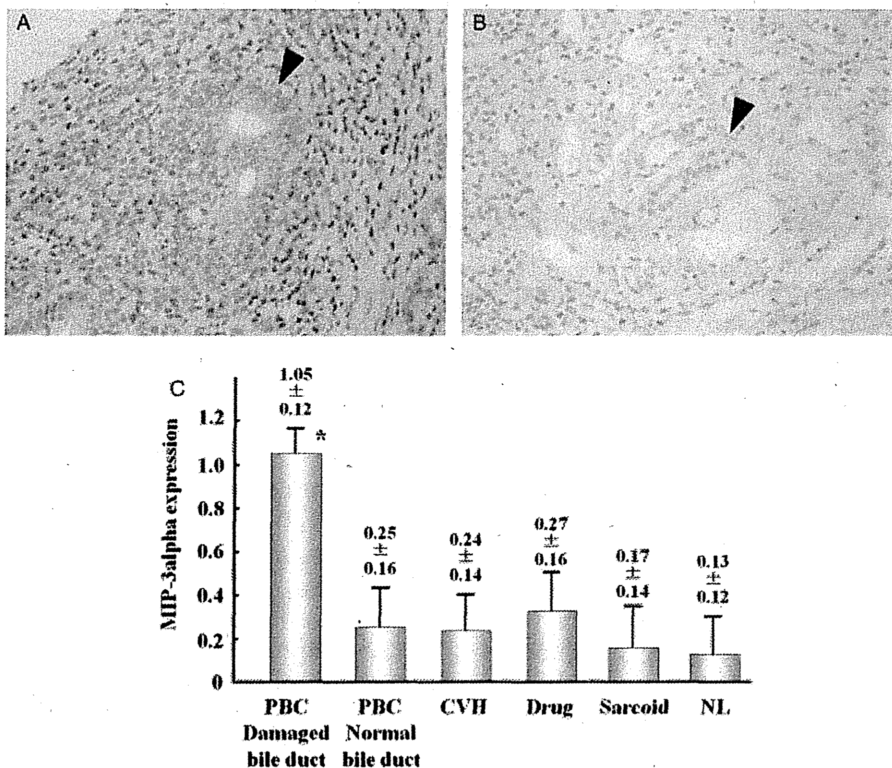

Fig. 4. Detection of macrophage inflammatory protein-3α (MIP-3α) in liver sections. MIP-3α was expressed in infiltrating mononuclear cells and bile ducts. In primary biliary cirrhosis (PBC), the damaged bile ducts show strong MIP-3α expression (A, arrowheads). In chronic viral hepatitis (CVH), a few MIP-3α$^+$ mononuclear cells were found, but bile ducts show no expression (B, arrowheads). (A) and (B) show serial sections of Figures 2A and B respectively. Semiquantitative evaluation also shows that the degree of MIP-3α expression in the damaged bile ducts of PBC is significantly higher than that in normal bile ducts in PBC or interlobular bile ducts in chronic viral hepatitis (CVH), drug-induced liver injury (Drug), hepatic sarcoidosis (Sarcoid) and normal livers (NL) (C, $P < 0.05$). Bars indicate the mean ± SEM. $^*P < 0.05$. (A and B) Immunohistochemistry for MIP-3α using formalin-fixed, paraffin-embedded sections.

© 2010 John Wiley & Sons A/S

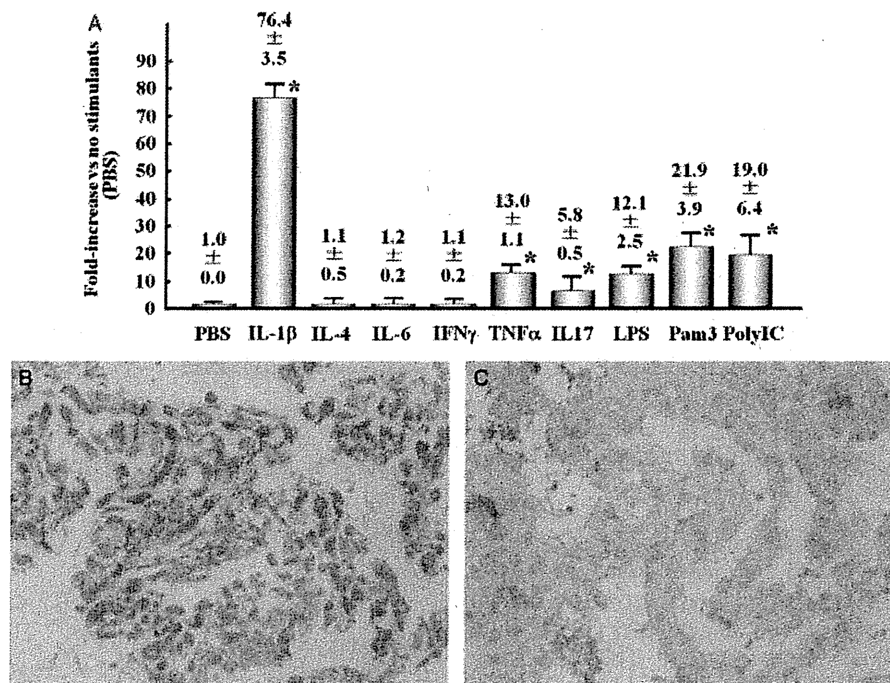

Fig. 5. Detection of macrophage inflammatory protein-3α (MIP-3α) mRNA in cultured human biliary epithelial cells (BECs). A: Quantitative analyses using real-time polymerase chain reaction in two independent experiments revealed that IL-1β, TNF-α, IL-17, LPS, Pam3SCK4 and poly(I:C) significantly upregulated MIP-3α mRNA expression. There were no differences between the two BEC lines. Data are shown relative (fold-increase) to the value for unstimulated cells (PBS). Bars indicate the mean ± SEM. $^{*}P < 0.05$. (B and C) Immunocytochemistry also demonstrated that MIP-3α protein was strongly expressed in the LPS-stimulated BECs (B), compared with the unstimulated BECs (C). IL, interleukin; LPS. lipopolysaccharide; poly(I:C), polyinosinic–polycytidylic acid; TNF, tumour necrosis factor;

