partly explain the high frequency of a chronic disease course in HCV infection, particularly if they can be confirmed to be transient in acute self-limiting infection, but persistent in the development of chronic disease.

As Ward et al. [50] so aptly pointed out, "too many studies have focused on too few epitopes" in the analysis of immune responses to HCV. A recent investigation identified 19 different CTL epitopes in 22 subjects, and only one epitope was recognized by T cell clones from more than one patient [51]. In another study, CTL responses to 8 different epitopes were detected in a patient who cleared the virus, yet only one of these epitopes had previously been mapped [8]. Together, these observations indicate that too little is currently known about the breadth and specificity of the immune response to HCV that is required for elimination of the virus. In particular, the role that epitope selection plays in determining the outcome of HCV infection needs to be addressed further, since it is likely to influence the efficiency and breadth of the response, but also the frequency of escape mutations, which have been shown to greatly influence the outcome of HCV infection [52,53].

In addition, most studies have focused on the $CD8^+$ T cells in peripheral blood, but that compartment may not be representative of the frequencies and functions of virus-specific $CD8^+$ T cells in the liver. In acutely HCV-infected chimpanzees, resolution of infection was associated with a broad and multispecific hepatic CTL response, whereas the CTL response of animals developing chronic infection was absent or weak and narrowly focused [54]. The human hepatic $CD8^+$ T cells response has only been investigated in chronically infected patients. Several reports document that virus-specific CTL could be expanded without viral antigenic stimulation from liver, but not from PBMC, which suggested that they were considerably more frequent in the liver than in the peripheral compartment [43,51,55]. Direct analyses with MHC-tetrameric complexes confirmed that the frequency of virus-specific $CD8^+$ T cells is one or two orders of magnitude higher in the liver than in PBMC of the same patient with chronic HCV infection [40,56]. In addition, almost all intrahepatic virus-specific, but only few peripheral, $CD8^+$ T cells expressed the activation marker CD69 [40,56]. Hepatic CTL activity appears to be required for a sustained response to IFN-α treatment, since it was reported that 7 or 9 patients with a detectable hepatic HCV-specific CTL response before treatment had a complete response to treatment, but only 1 of 10 patients without such a response did [57].

3.2. HBV

Adoptive transfer experiment in an HBV transgenic mouse model has demonstrate the role of virus-specific $CD8^+$ T cells in the elimination of HBV [58]. Experiments in chimpanzees provided further important insights for the role of $CD8^+$ T cell response in HBV infection. Depletion of $CD8^+$ T cells in a chimpanzee 6 weeks after inoculation with HBV prolonged the infection until the number of $CD8^+$ T cells had returned to baseline levels, demonstrating a central role for $CD8^+$ T cells in viral elimination [59].

In humans, tetramer binding analysis in 3 HLA-A2-positive patients seen during the incubation phase revealed that HBV-specific $CD8^+$ T cells were detectable in the circulation before the onset of symptoms and their frequency increased even after a pronounced reduction in serum HBVDNA levels had occurred, then decreased during recovery, but remained detectable even after viral clearance [20]. Other ex vivo analyses using MHC-peptide tetrameric complexes confirmed not only the high frequency of virus-specific $CD8^+$ T cell in patients with acute HBV infection, but also the decline in their numbers during the recovery phase [60,61]. Despite this decline, it has been found that subjects recovered from HBV infection could mount a strong and multispecific CTL response long after recovery [37]. In contrast to the resting memory phenotype of HCV-specific cells analyzed long after resolution, the responding HBV-specific $CD8^+$ T cells expressed the activation markers HLA-DR and CD69, suggesting that they were activated by trace amounts of persisting virus, as had been detected in other studies [31,36].

In contrast to acute HBV infection, $tetramer^+$ cells at levels exceeding those of normal controls could generally not be detected in PBMC from patients with chronic HBV infection in at least one study [61], although others reported that they were detectable in chronic HBV patients with low HBV DNA levels, but not in those with high HBV DNA levels [62,63]. Irrespective of detectable tetramer binding, virus-

specific $CD8^+$ T cells could be expanded via in vitro peptide stimulation only in those patients with low viral load, but not in those with high viral load [61,62].

These findings essentially agree with earlier results indicating that HBV-infected patients with an acute self-limiting disease course showed strong polyclonal and multispecific peripheral $CD8^+$ T cytotoxic responses cells against HBsAg and HBcAg [36,64,65]. In contrast, and somewhat different from what is observed in chronic HCV infection, virus-specific CTL responses were found to be absent in most patients with chronic HBV infection and weak in those in whom they could be detected [36,65]. Similarly, intracellular IFN-γ and TNF-α staining of T cells incubated with recombinant HBcAg revealed significantly more frequent and more vigorous $CD8^+$ T cell responses in patients who had cleared the virus compared to chronically infected patients [26].