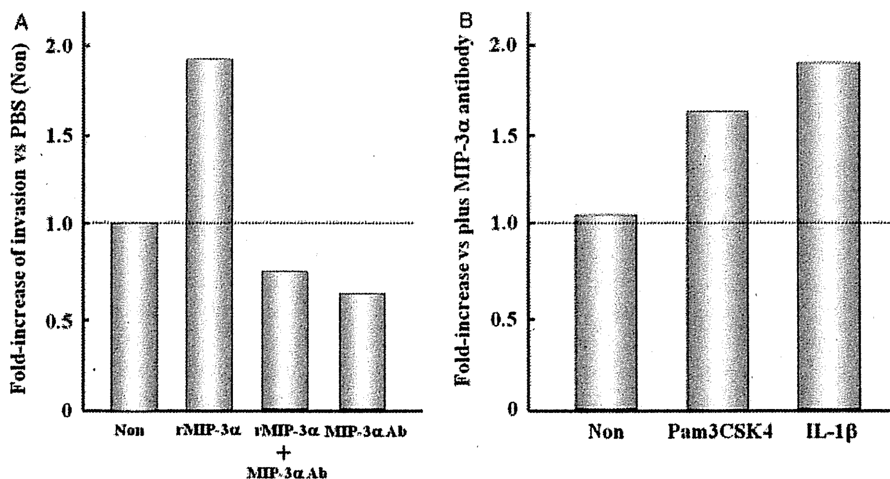

Fig. 6. Chemotactic effects of recombinant human macrophage inflammatory protein-3α (rMIP-3α) and secreted MIP-3α from cultured human biliary epithelial cells (BECs) on Langerhans cells (LCs). The representative data are shown in two independent experiments. (A) Data are shown as a relative degree of cell invasion of LCs vs that without rMIP-3α or MIP-3 antibody (Non). The migration of LCs was induced by rMIP-3α and inhibited by an additional neutralizing MIP-3α antibody (rMIP-3α+MIP-3α Ab). More precisely, the MIP-3α antibody decreased the migration at a level lower than the standard level (Non), suggesting that the standard medium for LCs originally contained MIP-3α. (B) Cultured BECs were stimulated with Pam3CSK4 or interleukin (IL)-1β for 2 days and these supernatants were added to a medium for LCs in the assay chamber at a concentration of 5%. Data are shown as the relative fold-increase vs those with additional neutralizing MIP-3α Ab, because the standard medium for LCs contains MIP-3α. Consequently, the additional supernatant of Pam3CSK4- or IL-1β-treated BECs upregulated the migration of LCs, compared with the non-treated medium (Non).

© 2010 John Wiley & Sons A/S

MIP-3α (CCL20) could migrate LCs. Dieu-Nosjean *et al.*(13) reported that MIP-3α plays a central role in recruiting LC precursors into the epithelium during inflammation. Langerin$^+$ DCs, moreover, were localized subepithelially adjacent to MIP-3α-expressing epithelium (21) and, in the colon of ulcerative colitis patients, MIP-3α plays an important role in the disease's pathogenesis (22). Interestingly, in the present study, MIP-3α-expressing BECs and Langerin$^+$ LCs were frequently co-localized in the same bile ducts showing cholangitis. A previous study demonstrated that MIP-3α was produced by keratinocytes and that the migration of LC precursors induced by the supernatant of activated skin keratinocytes was completely blocked with an antibody against MIP-3α (13). Therefore, the presence of BECs producing MIP-3α suggests that BECs play an important role in the migration of LCs in biliary layers of PBC patients.

Recently obtained evidence indicates that bile duct-related acquired immunity is closely associated with the periductal cytokine milieu and biliary innate immunity (4, 23–26). We have reported previously that human BECs possess receptors for some cytokines (IL-1β, IL-6, IL-4, IFN-γ, TNF-α and IL-17) and TLRs for some PAMPS [LPS, Pam3CSK4 and poly(I:C)] (24, 27), suggesting that they could show a response to these cytokines and PAMPs and induce various immunological reactions. In fact, the present study demonstrated that IL-17 as well as inflammatory cytokines (IL-1β and TNF-alpha) significantly upregulated the expression of MIP-3α. We demonstrated previously that IL-17-positive cells (Th17 cells) were accumulated around the damaged bile ducts in PBC and that BECs possessed the ability to produce Th17-inducible cytokines including IL-1β as a result of the innate immune response (27). Our previous studies, moreover, have found that the expression of IL-1β and TNF-α was increased in pathologic BECs of PBC patients (27, 28). The present study demonstrated that the expression of MIP-3α was selectively restricted at sites of inflammation including cholangitis in PBC, suggesting the production of MIP-3α to be closely associated with periductal cytokines including IL-1β, TNF-α and IL-17. In addition to these cytokine milieus, the damaged bile ducts and/or portal tracts show an increased susceptibility to PAMPs and enhanced production and secretion of innate inflammatory mediators in PBC (23, 29). All PAMPs examined in this study significantly upregulated the production of MIP-3α in human BECs. Therefore, BEC-derived MIP-3α production caused by PBC-specific cytokine and innate immune responses facilitates the migration of LCs as biliary epithelium-specific APCs, followed by the aggravating and continuous cholangitis in PBC.

In summary, this study provides the following key findings: the frequent clustering of mDCs, in particular LCs, around or within the damaged bile ducts in PBC; a significant positive correlation between the frequency of LCs and the degree of MIP-3α expression in bile ducts; and the induction of MIP-3α expression in BECs caused by the biliary innate immune and cytokine responses. These findings suggest that LCs existing around or within biliary epithelial layers are important as APCs in the damaged bile ducts in PBC and directed in their migration by BEC-derived MIP-3α induced via a PBC-specific periductal cytokine milieu and biliary innate immunity. An understanding of the specific functions of LCs and MIP-3α in biliary inflammatory and immune disorders might provide new strategies to attenuate the cholangiopathy in PBC.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare of Japan and Grants-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Kaplan MM. Primary biliary cirrhosis. *N Engl J Med* 1996; **335**: 1570–80.
2. Nakanuma Y, Ohta G. Histometric and serial section observations of the intrahepatic bile ducts in primary biliary cirrhosis. *Gastroenterology* 1979; **76**: 1326–32.
3. Gershwin ME, Ansari AA, Mackay IR, *et al.* Primary biliary cirrhosis: an orchestrated immune response against epithelial cells. *Immunol Rev* 2000; **174**: 210–25.
4. Harada K, Van de Water J, Leung PS, *et al.* In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. *Hepatology* 1997; **25**: 791–6.
5. Shimoda S, Harada K, Niiro H, *et al.* Biliary epithelial cells and primary biliary cirrhosis: the role of liver-infiltrating mononuclear cells. *Hepatology* 2008; **47**: 958–65.
6. Shimoda S, Nakamura M, Ishibashi H, *et al.* HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune diseases. *J Exp Med* 1995; **181**: 1835–45.
7. Demetris AJ, Sever C, Kakizoe S, *et al.* S100 protein positive dendritic cells in primary biliary cirrhosis and other chronic inflammatory liver diseases. Relevance to pathogenesis? *Am J Pathol* 1989; **134**: 741–7.
8. Tanimoto K, Akbar SM, Michitaka K, Onji M. Immunohistochemical localization of antigen presenting cells in liver from patients with primary biliary cirrhosis; highly restricted distribution of CD83-positive activated dendritic cells. *Pathol Res Pract* 1999; **195**: 157–62.
9. Silberberg I, Baer RL, Rosenthal SA. The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. *J Invest Dermatol* 1976; **66**: 210–7.
10. Dzionek A, Sohma Y, Nagafune J, *et al.* BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin,

© 2010 John Wiley & Sons A/S

mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med* 2001; **194**: 1823–34.