A direct comparison of 5 patients with acute HBV infection and 7 patients with acute HCV infection indicated that both groups of patients had similarly high levels of tetramer$^+$$CD8^+$ T cells specific for HBV and HCV, respectively, and that these cells shared a $CCR7^-CD45RA^-$ effector memory phenotype [48]. Others also reported that both HBV- and HCV-specific $CD8^+$ T cells from acutely infected patients were predominantly of a $CD28^+CD45RA^-$ memory phenotype [39,61]. Despite these similarities, virus-specific $CD8^+$ T cells differed functionally between the two patient groups [48]. Whereas HBV-specific CD8 T cells could be easily expanded via specific peptide stimulation, exhibited strong cytotoxic activity and IFN-γ secretion and contained high levels of intracellular perforin, all of these functions were impaired in HCV-specific CD8 T cells. All of the HBV patients went on to clear the virus, but only 3 of the HCV patients cleared virus and only after recovery of CD8 T cell functions. There is, however, a report of a similar transient impairment in the expansion capacity and cytolytic activity in HBV-specific $CD8^+$ T cells and restoration of these functions during recovery [60].

Similar to observations in chronic HCV infection, the frequency of HBV-specific $CD8^+$ T cells was found to be significantly higher in the liver than in peripheral blood and easily detectable even in patients in whom no tetramer binding was observed in PBMC [62,63]. Interestingly, the proportion of virus-specific $CD8^+$ T cells among total liver-infiltrating $CD8^+$ T lymphocytes was significantly higher in patients with low serum ALT and HBV DNA levels than in patients with high levels of serum ALT and HBV DNA [62]. Yet, their absolute numbers were similar because the frequency of liver-infiltrating $CD8^+$ T cells overall was also much greater in liver specimens of viremic patients compared to patients with low levels of HBV DNA. Of note, only $CD8^+$ T cells specific for HBcAg18-27 were analyzed. Therefore, it cannot be ruled out that the frequencies of $CD8^+$ T cells specific for other viral antigens differed substantially between the two patient groups and that differential responses to other epitopes could explain the different levels of viral control.

Studies in the HBV transgenic mouse model also demonstrated that virus-specific CTL was capable of eliminating viral DNA from the cytoplasm of hepatocytes via a mechanism that was at least partly mediated by non-cytopathic antiviral activities of IFN-γ and TNF-α [58]. There are indications that such non-cytolytic antiviral effector mechanisms also play a role in viral elimination in HBV-infected chimpanzees [21,59].

4. The role of $CD8^+$ T cells in liver damage

It is believed that HBV- and HCV-specific $CD8^+$ T cells function as a double-edged sword. They play a critical role in the control and clearance of the viruses; on the other hand, when overall antiviral immunity is not vigorous enough to clear the viruses, they may also cause sustained liver tissue damage through different pathways including perforin-mediated cytotoxicity and Fas ligand/Fas-mediated apoptosis.

Immunohistochemical studies show that $CD8^+$ T cells in liver biopsy specimens from patients with chronic HCV infection can frequently be detected in lobules and particularly in areas of interface hepatitis ("piecemeal necrosis") [66–69]. A linear correlation has been reported between lobular CD8 expression and the histological activity index in liver biopsies from patients with chronic hepatitis C [66], and a similar correlation of CD8β mRNA expression with serum ALT levels and the histological activity index was reported by others [68]. Even though the

frequency of HCV-specific $CD8^+$ T cells is much higher in liver than in peripheral blood of patients with chronic hepatitis C, a single specificity generally constitutes only between ~0.1% and 1% of liver-infiltrating $CD8^+$ lymphocytes, although up to 4% have been reported in some patients [40,56]. This suggests that the majority of intrahepatic $CD8^+$ T cells is not virus-specific. Based on these findings and the observation that the frequency of peripheral virus-specific $CD8^+$ T cells identified by IFN-γ ELISPOT assay was not associated with liver damage [8], it has been proposed that virus-specific intrahepatic $CD8^+$ T cells initiate liver damage, but that this process is amplified by the non-virus-specific $CD8^+$ T lymphocytes making up the bulk of the liver infiltrate [68]. Of note, even though the frequencies detected in the IFN-γ ELISPOT assay did not correlate with serum ALT levels, the frequencies obtained with tetramer binding did [8]. This observation is in agreement with the identification of two different populations of virus-specific $CD8^+$ T cells, of which the $CD38^+IFN\text{-}\gamma^-$ population was temporally associated with serum ALT activity, whereas the $CD38^-IFN\text{-}\gamma^+CD8^+$ T cells emerged after serum ALT levels had declined, but correlated with viral clearance [7].

Several lines of evidence support the hypothesis that the recognition and subsequent destruction of HBV-infected hepatocytes by viral antigen-specific HLA class I-restricted cytotoxic T cells is the cause of liver damage. In HBV-infected chimpanzees, a marked increase in intrahepatic CD3 and CD8 mRNA coincided with peak serum ALT levels [21]. This study also showed that viral clearance preceded the rise in hepatic T cell mRNA and serum ALT, demonstrating that destruction of infected hepatocytes is not essential for the elimination of HBV. In another acutely HBV-infected chimpanzee depleted of $CD8^+$ T cells, serum ALT levels closely paralleled the reappearance of $CD8^+$ T cells [59]. Unfortunately, these studies did not examine the role of virus-specific T cells, and human studies of immune responses in acute human HBV infection are confined to the peripheral compartment. During the incubation and acute infection phases, the highest number of circulating core18-27-specific $CD8^+$ cells was observed at the same time as maximal ALT levels in one patient, but following peak ALT activity in another [27]. Elevations in ALT levels were also found to coincide with expansion of peripheral core18-27-specific $CD8^+$ T cells in chronically HBV-infected patients [63].