11. Boor PP, Ijzermans JN, van der Molen RG, *et al.* Immunomagnetic selection of functional dendritic cells from human lymph nodes. *Immunol Lett* 2005; **99**: 162–8.
12. de Witte L, Nabatov A, Pion M, *et al.* Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. *Nat Med* 2007; **13**: 367–71.
13. Dieu-Nosjean MC, Massacrier C, Homey B, *et al.* Macrophage inflammatory protein 3alpha is expressed at inflamed epithelial surfaces and is the most potent chemokine known in attracting Langerhans cell precursors. *J Exp Med* 2000; **192**: 705–18.
14. Liao F, Rabin RL, Smith CS, *et al.* CC-chemokine receptor 6 is expressed on diverse memory subsets of T cells and determines responsiveness to macrophage inflammatory protein 3 alpha. *J Immunol* 1999; **162**: 186–94.
15. Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. *J Hepatol* 1990; **11**: 272–6.
16. Nakanuma Y, Hoso M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. *Microsc Res Tech* 1997; **38**: 552–70.
17. Harada K, Ohba K, Ozaki S, *et al.* Peptide antibiotic human beta-defensin-1 and -2 contribute to antimicrobial defense of the intrahepatic biliary tree. *Hepatology* 2004; **40**: 925–32.
18. Geissmann F, Dieu-Nosjean MC, Dezutter C, *et al.* Accumulation of immature Langerhans cells in human lymph nodes draining chronically inflamed skin. *J Exp Med* 2002; **196**: 417–30.
19. Tobe K, Endo H, Nagashima H, Kobayashi T. Langerhans cells among bile duct epithelial cells in chronic liver disease. *Acta Pathol Jpn* 1982; **32**: 93–9.
20. Ballardini G, Fallani M, Bianchi FB, Pisi E. Antigen presenting cells in liver biopsies from patients with primary biliary cirrhosis. *Autoimmunity* 1989; **3**: 135–44.
21. Kaser A, Ludwiczek O, Holzmann S, *et al.* Increased expression of CCL20 in human inflammatory bowel disease. *J Clin Immunol* 2004; **24**: 74–85.
22. Watanabe S, Yamakawa M, Hiroaki T, *et al.* Correlation of dendritic cell infiltration with active crypt inflammation in ulcerative colitis. *Clin Immunol* 2007; **122**: 288–97.
23. Harada K, Isse K, Kamihira T, *et al.* Th1 cytokine-induced downregulation of PPARgamma in human biliary cells relates to cholangitis in primary biliary cirrhosis. *Hepatology* 2005; **41**: 1329–38.
24. Harada K, Isse K, Nakanuma Y. Interferon gamma accelerates NF-kappaB activation of biliary epithelial cells induced by Toll-like receptor and ligand interaction. *J Clin Pathol* 2006; **59**: 184–90.
25. Harada K, Ohira S, Isse K, *et al.* Lipopolysaccharide activates nuclear factor-kappaB through toll-like receptors and related molecules in cultured biliary epithelial cells. *Lab Invest* 2003; **83**: 1657–67.
26. Isse K, Harada K, Zen Y, *et al.* Fractalkine and CX3CR1 are involved in the recruitment of intraepithelial lymphocytes of intrahepatic bile ducts. *Hepatology* 2005; **41**: 506–16.
27. Harada K, Shimoda S, Sato Y, *et al.* Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. *Clin Exp Immunol* 2009; **157**: 261–70.
28. Yasoshima M, Kono N, Sugawara H, *et al.* Increased expression of interleukin-6 and tumor necrosis factor-alpha in pathologic biliary epithelial cells: in situ and culture study. *Lab Invest* 1998; **78**: 89–100.
29. Takii Y, Nakamura M, Ito M, *et al.* Enhanced expression of type I interferon and toll-like receptor-3 in primary biliary cirrhosis. *Lab Invest* 2005; **85**: 908–20.

© 2010 John Wiley & Sons A/S

Pathogenesis of IgG4-related disease

Yoh Zen[a] and Yasuni Nakanuma[b]

[a]Institute of Liver Studies, King's College Hospital, London, UK and [b]Department of Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

Correspondence to Yoh Zen, MD, PhD, FRCPath, Institute of Liver Studies, King's College Hospital, Denmark Hill, London, SE5 9RS, UK
Tel: +44 20 3299 3734; fax: +44 20 3299 3125; e-mail: yoh.zen@kcl.ac.uk

Current Opinion in Rheumatology 2011, 23:114–118

Purpose of review

To review studies that have examined underlying genetic and immunological aspects of IgG4-related disease.

Recent findings

Genetic studies have suggested that several human leukocyte antigen (HLA) and non-HLA haplotypes/genotypes are associated with susceptibility to IgG4-related disease or to disease relapse after steroid therapy. Among several autoantibodies identified so far, autoantibodies against lactoferrin and carbonic anhydrase II are most frequently detected in serum of IgG4-disease patients. However, it has not been well clarified whether or not those autoantibodies belong to an IgG4 subclass. Studies that have demonstrated molecular mimicry between *Helicobacter pylori* and constituents of pancreatic epithelial cells suggest that gastric *H. pylori* infection triggers autoimmune pancreatitis in genetically predisposed individuals through antibody cross-reactivity. Recently, T-helper 2 immune reaction has been suggested to be predominant in IgG4-related disease. Interestingly, regulatory immune reactions are activated in IgG4-related disease, and regulatory cytokines interleukin-10 and transforming growth factor-β have been suggested, respectively, to play important roles in IgG4 class switch and fibroplasia.

Summary

Autoimmunity has been considered the most probable pathogenesis of IgG4-related disease, but has not been completely proved so far. A breakthrough study to detect a specific autoantigen, autoantibody, or pathogen is necessary.

Keywords

autoimmune pancreatitis, cytokine, IgG4, pathology, regulatory T cells

Curr Opin Rheumatol 23:114–118
© 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins
1040-8711

Introduction

IgG4-related disease is a unique inflammatory condition characterized by tumorous swelling of affected organs and high serum IgG4 concentrations [1]. Histological characteristics are diffuse lymphoplasmacytic infiltration, occasional eosinophils, storiform fibrosis, obliterative phlebitis, and infiltration by numerous IgG4-bearing plasma cells [2•]. That autoimmunity may participate in pathogenesis is suggested, particularly by serologic abnormalities (e.g. presence of autoantibodies and elevated levels of γ-globulin) and by a dramatic response to steroid therapy [3,4••]. However, the detailed pathogenetic mechanism and underlying immune response have not been well clarified. Especially mysterious is why IgG4 is specifically elevated in this disease.

In this article, we review reports that have examined underlying genetic and immunological aspects of IgG4-related disease. A caveat is that most studies on the pathogenesis of IgG4-related disease have examined only pancreatitis and sialodacryoadenitis.

Genetic backgrounds

Several genetic susceptibility factors for IgG4-related disease have been identified. Most studies so far have examined only Asian patients with autoimmune pancreatitis (AIP), a pancreatic manifestation of IgG4-related disease. It is still unknown whether or not these data apply to Western people because susceptibility factors often vary among different ethnic groups. In 2002, Kawa *et al.* [5] reported that the frequencies of human leukocyte antigen (HLA) serotypes DRB1*0405 and DQB1*0401 were significantly higher in Japanese patients with AIP than in healthy individuals and in patients with chronic calcifying pancreatitis. In contrast, a Korean study [6] could not show significant associations between HLA type and development of AIP in Korean patients. Interestingly, this study revealed that the nonaspartic acid at DQβ1 57 was significantly associated with relapse of AIP. All Korean patients who experienced a relapse during or after steroid treatment had nonaspartic acid at DQβ1 57 in contrast to 30% of AIP patients without a relapse [6]. However, this result could not be confirmed in Japanese patients [7•].

DOI:10.1097/BOR.0b013e3283412f4a

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Non-HLA genes have been also investigated regarding susceptibility to AIP. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), expressed on activated T cells, plays an important role in the regulation of T-cell stimulation. Many single-nucleotide polymorphisms (SNPs) have been identified in *CTLA-4*, encoding CTLA-4, and some are associated with susceptibility to autoimmune diseases [8]. It was reported that the frequency of a *CTLA-4* 49A haplotype was significantly higher in Chinese AIP patients than in healthy individuals [9]. The −318C/+49A/CT60G haplotype increased susceptibility to AIP. The investigators also found that the tumor necrosis factor-α (TNF-α) promoter −863A haplotype was related to extrapancreatic involvement in Chinese patients with AIP [9]. A Japanese study [10] showed that a SNP at *CTLA-4* +6233 in the 3′-untranslated region contributes to disease susceptibility. A +6230G/G genotype increased susceptibility to AIP, whereas a +6230A haplotype was associated with AIP resistance [10]. In addition, the +49A/A and +6230A/A genotypes were related to an increased risk of relapse [10].

Fc receptor-like 3 (FCRL3) molecule, expressed on B cells, is known to augment autoantibody production in individuals with disease-susceptible genotypes [11]. *FCRL3* polymorphisms have been shown to contribute to susceptibility to autoimmune diseases. Umemura *et al.* [12] reported that the −110A/A genotype is associated with AIP in the Japanese population. Interestingly, serum IgG4 concentrations were positively correlated with the number of susceptible alleles [12]. The same group also examined SNPs in the gene encoding Toll-like receptor 4 (*TLR4*), but they were not associated with susceptibility to or relapse of AIP [13•].

Target organs

IgG4-related disease can develop in almost all organs. The pancreatic lesion (AIP) is the prototype of IgG4-related disease. After the landmark study by Hamano *et al.* [1], IgG4-related lesions histologically similar to AIP have been identified in various organs such as bile duct (sclerosing cholangitis) [14], salivary gland (chronic sclerosing sialadenitis) [15•], lacrimal gland (chronic sclerosing dacryoadenitis or Mikulicz's disease) [16•], retroperitoneum (retroperitoneal fibrosis) [17•], aorta (inflammatory aneurysm) [18], lung (nodular or diffuse inflammation) [19•], and kidney (tubulointerstitial nephritis) [20••]. Multiple lesions can develop in different organs in a patient simultaneously or metachronously. Interestingly, kidney lesions have always been associated with extrarenal disease in personally studied cohorts. This may suggest that kidneys are not a primary site of IgG4-related disease.

IgG4-related lesions in various organs are now considered one of the manifestations of systemic IgG4-related disease based on similar clinical and histological features [21•,22••]. However, clinicopathological features are not completely identical among manifestations. One of the differences is that the proportion of women is significantly higher in patients with sialadenitis or dacryoadenitis (approximately 50%) than in patients with involvement of other organs (15–25%) (Y. Zen, unpublished data). Although IgG4-related disease has been thought of as a single disease entity, different causative factors may be involved in different organs.

Potential autoantigens

Several groups have tried to identify autoantigens based on the hypothesis that IgG4-related disease is an autoimmune disorder. Nonspecific antinuclear antibodies are identified in more than half of patients with IgG4-related disease. Antibodies against lactoferrin and carbonic anhydrase (CA)-II are the most frequently detected autoantibodies in AIP (73 and 54%, respectively) [23]. Interestingly, a strong positive correlation between increase in serum IgG4 levels and anti-CA-II antibody levels has been reported in AIP [24]. More recently, it was reported that anti-CA-IV antibodies were detected in 10 of 29 (34%) patients with AIP [25]. Lactoferrin and carbonic anhydrases are expressed in some exocrine organs including the pancreas, salivary gland, bile duct, and renal tubules. The distribution of these antigens may be related to systemic manifestations of IgG4-related disease.

Pancreatic secretory trypsin inhibitor (PSTI) is another potential autoantigen. Antibodies against this molecule were detected in 30–40% of patients with AIP [26]. In a study by Asada *et al.* [26], 23 of 26 patients with AIP had antibodies against either CA-II or lactoferrin. Two of three patients in whom neither of these autoantibodies could be demonstrated had anti-PSTI antibodies. Interestingly, these autoantibodies were of the IgG1, not the IgG4 subclass [26]. A recent European study [27•] also revealed autoantibodies against trypsinogens, as well as anti-PSTI antibodies in AIP patients.

In 2010, Yamamoto *et al.* [28•] performed a proteomics study to identify potential autoantigens for IgG4-related diseases in 28 patients with IgG4-related dacryoadenitis, sialadenitis, pancreatitis (AIP), or tubulointerstitial nephritis. Immune complexes taken from patients' sera were divided into immunoglobulin and antigens and were analyzed by surface-enhanced laser desorption/ionization–time-of-flight mass spectrometry. They found an antigen, a 13.1-kDa protein that was as a potential candidate autoantigen, in all samples from patients with IgG4-related diseases, but not in samples from patients in the normal and disease-control cohorts [28•].

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

To the best of our knowledge, IgG4 type autoantibodies have not been detected in IgG4-related disease. It remains to be answered whether IgG4 is an autoantibody in IgG4-related disease or is overexpressed secondarily in response to an unknown primary inflammatory stimulus. Aoki *et al.* [29] examined whether or not IgG4 from patients with IgG4-related disease can bind to human tissue. Histological sections of normal human tissue were incubated with patients' serum. IgG4 bound to a tissue antigen was then detected by secondary antibodies against human IgG4. Interestingly, IgG4 from the patients was found to be able to bind to the normal epithelia of the pancreatic ducts, bile ducts, gallbladder, and salivary gland ducts [29].

Involvement of *Helicobacter pylori*

The possible involvement of *Helicobacter pylori* in the pathogenesis of AIP was reported in 2005 [30]. Guarneri *et al.* [31] found a significant homology between human CA-II and α-carbonic anhydrase of *H. pylori*. The homologous segments contain the binding motif of the HLA molecule DRB1*0405. This study suggests that gastric *H. pylori* infection triggers AIP in genetically predisposed individuals through molecular mimicry [31]. More recently, Frulloni *et al.* [32••] found that 94% of patients with AIP had antibodies against plasminogen-binding protein of *H. pylori*. The amino acid sequence of plasminogen-binding protein exhibits homology with that of ubiquitin–protein ligase E3 component n-recognin 2, an enzyme expressed in pancreatic acinar cells. However, serum IgG4 levels were elevated in only 53% of patients in this study, suggesting that the cohort assessed might in substantial part represent non–IgG4-related AIP (type II AIP [4••]) [32••].