Although these results suggest that virus-specific $CD8^+$ T cells mediate liver damage, they also raise the question of how representative the peripheral compartment is of the intrahepatic immune response. Whereas core18-27-specific CD8 T cells were found with a frequency of <0.1% in blood, they constituted up to 9% of the total intrahepatic CD8 population in patients with serum ALT levels of <35 UL, whereas intrahepatic frequencies in patients with higher serum ALT activity were markedly lower (0% to 0.24%) [62]. Moreover, the absolute number of these cells among the liver infiltrate was the same in both patient groups because of significant dilution of virus-specific T cells in the total infiltrate of patients with inflammatory activity in the liver. Since only a single epitope was investigated, it cannot be ruled out that patients with hepatic immunopathology had higher frequencies of $CD8^+$ T cell responses with other specificities. Nonetheless, it could be speculated that the non-virus-specific infiltrating $CD8^+$ T lymphocytes play a major role in liver damage.

5. NKT cells

NKT cells constitute a lymphoid lineage that is distinct from classical T, B or natural killer (NK) cells, but expresses cell surface markers of both T cells and NK cells [70]. NKT cells recognize cell wall glycolipid antigens presented by the non-polymorphic, non-classical MHC class I-like CD1 molecules. Human CD1d-restricted NKT cells are characterized by the expression of an invariant Vα24JαQ TCR and stimulated human and murine NKT cells secrete high amounts of both IFN-γ and interleukin (IL)-4. NKT cells have been implicated in other inflammatory liver diseases, such as primary biliary cirrhosis (PBC) [71,72] and hepatic graft-versus-host disease [73], and are part of the infiltrate in chronically HCV-infected liver [74]. Like NK cells, NKT cells are believed to play an important role in killing HBV- or HCV-infected cells. In agreement with such a role, Vα24Vβ11 double-positive NKT cells in the liver, but not in the blood, of HCV patients were found to express CD69 and CD45RO, indicating their recent activation [75]. Interestingly, their frequency was

lower in the blood of HCV-positive patients than in controls. A recent study reported significant enrichment of Vα24$^+$ T cells in liver compared to peripheral blood of 12 patients with chronic HCV infection, but in most cases, only between 2% and 5% of intrahepatic CD3$^+$ cells were also positive fore Vα24 [76]. However, in an analysis of mRNA expression of a panel of phenotypic markers of intrahepatic lymphocyte subsets, transcripts for Vα24αJQ were detectable in only 20 of 48 patients with chronic hepatitis C [68].

In a transgenic mouse model of HBV infection, activation of NKT was shown to result in clearance of HBV through largely non-cytolytic IFN-γ-dependent mechanisms [77,78]. Note that, compared to human liver, mouse liver contains significantly higher proportions of NKT cells (~4% vs. ~30%). Thus, the relevance of these results to human patients with viral hepatitis remains to be established.

6. Antigen presenting cells (APC)

APC are critical for the induction and development of T cell immunity. Therefore understanding the function of APC may hold the key for understanding the T cell immunity in viral hepatitis, especially why this immunity appears to be suboptimal in clearing HBV and HCV during chronic infections of these viruses (Table 2).

Dendritic cells (DC) are the most potent APC, and their antigen uptake and presentation, along with the costimulatory signals they provide, are crucial for the initiation of cell-mediated immune responses to microbial antigens. Antigen uptake by immature DC triggers maturation, including upregulation of MHC, costimulatory and adhesion molecule expression, and this results in efficient antigen presentation to T cells. Mature DC also secrete high levels of IL-12, which in turn promotes CTL maturation.

Table 2
Potential defects in cellular immunity in chronic HCV infection

Immune cell subsets	Abnormality	Cause
CD8$^+$ T cells	Proliferation, cytokine production, cytotoxicity	?
NK cells	Proliferation, cytokine production, cytotoxicity	Interaction between HCV E2 and CD81?
DC	Allostimulatory, maturity	Interaction between HCV E1/E2 and DC-SIGN/DC-SIGNR?

DC are constituents of the inflammatory infiltrate in the liver from patients with chronic hepatitis C and have been described as forming ordered arrays in which mostly CD8$^+$ lymphocytes and a few plasma cells are embedded [67]. These networks were in contact with hepatocytes at the periphery of lobules, but did not extend to the basement membrane of bile ducts. This localization suggest that DC, by stimulating CD8$^+$ T cells, could play a role in the destruction of hepatocytes in chronic hepatitis C.

Most research, however, has focused on possible defects in DC function and their contribution to viral persistence and the development of a chronic disease course during HCV infection. Several groups of researchers reported that monocyte-derived DC from patients with chronic HCV infection were impaired in their allostimulatory capacity compared to healthy controls or individuals who had cleared the virus [79–82]. A qualitatively similar, but somewhat lesser, impairment was found in DC generated in the presence of HCV core or NS3, but not E2 [82]. Supernatants from mixed lymphocyte reactions contained significantly less IL-12 in the presence of DC from patients with chronic HCV infection compared to control DC, but this was not observed with HCV-antigen-treated DC, suggesting that the presence of these viral antigens does not fully reproduce the functional abnormalities of DC from chronically HCV-infected subjects [82]..

Differentiation of monocytes into immature DC as assessed by surface marker expression was generally similar between HCV patients and controls [79–81]. Antigen uptake and presentation by immature DC also did not differ significantly [79–81]. In contrast, there was a marked reduction in the proportion of HCV DC positive for mature DC markers compared to healthy controls [80]. Furthermore, the decrease in antigen uptake that normally accompanies DC maturation was not observed in DC from chronic HCV patients [80]. These results suggest that the reduced allostimulatory capacity of DC in patients with chronic HCV infection is attributable to impaired DC maturation. This is, however, not a consistent finding [83], and DC maturation and functions in chronically HCV-infected

chimpanzees were also found to be normal [84]. Methodological differences in the protocols used for the generation and maturation of DC may largely account for these discrepancies.

As in chronic HCV infection, phenotypically normal immature DC could be generated from patients with chronic HBV infection, although in this case they were derived from $CD34^+$ (stem) cells incubated with GM-CSF and IL-6IL-6R fusion protein [85]. T cell proliferation in the presence of autologous DC pulsed with HBeAg or HBsAg was observed in significantly fewer chronically infected patients than in subjects with resolved HCV infection or healthy controls. The secretion of IFN-γ was also significantly reduced, whereas that of IL-10 was unaffected. Proliferation and cytokine production in response to the recall antigen, tetanus toxoid, did not differ significantly between the groups, suggesting that the defect was HCV antigen-specific. Although the study design makes it difficult to determine whether the defect is localized to T cells or DC, one possible interpretation of these results is that impaired priming by DC contributes to the decreased virus-specific T cell responses consistently observed in chronic HBV infection.

7. Autoimmunity in HBV and HCV infection

Both chronic hepatitis B and C have been associated with autoimmune phenomena. Autoantibodies, such as antibodies to nuclei (ANA), smooth muscle (SMA), gastric parietal cells (GPC), etc., have been detected in patients with chronic HCV or HCV infection, with prevalence varied widely in different studies. Based on the sequence homology and antibody cross-reactivity, molecular mimicry between viral antigens and self proteins is thought to play a role in the viral hepatitis-related autoimmunity. However, the mechanism underlying the autoimmune phenomena in chronic viral hepatitis is poorly understood. Little is known regarding the involvement of T cell immunity in the induction of autoreactive antibody response in HBV and HCV infection. Considering the critical role of T cell immunity in both infectious diseases and autoimmune diseases, a thorough understanding of T cell immune responses during HBV and HCV infection is likely to provide important insight for elucidating the potential link between autoimmune diseases and viral infection as well as vaccination.

8. Conclusion

The nature of T cell immunity against hepatitis B virus (HBV) and hepatitis C virus (HCV) under different clinical situations is beginning to be appreciated as the result of the development and application of novel assays over the last decade to study T cell immunity, which has important impact on the studies on T cell immunity in autoimmune liver diseases as well. However, many key steps in the life cycle of virus-specific T cells are still poorly understood. Active research is underway to elucidate the function of antigen presenting cells for HBV and HCV antigens, priming of naïve T cells, clonal expansion and differentiation of antigen-specific T cells to effector cells and memory cells, migration of the immune T cells to the liver, and the number and characteristics of effector functions of virus-specific T cells in the liver at different disease stages, especially the early phase of infection. Understanding of the overall T cell response requires a comprehensive approach using multiple laboratory techniques. This strategy has the opportunity to elucidate the role of T cell responses in the clearance or persistence of virus and development of liver disease, which will greatly enhance the development of immunotherapy and vaccines for chronic viral hepatitis, as well as the understanding of T cell immunity in autoimmune liver diseases.

References

[1] Lai CL, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. Lancet 2003;362:2089.
[2] European Association for the Study of the Liver. Consensus statement. EASL International Consensus Conference on Hepatitis C. Paris, 26–28, February 1999. J Hepatol, vol. 30, p. 956.
[3] Cramp ME, Carucci P, Underhill J, Naoumov NV, Williams R, Donaldson PT. Association between HLA class II genotype and spontaneous clearance of hepatitis C viraemia. J Hepatol 1998;29:207.
[4] Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C