Cytokines and chemokines

In 2000, Okazaki *et al.* [23] serologically examined T-helper 1 (Th1)/Th2 balance in patients with AIP. Numbers of interferon-γ (IFN-γ)-producing peripheral $CD4^+$ cells and secreted levels of IFN-γ were significantly increased compared with those in controls, whereas numbers of interleukin (IL)-4-producing cells and serum levels of IL-4 were not increased. Yamamoto *et al.* [33] similarly reported that IFN-γ/IL-4 ratios were significantly higher in IgG4-related dacryoadenitis and sialadenitis than in Sjögren's syndrome. In contrast, five recent studies provided data suggesting that the Th2-dominant immune response is more activated in IgG4-related disease [34,35•,36•,37•,38•]. Peripheral blood mononuclear cells collected from patients with IgG4-related disease produced predominantly Th2-type cytokines such as IL-4, IL-5, IL-10, and IL-13 after T-cell stimulation. Interestingly, peripheral blood mononuclear cells of one patient exhibited increased production of IgG4, as well as IL-10 upon stimulation with TLR ligands [36•].

In 2007, we histopathologically examined cytokine production *in situ* in materials from patients with IgG4-related disease [39]. Quantitative real-time PCR using RNA extracted from frozen tissue of affected organs (pancreas, bile duct, salivary gland, and lacrimal gland) revealed significantly higher ratios of IL-4/IFN-γ (45.8-fold), IL-5/IFN-γ (18.7-fold), and IL-13/IFN-γ (20.7-fold) in IgG4-related disease tissues than in tissues from patients with classical autoimmune diseases [39]. Lymphocytes expressing IL-4 were clearly demonstrated by in-situ hybridization. Similarly, Th2-dominant cytokine production was also reported in salivary and lacrimal gland lesions [34]. Th2-type cytokines play an important role in inflammatory reaction in the organs affected by IgG4-related disease.

A chemokine expression profile in IgG4-related disease has not been well characterized. One study [40] showed that in IgG4-related pancreatitis and cholangitis expression levels of CXC chemokine receptor-5 (CXCR5) and CXC chemokine ligand-13 (CXCL13) mRNAs were higher than in chronic pancreatitis and primary sclerosing cholangitis. In addition, $CXCR5^+$ or $CXCL13^+$ lymphocytes were noted in periductal areas with or without parenchymal localization [40]. Another study [35•] showed expression of CD40 and its ligand in infiltrating lymphocytes and plasmacytes. Interaction of CXCR5–CXCL13 or of CD40–CD40 ligand may be involved in lymphocyte trafficking, B-cell activation, and immunoglobulin production in IgG4-related disease.

Role of regulatory T cells

Impaired function or a decreased number of regulatory T cells (Tregs) is believed to be an important step in the development of autoimmune disorders. However, of interest is that the number of Tregs is not decreased – rather, characteristically increased – in both tissue and blood of patients with IgG4-related disease. Our investigation revealed that mRNA of forkhead box P3 (Foxp3, a Tregs-specific transcriptional factor) in IgG4-related disease was expressed 36.4-fold higher than in classical autoimmune diseases. Two regulatory cytokines, IL-10 and transforming growth factor-β (TGF-β), were also significantly overexpressed (45.3-fold and 39.4-fold, respectively) [39]. Furthermore, $CD4^+CD25^+Foxp3^+$ Tregs could be detected within affected tissues by immunohistochemistry, in numbers significantly higher than in autoimmune and nonautoimmune disease controls. Overexpression of IL-10 and TGF-β was also confirmed in IgG4-related tubulointerstitial nephritis [20••]. Koyabu *et al.* [41•] reported that the number of $Foxp3^+$ cells was significantly correlated with the

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

number of IgG4+ plasma cells in IgG4-related cholangitis. Miyoshi *et al.* [42] examined the number of Tregs in the blood and reported that the number of CD4+CD25high Tregs was significantly higher in patients with AIP than in patients with chronic pancreatitis and was correlated with the level of serum IgG4. The number of naive Tregs was significantly decreased. They speculated that hyporeaction of naive Tregs might be involved in the development of IgG4-related disease, whereas hyperreaction of CD4+CD25high Tregs could reflect IgG4-related disease progression [42].

Histology-immunology correlations

Two key histological features of IgG4-related disease are infiltration of IgG4+ plasma cells and storiform fibrosis. IL-10 produced by Th2 cells or Tregs may participate in IgG4+ plasma cell infiltration because IL-10 has a potent function in directing B cells to produce IgG4. TGF-β secreted from Tregs and macrophages may induce fibroplasia, given the strong fibrogenic function of TGF-β and the expression of a TGF-β receptor in myofibroblasts in AIP [39,43]. On occasion, IgG4-related disease histologically shows eosinophilic infiltration. In addition to IgG4, serum eosinophilia and high serum IgE levels are frequently seen in patients with IgG4-related disease. These pathological and immunological features may be mediated by IL-4, IL-5, and IL-13 because these cytokines are important for class switch to IgE production and for eosinophil migration or activation. IgG4-related disease commonly develops in people with a predisposition to allergy [44•,45•]. About 20% of patients in our studied cohorts have allergic disorders (e.g. bronchial asthma or drug allergy). One attractive hypothesis is a possible involvement of chronic allergic reaction, but not autoimmunity, in the pathogenesis of IgG4-related disease. However, a specific allergen must be identified to confirm this hypothesis.

Conclusion

We have briefly described data on the pathogenesis of IgG4-related disease. Because most of the research is based on only AIP or sialadenitis/dacryoadenitis, it is still unknown whether the same immune reaction takes place in other organs. A point of particular importance is that potential autoantigens are not identified in nonglandular tissues (e.g. retroperitoneum or artery). Regarding Th1/Th2 balance, recent studies have accumulated evidence that suggests an involvement of Th2-dominant reaction in the pathogenesis of IgG4-related disease. Autoimmunity has been suggested as the most possible pathogenetic cause, but allergic cause is another attractive hypothesis given observed cytokine expression profiles and the frequent association of IgG4-related disease with a predisposition to allergy. A breakthrough study to detect a specific autoantigen, autoantibody, pathogen, or allergen is necessary.

Acknowledgements

The authors thank Dr A.S. Knisely (Institute of Liver Studies, King's College Hospital, UK) for helpful comments and suggestions in this article. Our study was supported in part by a research grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology (no. 17790231).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest
Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 129–130).

1 Hamano H, Kawa S, Horiuchi A, *et al.* High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 2001; 344:732–738.

2 Zhang L, Smyrk TC. Autoimmune pancreatitis and IgG4-related systemic
• diseases. Int J Clin Exp Pathol 2010; 3:491–504.
A review of clinicopathological characteristics of IgG4-related disease, particularly emphasizing the pancreatic lesion.

3 Okazaki K, Uchida K, Fukui T. Recent advances in autoimmune pancreatitis: concept, diagnosis, and pathogenesis. J Gastroenterol 2008; 43:409–418.

4 Park DH, Kim MH, Chari ST. Recent advances in autoimmune pancreatitis.
•• Gut 2009; 58:1680–1689.
A comprehensive review of AIP, especially providing substantial data on a new classification system of AIP. Data on animal models of AIP have also been summarized.

5 Kawa S, Ota M, Yoshizawa K, *et al.* HLA DRB10405-DQB10401 haplotype is associated with autoimmune pancreatitis in the Japanese population. Gastroenterology 2002; 122:1264–1269.

6 Park do H, Kim MH, Oh HB, *et al.* Substitution of aspartic acid at position 57 of the DQbeta1 affects relapse of autoimmune pancreatitis. Gastroenterology 2008; 134:440–446.

7 Hirano K, Asaoka Y, Tada M, *et al.* No significant relation between relapse of
• autoimmune pancreatitis and substitution of aspartic acid at position 57 of DQbeta1. J Gastroenterol 2009; 44:799–800.
This study provided negative data that amino acid sequences at position 57 of DQbeta1 are not associated with relapse of AIP in Japanese patients.

8 Scalapino KJ, Daikh DI. CTLA-4: a key regulatory point in the control of autoimmune disease. Immunol Rev 2008; 223:143–155.

9 Chang MC, Chang YT, Tien YW, *et al.* T-cell regulatory gene CTLA-4 polymorphism/haplotype association with autoimmune pancreatitis. Clin Chem 2007; 53:1700–1705.

10 Umemura T, Ota M, Hamano H, *et al.* Association of autoimmune pancreatitis with cytotoxic T-lymphocyte antigen 4 gene polymorphisms in Japanese patients. Am J Gastroenterol 2008; 103:588–594.

11 Kochi Y, Yamada R, Suzuki A, *et al.* A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. Nat Genet 2005; 37:478–485.

12 Umemura T, Ota M, Hamano H, *et al.* Genetic association of Fc receptor-like 3 polymorphisms with autoimmune pancreatitis in Japanese patients. Gut 2006; 55:1367–1368.

13 Umemura T, Katsuyama Y, Hamano H, *et al.* Association analysis of Toll-like
• receptor 4 polymorphisms with autoimmune pancreatitis. Hum Immunol 2009; 70:742–746.
This study examined TLR4 gene polymorphisms in Japanese patients with AIP and concluded that such polymorphisms are not associated with susceptibility to or relapse of the disease.

14 Zen Y, Harada K, Sasaki M, *et al.* IgG4-related sclerosing cholangitis with and without hepatic inflammatory pseudotumor, and sclerosing pancreatitis-associated sclerosing cholangitis: do they belong to a spectrum of sclerosing pancreatitis? Am J Surg Pathol 2004; 28:1193–1203.

15 Geyer JT, Ferry JA, Harris NL, *et al.* Chronic sclerosing sialadenitis (Küttner
• tumor) is an IgG4-associated disease. Am J Surg Pathol 2010; 34:202–210.
This study clearly demonstrated that chronic sclerosing sialadenitis belongs to a spectrum of systemic IgG4-related disease based on characteristic pathological features including numerous infiltrating IgG4+ plasma cells.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

16 Takano KI, Yamamoto M, Takahashi H, *et al.* Clinicopathologic similarities
• between Mikulicz disease and Küttner tumor. Am J Otolaryngol 2009. doi:10.1016/j.amjoto.2009.08.002. [Epub ahead of print]
A study showing clinicopathological similarities of two characteristic salivary or lacrimal gland lesions.

17 Zen Y, Onodera M, Inoue D, *et al.* Retroperitoneal fibrosis: a clinicopathologic
• study with respect to immunoglobulin G4. Am J Surg Pathol 2009; 33:1833–1839.
A clinicopathological study that revealed that retroperitoneal fibrosis can be classified into IgG4-related and nonrelated forms.

18 Kasashima S, Zen Y, Kawashima A, *et al.* Inflammatory abdominal aortic aneurysm: close relationship to IgG4-related periaortitis. Am J Surg Pathol 2008; 32:197–204.

19 Zen Y, Inoue D, Kitao A, *et al.* IgG4-related lung and pleural disease: a
• clinicopathologic study of 21 cases. Am J Surg Pathol 2009; 33:1886–1893.
This study provided data that IgG4-related lung lesions can manifest various morphological changes.

20 Nakashima H, Miyake K, Moriyama M, *et al.* An amplification of IL-10 and TGF-
•• beta in patients with IgG4-related tubulointerstitial nephritis. Clin Nephrol 2010; 73:385–391.
This study examined expression levels of cytokines using tissue samples of IgG4-related tubulointerstitial nephritis and concluded that expression levels of IL-4, IL-10, and TGF-β in this disease were significantly higher than in other tubulointerstitial nephritis.

21 Kamisawa T, Takuma K, Egawa N, *et al.* Autoimmune pancreatitis and IgG4-
• related sclerosing disease. Nat Rev Gastroenterol Hepatol 2010; 7:401–409.
A review article of clinical features and differential diagnosis of AIP and IgG4-related disease.

22 Masaki Y, Dong L, Kurose N, *et al.* Proposal for a new clinical entity, IgG4-
•• positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders. Ann Rheum Dis 2009; 68:1310–1315.
A Japanese multiinstitutional study comparing clinical and pathological features of IgG4-related sialadenitis/dacryoadenitis and Sjögren syndrome. A new concept of $IgG4^+$ multiorgan lymphoproliferative syndrome proposed.

23 Okazaki K, Uchida K, Ohana M, *et al.* Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. Gastroenterology 2000; 118:573–581.

24 Aparisi L, Farre A, Gomez-Cambronero L, *et al.* Antibodies to carbonic anhydrase and IgG4 levels in idiopathic chronic pancreatitis: relevance for diagnosis of autoimmune pancreatitis. Gut 2005; 54:703–709.

25 Nishimori I, Miyaji E, Morimoto K, *et al.* Serum antibodies to carbonic anhydrase IV in patients with autoimmune pancreatitis. Gut 2005; 54:274–281.

26 Asada M, Nishio A, Uchida K, *et al.* Identification of a novel autoantibody against pancreatic secretory trypsin inhibitor in patients with autoimmune pancreatitis. Pancreas 2006; 33:20–26.

27 Löhr JM, Faissner R, Koczan D, *et al.* Autoantibodies against the exocrine
• pancreas in autoimmune pancreatitis: gene and protein expression profiling and immunoassays identify pancreatic enzymes as a major target of the inflammatory process. Am J Gastroenterol 2010; 105:2060–2071.
Potential autoantibodies against pancreatic enzymes were identified in AIP patients through RNA microarrays of pancreatic tissue and immunoassays.

28 Yamamoto M, Naishiro Y, Suzuki C, *et al.* Proteomics analysis in 28 patients
• with systemic IgG4-related plasmacytic syndrome. Rheumatol Int 2010; 30:565–568.
A proteomics study to identify autoantigens in IgG4-related disease. A 13.1-kDa candidate protein was identified, but its sequence was not clarified.

29 Aoki S, Nakazawa T, Ohara H, *et al.* Immunohistochemical study of autoimmune pancreatitis using anti-IgG4 antibody and patients' sera. Histopathology 2005; 47:147–158.

30 Kountouras J, Zavos C, Chatzopoulos D. Autoimmune pancreatitis, Helicobacter pylori infection, and apoptosis: a proposed relationship. Pancreas 2005; 30:192–193.

31 Guarneri F, Guarneri C, Benvenga S. Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? J Cell Mol Med 2005; 9:741–744.

32 Frulloni L, Lunardi C, Simone R, *et al.* Identification of a novel antibody
•• associated with autoimmune pancreatitis. N Engl J Med 2009; 361:2135–2142.
An antibody against plasminogen-binding protein of *H. pylori*, which shows sequence homology to an enzyme highly expressed in pancreatic acinar cells, was identified in 94% of patients with AIP and in only 5% of patients with pancreatic cancer.