European Network for Cooperative Research. Lancet 1999; 354:2119.
[5] Mangia A, Gentile R, Cascavilla I, Margaglione M, Villani MR, Stella F, et al. HLA class II favors clearance of HCV infection and progression of the chronic liver damage. J Hepatol 1999; 30:984.
[6] Harcourt G, Hellier S, Bunce M, Satsangi J, Collier J, Chapman R, et al. Effect of HLA class II genotype on T helper lymphocyte responses and viral control in hepatitis C virus infection. J Viral Hepatitis 2001;8:174.
[7] Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 2001;194:1395.
[8] Lechner F, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000; 191:1499.
[9] Wedemeyer H, He XS, Nascimbeni M, Davis AR, Greenberg HB, Hoofnagle JH, et al. Impaired effector function of hepatitis C virus-specific $CD8^+$ T cells in chronic hepatitis C virus infection. J Immunol 2002;169:3447.
[10] Ulsenheimer A, Gerlach JT, Gruener NH, Jung MC, Schirren CA, Schraut W, et al. Detection of functionally altered hepatitis C virus-specific CD4 T^+ cells in acute and chronic hepatitis C. Hepatology 2003;37:1189.
[11] Diepolder HM, Zachoval R, Hoffmann RM, Wierenga EA, Santantonio T, Jung MC, et al. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. Lancet 1995; 346:1006.
[12] Missale G, Bertoni R, Lamonaca V, Valli A, Massari M, Mori C, et al. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. J Clin Invest 1996;98:706.
[13] Gerlach JT, Diepolder HM, Jung MC, Gruener NH, Schraut WW, Zachoval R, et al. Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. Gastroenterology 1999;117:933.
[14] Lamonaca V, Missale G, Urbani S, Pilli M, Boni C, Mori C, et al. Conserved hepatitis C virus sequences are highly immunogenic for CD4(+) T cells: implications for vaccine development. Hepatology 1999;30:1088.
[15] Chang KM, Thimme R, Melpolder JJ, Oldach D, Pemberton J, Moorhead-Loudis J, et al. Differential $CD4^+$ and $CD8^+$ T-cell responsiveness in hepatitis C virus infection. Hepatology 2001;33:267.
[16] Tsai SL, Liaw YF, Chen MH, Huang CY, Kuo GC. Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. Hepatology 1997; 25:449.
[17] Cramp ME, Carucci P, Rossol S, Chokshi S, Maertens G, Williams R, et al. Hepatitis C virus (HCV) specific immune responses in anti-HCV positive patients without hepatitis C viraemia. Gut 1999;44:424.
[18] Grakoui A, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghrayeb J, et al. HCV persistence and immune evasion in the absence of memory T cell help. Science 2003;302:659.
[19] Thimme R, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. Proc Natl Acad Sci U S A 2002;99:15661.
[20] Webster GJ, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, et al. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. Hepatology 2000; 32:1117.
[21] Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999;284:825.
[22] Thursz MR, Kwiatkowski D, Allsopp CE, Greenwood BM, Thomas HC, Hill AV. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. N Engl J Med 1995;332:1065.
[23] Höhler T, Gerken G, Notghi A, Lubjuhn R, Taheri H, Protzer U, et al. HLA-DRB1*1301 and *1302 protect against chronic hepatitis B. J Hepatol 1997;26:503.
[24] Diepolder HM, Jung MC, Keller E, Schraut W, Gerlach JT, Grüner N, et al. A vigorous virus-specific $CD4^+$ T cell response may contribute to the association of HLA-DR13 with viral clearance in hepatitis B. Clin Exp Immunol 1998; 113:244.
[25] Franco A, Guidotti LG, Hobbs MV, Pasquetto V, Chisari FV. Pathogenetic effector function of CD4-positive T helper 1 cells in hepatitis B virus transgenic mice. J Immunol 1997;159: 2001.
[26] Matsumura S, Yamamoto K, Shimada N, Okano N, Okamoto R, Suzuki T, et al. High frequency of circulating HBcAg-specific CD8 T cells in hepatitis B infection: a flow cytometric analysis. Clin Exp Immunol 2001;124:435.
[27] Webster G, Bertoletti A. Quantity and quality of virus-specific CD8 cell response: relevance to the design of a therapeutic vaccine for chronic HBV infection. Mol Immunol 2001;38: 467.
[28] Ferrari C, Penna A, Bertoletti A, Valli A, Antoni AD, Giuberti T, et al. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. J Immunol 1990;145:3442.
[29] Jung MC, Diepolder HM, Spengler U, Wierenga EA, Zachoval R, Hoffmann RM, et al. Activation of a heterogeneous hepatitis B (HB) core and e antigen-specific $CD4^+$ T-cell population during seroconversion to anti-HBe and anti-HBs in hepatitis B virus infection. J Virol 1995;69: 3358.
[30] Löhr HF, Weber W, Schlaak J, Goergen B, Meyer zum Büschenfelde KH, Gerken G. Proliferative response of CD4+ T cells and hepatitis B virus clearance in chronic hepatitis with or without hepatitis B e-minus hepatitis B virus mutants. Hepatology 1995;22:61.
[31] Penna A, Artini M, Cavalli A, Levrero M, Bertoletti A, Pilli M, et al. Long-lasting memory T cell responses following self-limited acute hepatitis B. J Clin Invest 1996;98:1185.
[32] Löhr HF, Krug S, Herr W, Weyer S, Schlaak J, Wolfel T, et al. Quantitative and functional analysis of core-specific T-helper cell and CTL activities in acute and chronic hepatitis B. Liver 1998;18:405.

[33] Sugimoto K, Stadanlick J, Ikeda F, Brensinger C, Furth EE, Alter HJ, et al. Influence of ethnicity in the outcome of hepatitis C virus infection and cellular immune response. Hepatology 2003;37:590.
[34] Rico MA, Quiroga JA, Subirá D, Castañón S, Esteban JM, Pardo M, et al. Hepatitis B virus-specific T-cell proliferation and cytokine secretion in chronic hepatitis B e antibody-positive patients treated with ribavirin and interferon alfa. Hepatology 2001;33:295.
[35] Tsai SL, Chen PJ, Lai MY, Yang PM, Sung JL, Huang JH, et al. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. J Clin Invest 1992;89:87.
[36] Rehermann B, Fowler P, Sidney J, Person J, Redeker A, Brown M, et al. The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. J Exp Med 1995;181:1047.
[37] Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med 1996;2:1104.
[38] Grüner NH, Gerlach TJ, Jung MC, Diepolder HM, Schirren CA, Schraut WW, et al. Association of hepatitis C virus-specific CD8$^+$ T cells with viral clearance in acute hepatitis C. J Infect Dis 2000;181:1528.
[39] Sobao Y, Tomiyama H, Nakamura S, Sekihara H, Tanaka K, Takiguchi M. Visual demonstration of hepatitis C virus-specific memory CD8$^+$ T-cell expansion in patients with acute hepatitis C. Hepatology 2001;33:287.
[40] He XS, Rehermann B, López-Labrador FX, Boisvert J, Cheung R, Mumm J, et al. Quantitative analysis of hepatitis C virus-specific CD8$^+$ T cells in peripheral blood and liver using peptide-MHC tetramers. Proc Natl Acad Sci U S A 1999;96:5692.
[41] Rehermann B, Chang KM, McHutchison JG, Kokka R, Houghton M, Chisari FV. Quantitative analysis of the peripheral blood cytotoxic T lymphocyte response in patients with chronic hepatitis C virus infection. J Clin Invest 1996;98:1432.
[42] Cerny A, McHutchison JG, Pasquinelli C, Brown ME, Brothers MA, Grabscheid B, et al. Cytotoxic T lymphocyte response to hepatitis C virus-derived peptides containing the HLA A2.1 binding motif. J Clin Invest 1995;95:521.
[43] Koziel MJ, Dudley D, Afdhal N, Grakoui A, Rice CM, Choo QL, et al. HLA class I-restricted cytotoxic T lymphocytes specific for hepatitis C virus. Identification of multiple epitopes and characterization of patterns of cytokine release. J Clin Invest 1995;96:2311.
[44] Hiroishi K, Kita H, Kojima M, Okamoto H, Moriyama T, Kaneko T, et al. Cytotoxic T lymphocyte response and viral load in hepatitis C virus infection. Hepatology 1997; 25:705.
[45] Shoukry NH, Grakoui A, Houghton M, Chien DY, Ghrayeb J, Reimann KA, et al. Memory CD8$^+$ T cells are required for protection from persistent hepatitis C virus infection. J Exp Med 2003;197:1645.
[46] Lechner F, Gruener NH, Urbani S, Uggeri J, Santantonio T, Kammer AR, et al. CD8$^+$ T lymphocyte responses are induced during acute hepatitis C virus infection but are not sustained. Eur J Immunol 2000;30:2479.
[47] Nakamoto Y, Kaneko S, Takizawa H, Kikumoto Y, Takano M, Himeda Y, et al. Analysis of the CD8-positive T cell response in Japanese patients with chronic hepatitis C using HLA-A*2402 peptide tetramers. J Med Virol 2003;70:51.
[48] Urbani S, Boni C, Missale G, Elia G, Cavallo C, Massari M, et al. Virus-specific CD8$^+$ lymphocytes share the same effector-memory phenotype but exhibit functional differences in acute hepatitis B and C. J Virol 2002;76:12423.
[49] Gruener NH, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, et al. Sustained dysfunction of antiviral CD8$^+$ T lymphocytes after infection with hepatitis C virus. J Virol 2001;75:5550.
[50] Ward S, Lauer G, Isba R, Walker B, Klenerman P. Cellular immune responses against hepatitis C virus: the evidence base 2002. Clin Exp Immunol 2002;128:195.
[51] Wong DK, Dudley DD, Afdhal NH, Dienstag J, Rice CM, Wang L, et al. Liver-derived CTL in hepatitis C virus infection: breadth and specificity of responses in a cohort of persons with chronic infection. J Immunol 1998;160:1479.
[52] Chang KM, Rehermann B, McHutchison JG, Pasquinelli C, Southwood S, Sette A, et al. Immunological significance of cytotoxic T lymphocyte epitope variants in patients chronically infected by the hepatitis C virus. J Clin Invest 1997; 100:2376.
[53] Erickson AL, Kimura Y, Igarashi S, Eichelberger J, Houghton M, Sidney J, et al. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. Immunity 2001;15:883.
[54] Cooper S, Erickson AL, Adams EJ, Kansopon J, Weiner AJ, Chien DY, et al. Analysis of a successful immune response against hepatitis C virus. Immunity 1999;10:439.
[55] Nelson DR, Marousis CG, Davis GL, Rice CM, Wong J, Houghton M, et al. The role of hepatitis C virus-specific cytotoxic T lymphocytes in chronic hepatitis C. J Immunol 1997;158:1473.
[56] Grabowska AM, Lechner F, Klenerman P, Tighe PJ, Ryder S, Ball JK, et al. Direct ex vivo comparison of the breadth and specificity of the T cells in the liver and peripheral blood of patients with chronic HCV infection. Eur J Immunol 2001; 31:2388.
[57] Nelson DR, Marousis CG, Ohno T, Davis GL, Lau JY. Intrahepatic hepatitis C virus-specific cytotoxic T lymphocyte activity and response to interferon alfa therapy in chronic hepatitis C. Hepatology 1998;28:225.
[58] Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity 1996;4:25.
[59] Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, et al. CD8$^+$ T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol 2003;77:68.
[60] Maini MK, Boni C, Ogg GS, King AS, Reignat S, Lee CK, et al. Direct ex vivo analysis of hepatitis B virus-specific