33 Yamamoto M, Harada S, Ohara M, *et al.* Clinical and pathological differences between Mikulicz's disease and Sjogren's syndrome. Rheumatology 2005; 44:227–234.

34 Miyake K, Moriyama M, Aizawa K, *et al.* Peripheral CD4+ T cells showing a Th2 phenotype in a patient with Mikulicz's disease associated with lymphadenopathy and pleural effusion. Mod Rheumatol 2008; 18:86–90.

35 Kudo-Tanaka E, Nakatsuka S, Hirano T, *et al.* A case of Mikulicz's disease with
• Th2-biased cytokine profile: possible feature discriminable from Sjögren's syndrome. Mod Rheumatol 2009; 19:691–695.
This study described a case of IgG4-related disease with overproduction of IL-4, IL-5, and IL-13 from peripheral blood mononuclear cells; expression of CD40 and its ligand in sialadenitis is also described.

36 Akitake R, Watanabe T, Zaima C, *et al.* Possible involvement of T helper type 2
• responses to Toll-like receptor ligands in IgG4-related sclerosing disease. Gut 2010; 59:542–545.
A case report in which $IgG4^+$ plasma cells were identified in colonic mucosa and production of IgG4 or IL-10 from peripheral blood cells was enhanced upon stimulation with TLR ligands.

37 Suzuki K, Tamaru JI, Okuyama A, *et al.* IgG4-positive multiorgan lympho-
• proliferative syndrome manifesting as chronic symmetrical sclerosing dacryo-sialadenitis with subsequent secondary portal hypertension and remarkable IgG4-linked IL-4 elevation. Rheumatology (Oxford) 2010; 49:1789–1791.
This study described a case of IgG4-related disease in multiple organs with a significantly increased level of serum IL-4.

38 Kanari H, Kagami S, Kashiwakuma D, *et al.* Role of Th2 cells in IgG4-related
• lacrimal gland enlargement. Int Arch Allergy Immunol 2010; 152 (Suppl 1): 47–53.
A study on cytokine expression levels on $CD4^+$ T cells in patients with IgG4-dacryoadenitis that showed significantly higher levels of IL-4, IL-5, and IL-10 than in disease-control patients and healthy persons.

39 Zen Y, Fujii T, Harada K, *et al.* Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology 2007; 45:1538–1546.

40 Esposito I, Born D, Bergmann F, *et al.* Autoimmune pancreatocholangitis, nonautoimmune pancreatitis and primary sclerosing cholangitis: a comparative morphological and immunological analysis. PLoS One 2008; 3:e2539.

41 Koyabu M, Uchida K, Miyoshi H, *et al.* Analysis of regulatory T cells and IgG4-
• positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases. J Gastroenterol 2010; 45:732–741.
This study revealed that the $IgG4^+/IgG1^+$ cell ratio in IgG4-cholangitis is significantly higher than in classical autoimmune liver diseases and the number of $IgG4^+$ cells correlated with the number of $Foxp3^+$ cells.

42 Miyoshi H, Uchida K, Taniguchi T, *et al.* Circulating naïve and CD4+CD25high regulatory T cells in patients with autoimmune pancreatitis. Pancreas 2008; 36:133–140.

43 Detlefsen S, Sipos B, Zhao J, *et al.* Autoimmune pancreatitis: expression and cellular source of profibrotic cytokines and their receptors. Am J Surg Pathol 2008; 32:986–995.

44 Kamisawa T, Anjiki H, Egawa N, Kubota N. Allergic manifestations in auto-
• immune pancreatitis. Eur J Gastroenterol Hepatol 2009; 21:1136–1139.
This study identified allergic predisposition in 20 of 45 patients with AIP and compared clinical features between patients with and without allergic disorders.

45 Sah RP, Pannala R, Zhang L, *et al.* Eosinophilia and allergic disorders in
• autoimmune pancreatitis. Am J Gastroenterol 2010. doi:10.1038/ajg.2010.236. [Epub ahead of print]
This study examined peripheral eosinophilia, allergic disorders, and pancreatic eosinophil infiltration in patients with AIP and revealed that patients with and without peripheral eosinophilia were similar in clinical profile.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

201324028B（別刷 2/6）

厚生労働科学研究費補助金　難治性疾患等克服研究事業

（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

平成23～25年度　総合研究報告書

研究成果の刊行物・別刷（平成23年度）

分冊６－２

研究代表者　坪内　博仁

平成26（2014）年３月

Ｖ．研究成果の刊行物・別刷
（平成23年度）

分冊６－２

研究成果の刊行に関する一覧表（平成23年度）

雑 誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Zen Y, Nakanuma Y.	Pathogenesis of IgG4-related disease.	Curr Opin Rheumatol	23	114-118	2011
Umemura T, Zen Y, Hamano H, Joshita S, Ichijo T, Yoshizawa K, Kiyosawa K, Ota M, Kawa S, Nakanuma Y, Tanaka E.	Clinical significance of immunoglobulin G4- associated autoimmune hepatitis.	J Gastroenterol	46	48-55	2011
Takeyama Y, SakisakaS.	Hepatobiliary membrane transporters in primary biliary cirrhosis.	Hepatol Res	42	120-130	2012
Honda A, Miyazaki T, Ikegami T, Iwamoto J, Maeda T, Hirayama T, Saito Y, Teramoto T, Matsuzaki Y.	Cholesterol 25-hydroxylation activity of CYP3A.	J Lipid Res	52	1509-1516	2011
Toda K, Hayashi Y, Ono M, Saibara T.	Impact of ovarian sex steroids on ovulation and ovulatory gene induction in aromatase- null mice.	Endocrinology	153	386-394	2012
Yoneda M, Fujii H, Sumida Y, Hyogo H, Itoh Y, Ono M, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Imajo K, Chayama K, Saibara T, Kawada N, Fujimoto K,Kohgo Y, Yoshikawa T, Okanoue T.	Platelet count for predicting fibrosis in nonalcoholic fatty liver disease.	J Gastroenterol	46	1300-1306	2011
Ono M, Ochi T, Munekage K, Ogasawara M, HiroseA, Nozaki Y, Takahashi M, Okamoto N, Saibara T.	Angiotensinogen gene haplotype is associated with the prevalence of Japanese non-alcoholic steatohepatitis.	Hepatol Res	41	1223-1229	2011
Taniuchi K, Cemy RL, Tanouchi A, Kohno K, Kotani N, Saibara T, Hollingsworth MA.	Overexpression of GalNAc-transferase GalNAc-T3 promotes pancreatic cancer cell growth.	Oncogene	30	4843-4854	2011
Taniuchi K, Iwasaki S, Saibara T.	BART inhibits pancreatic cancer cell invasion by inhibiting ARL2-mediated RhoA inactivation.	Int J Oncol	39	1243-1252	2011

Koga M, Saito H, Mukai M, Saibara T, Kasayama S.	Serum dehydroepiandrosterone sulphate levels in patients with non-alcoholic fatty liver disease.	Internal Med	50	1657-1661	2011
Yasui K, Hashimoto E, Komorizono Y, Koike K, Arii S, Imai Y, ShimaT, Kanbara Y, SaibaraT, Mori T, KawataS, Uto H, Takami S,Sumida Y, Takamura T, Kawanaka M, OkanoueT.	Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma.	Clin Gastroenterol Hepatol	9	428-433	2011
Ogasawara M, Hirose A, Ono M, Aritake K, Nozaki Y, Takahashi M, Okamoto N, SakamotoS, Iwasaki S, Asanuma T, Taniguchi T, Urade Y, Onishi S, Saibara T, Oben JA.	A novel and comprehensive mouse model of human non-alcoholic steatohepatitis with the full range of dysmetabolic and histological abnormalities induced by gold thioglucose and a high-fat diet.	Liver Int	31	542-551	2011
Sumida Y, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, Eguchi Y, Suzuki Y, Imai S, Kanemasa K, Fujita K, Chayama K, Yasui K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Okanoue T.	A simple clinical scoring system using ferritin, fasting insulin, and type IVcollagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease.	J Gastroenterol	46	257-268	2011
宗景玄祐，小野正文，西原利治	NASH に関係するリポ蛋白遺伝子	肝胆膵	62	977-984	2011
小野正文，宗景玄祐，越智経浩，西原利治	全身疾患としての非アルコール性脂肪性肝疾患　疾患概念の変遷と国内外の疫学	診断と治療	99	1461-1467	2011
小野正文，岡本宣人，西原利治	今日の NAFLD・NASH 治療法	Pharma Medica	29	47-52	2011
小野正文，麻植啓輔，西原利治	インクレチンの肝細胞への脂肪蓄積抑制作用－NASH 治療薬創薬への期待－	日本臨牀	69	853-858	2011
増田弘誠，小野正文，西原利治	身体合併症	精神科	18	629-633	2011

岩﨑信二，高橋昌也，西原利治	薬物治療法：UDCA とベザフィブレートを中心に	肝胆膵	62	729-736	2011
増田弘誠，小野正文，西原利治	脂肪肝・非アルコール性脂肪肝炎（NASH）に対する栄養療法	肝胆膵	63	445-451	2011
Ninomiya M, Ueno Y, Shimosegawa T.	PBC: animal models of cholangiopathies and possible endogenous viral infections.	Int J Hepatol	2012	ID649290	2012
Ninomiya M, Ueno Y, Funayama R, Nagashima T, Nishida Y, Kondo Y, Inoue J, Kakazu E, KimuraO, Nakayama K, Shimosegawa T.	Use of Illumina deep sequencing technology to differentiate hepatitis C virus variants.	J Clin Microbiol	2012	ID649290	2012
Yang F, Priester S, Onori P, Venter J, Renzi A, Franchitto A, Munshi MK, Wise C, Dostal DE, Marzioni M, Saccomanno S, Ueno Y, Gaudio E, Glaser S.	Castration inhibits biliary proliferation induced by bile duct obstruction: novel role for the autocrine trophic effect of testosterone.	Am J Physiol Gastrointest Liver Physiol	301	981-991	2011
Renzi A, Glaser S, Demorrow S, Mancinelli R, Meng F, FranchittoA, Venter J, White M, Francis H, Han Y, Alvaro D, Gaudio E, Carpino G, Ueno Y, Onori P, Alpini G.	Melatonin inhibits cholangiocyte hyperplasia in cholestatic rats by interaction with MT1 but not MT2 melatonin receptors.	Am J Physiol Gastrointest Liver Physiol	301	634-643	2011
Alpini G, Franchitto A, Demorrow S, Onori P, Gaudio E, Wise C, Francis H, Venter J, Kopriva S, Mancinelli R, Carpino G, Stagnitti F, Ueno Y, Han Y, Meng F, Glaser S.	Activation of alpha(1) -adrenergic receptors stimulate the growth of small mouse cholangiocytes via calcium-dependent activation of nuclear factor of activated T cells 2 and specificity protein 1.	Hepatology	53	628-639	2011
二宮匡史，福島耕二，上野義之，下瀬川徹	PBC とその類縁疾患，オーバーラップス 基礎 ウイルス感染と PBC	肝胆膵	62	665-669	2011
Kuroda, H, TakikawaY, Onodera M, Kakisaka K, Yoshida Y, Kataoka K, Sawara K, Miyamoto Y, Oikawa K, Endo R,	Serial changes of liver stiffness measured by acoustic radiation force impulse imaging in acute liver failure: a case report.	J Clin Ultrasound	40 (2)	99-104	2012

Suzuki K.					
Kuroda H,Takikawa Y, Suzuki K.	Non-invasive markers of liver fibrosis.	Eur Gastroenterol Hepatol	7 (4)	264-267	2011
Naiki T,Nakayama N, Mochida S,Oketani M, Takikawa Y,Suzuki K, Tada S,Ichida T, Moriwaki H,Tsubuchi H.	Novel scoring system as a useful model to predict the outcome of patients with acute liver failure: Application to indication criteria for liver transplantation.	Hepatol Res	42 (1)	68-75	2012
Shimizu M, Sakai H, Shirakami Y, Iwasa J, Yasuda Y, Kubota M, Takai K, Tsurumi H, Tanaka T, Moriwaki H.	Acyclic retinoid inhibits diethylnitrosamine- induced liver tumorigenesis in obese and diabetic C57BLKS/J- +(db)/+Lepr(db) mice.	Cancer Prev Res	4	128-136	2011
Shimizu M, Sakai H, Shirakami Y, Yasuda Y, Kubota M, Terakura D, Baba A, Ohno T,Hara Y, Tanaka T, Moriwaki H.	Preventive effects of (-)-epigallocatechin gallate on diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BL/ KsJ-db/db Mice.	Cancer Prev Res	4	396-403	2011
Shimizu M, Yasuda Y, Sakai H, Kubota M, Terakura D, Baba A, Ohno T, Kochi T, Tsurumi H, Tanaka T, Moriwaki H.	Pitavastatin suppresses diethylnitrosamine- induced liver preneoplasms in male C57BL/ KsJ-db/db obese mice.	BMC Cancer	11	281	2011
Shimizu M, Adachi S, Masuda M, Kozawa O, Moriwaki H.	Cancer chemoprevention with green tea catechins by targeting receptor tyrosine kinases.	Mol Nutr Food Res	55	832-843	2011
Shimizu M, Sakai H, Moriwaki H.	Chemoprevention of hepatocellular carcinoma by acyclic retinoid.	Front Biosci	16	759-769	2011
Osawa Y, Kanamori H, Seki E, Hoshi M, Ohtani H, Yasuda Y, Ito H, Suetsugu A, Nagaki M, Moriwaki H, Saito K, Seishima M.	L-tryptophan-mediated enhancement of susceptibility to nonalcoholic fatty liver disease is dependent on the mammalian target of rapamycin.	J Biol Chem	286	34800-34808	2011
Osawa Y, Seki E, Kodama Y, Suetsugu A, Miura K, Adachi M, Ito H, Shiratori Y, Banno Y, Olefsky JM, Nagaki M, Moriwaki H, Brenner DA, Seishima M.	Acid sphingomyelinase regulates glucoseand lipid metabolism in hepatocytes throughAKT activation and AMP-activated proteinkinase suppression.	FASEB J	25	1133-1144	2011