CD8(+) T cells associated with the control of infection. Gastroenterology 1999;117:1386.
[61] Sobao Y, Tomiyama H, Sugi K, Tokunaga M, Ueno T, Saito S, et al. The role of hepatitis B virus-specific memory CD8 T cells in the control of viral replication. J Hepatol 2002;36:105.
[62] Maini MK, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, et al. The role of virus-specific $CD8^+$ cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med 2000;191:1269.
[63] Shimada N, Yamamoto K, Kuroda MJ, Terada R, Hakoda T, Shimomura H, et al. HBcAg-specific CD8 T cells play an important role in virus suppression, and acute flare-up is associated with the expansion of activated memory T cells. J Clin Immunol 2003;23:223.
[64] Bertoletti A, Ferrari C, Fiaccadori F, Penna A, Margolskee R, Schlicht HJ, et al. HLA class I-restricted human cytotoxic T cells recognize endogenously synthesized hepatitis B virus nucleocapsid antigen. Proc Natl Acad Sci U S A 1991; 88:10445.
[65] Nayersina R, Fowler P, Guilhot S, Missale G, Cerny A, Schlicht HJ, et al. HLA A2 restricted cytotoxic T lymphocyte responses to multiple hepatitis B surface antigen epitopes during hepatitis B virus infection. J Immunol 1993;150:4659.
[66] Fiore G, Angarano I, Caccetta L, Serrone M, Jirillo E, Schiraldi O, et al. In-situ immunophenotyping study of hepatic-infiltrating cytotoxic cells in chronic active hepatitis C. Eur J Gastroenterol Hepatol 1997;9:491.
[67] Gallè MB, DeFranco RM, Kerjaschki D, Romanelli RG, Montalto P, Gentilini P, et al. Ordered array of dendritic cells and CD8+ lymphocytes in portal infiltrates in chronic hepatitis C. Histopathology 2001;39:373.
[68] Leroy V, Vigan I, Mosnier JF, Dufeu-Duchesne T, Pernollet M, Zarski JP, et al. Phenotypic and functional characterization of intrahepatic T lymphocytes during chronic hepatitis C. Hepatology 2003;38:829.
[69] Harvey CE, Post JJ, Palladinetti P, Freeman AJ, Ffrench RA, Kumar RK, et al. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. J Leukoc Biol 2003;74:360.
[70] Kita H, Kronenberg M, Gershwin ME. Intrahepatic NKT cells. In: Gershwin ME, Vierling JM, Manns MP, editors. Liver immunology. Philadelphia: Hanley and Belfus, 2002. p. 85.
[71] Kita H, Naidenko OV, Kronenberg M, Ansari AA, Rogers P, He XS, et al. Quantitation and phenotypic analysis of natural killer T cells in primary biliary cirrhosis using a human CD1d tetramer. Gastroenterology 2002;123:1031.
[72] Harada K, Isse K, Tsuneyama K, Ohta H, Nakanuma Y. Accumulating CD57+CD3+ natural killer T cells are related to intrahepatic bile duct lesions in primary biliary cirrhosis. Liver Int 2003;23:94.
[73] Zeng D, Lewis D, Dejbakhsh-Jones S, Lan F, Garcia-Ojeda M, Sibley R, et al. Bone marrow $NK1.1^-$ and $NK1.1^+$ T cells reciprocally regulate acute graft versus host disease. J Exp Med 1999;189:1073.
[74] Boisvert J, Kunkel EJ, Campbell JJ, Keeffe EB, Butcher EC, Greenberg HB. Liver-infiltrating lymphocytes in end-stage hepatitis C virus: subsets, activation status, and chemokine receptor phenotypes. J Hepatol 2003;38:67.
[75] Lucas M, Gadola S, Meier U, Young NT, Harcourt G, Karadimitris A, et al. Frequency and phenotype of circulating Valpha24 Vbeta11 double-positive natural killer T cells during hepatitis C virus infection. J Virol 2003;77:2251.
[76] Nuti S, Rosa D, Valiante NM, Saletti G, Caratozzolo M, Dellabona P, et al. Dynamics of intra-hepatic lymphocytes in chronic hepatitis C: enrichment for $Va24^+$ T cells and rapid elimination of effector cells by apoptosis. Eur J Immunol 1998;28:3448.
[77] Kakimi K, Guidotti LG, Koezuka Y, Chisari FV. Natural killer T cell activation inhibits hepatitis B virus replication in vivo. J Exp Med 2000;192:921.
[78] Kakimi K, Lane TE, Chisari FV, Guidotti LG. Cutting edge: Inhibition of hepatitis B virus replication by activated NK T cells does not require inflammatory cell recruitment to the liver. J Immunol 2001;167:6701.
[79] Kanto T, Hayashi N, Takehara T, Tatsumi T, Kuzushita N, Ito A, et al. Impaired allostimulatory capacity of peripheral blood dendritic cells recovered from hepatitis C virus-infected individuals. J Immunol 1999;162:5584.
[80] Auffermann-Gretzinger S, Keeffe EB, Levy S. Impaired dendritic cell maturation in patients with chronic, but not resolved, hepatitis C virus infection. Blood 2001;97:3171.
[81] Bain C, Fatmi A, Zoulim F, Zarski JP, Trepo C, Inchauspe G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. Gastroenterology 2001;120:512.
[82] Dolganiuc A, Kodys K, Kopasz A, Marshall C, Do T, Romics Jr L, et al. Hepatitis C virus core and nonstructural protein 3 proteins induce pro- and anti-inflammatory cytokines and inhibit dendritic cell differentiation. J Immunol 2003;170: 5615.
[83] Longman RS, Talal AH, Jacobson IM, Albert ML, Rice CM. Presence of functional dendritic cells in patients chronically infected with hepatitis C virus. Blood 2004;103:1026.
[84] Larsson M, Babcock E, Grakoui A, Shoukry N, Lauer G, Rice C, et al. Lack of phenotypic and functional impairment in dendritic cells from chimpanzees chronically infected with hepatitis C virus. J Virol 2004;78:6151.
[85] Löhr HF, Pingel S, Böcher WO, Bernhard H, Herzog-Hauff S, Rose-John S, et al. Reduced virus specific T helper cell induction by autologous dendritic cells in patients with chronic hepatitis B-restoration by exogenous interleukin-12. Clin Exp Immunol 2002;130:107.

J Gastroenterol 2005; 40:223–224
DOI 10.1007/s00535-005-1548-5

Journal of Gastroenterology

Editorial

Interobserver variation in assessing small bile duct lesions in PBC, CVH, and AIH

Article on page 164
Are bile duct lesions of primary biliary cirrhosis distinguishable from those of autoimmune hepatitis and chronic viral hepatitis? Interobserver histological agreement on trimmed bile ducts
ZEN Y, HARADA K, SASAKI M, et al.

Histological features provide the basis for the diagnosis and classification of tumors and inflammatory disorders arising in various organs. Though the histological diagnosis may sometimes be a gold standard in some liver diseases, interobserver variation is always inevitable.

The diagnosis of primary biliary cirrhosis (PBC) has been largely dependent on histopathological diagnosis, especially in past days before the specific autoantibodies, antimitochondrial antibodies (AMA), had become available for daily practical use. The characteristic histopathological features of PBC are bile duct lesions, granuloma formation, eosinophilic infiltration, and copper accumulation, of which bile duct lesions are the foremost findings. These features include the characteristic features of cholangitis, i.e., bile duct loss and proliferation of bile ductules. Long-term, cholangitis shows pleomorphic and destructive features, and this is called chronic nonsuppurative destructive cholangitis (CNSDC).[1] The above histological changes are characteristic of PBC, but are not specific for this disease, as these changes are also seen in autoimmune hepatitis (AIH) and even in chronic viral hepatitis (CVH).

Biliary changes resembling CNSDC can occur in classic AIH,[2] and they are not associated with distinctive clinical features or treatment response.[3] The presence of lymphoid aggregates surrounding a bile duct (lymphocytic cholangitis) and/or a pleomorphic or mixed inflammatory infiltrate encircling a bile duct (pleomorphic cholangitis) constitutes an exuberant inflammatory reaction within the portal tract. However, these findings occur in fewer than 10% of AIH patients. Useful histological findings that discriminate ordinary PBC from ordinary AIH are bile duct destruction, bile duct loss, isolated granulomas, and copper accumulation.

Reprint requests to: H. Ishibashi

In CVH, particularly when it is hepatitis C virus (HCV)-related, the bile duct injury (called hepatitis-associated bile duct lesion) is not infrequently associated with the features of chronic hepatitis.[4] The histopathological features of early-stage PBC require differentiation from those of chronic hepatitis, especially those associated with HCV. In chronic hepatitis, the affected duct segment shows vacuolization and stratification of cholangiocytes, with preservation of the basement membrane without cholestatic features.

In this issue of the *Journal of Gastroenterology*, Zen et al.[5] report the interobserver variations on trimmed bile ducts obtained from PBC, AIH, and CVH. The observers consisted of general pathologists, local hepatopathologists, and special hepatopathologists. Bile ducts in a mixed sample of trimmed bile ducts from PBC and CVH were correctly diagnosed as PBC (more than 80%), although for mildly injured bile ducts, the diagnostic accuracy was worse (less than 50%). Zen et al. concluded that bile duct injury histologically indistinguishable from that of CNSDC in PBC could be encountered in AIH.

Although histological scoring systems for chronic hepatic diseases, including viral hepatitis,[6] steatohepatitis,[7] and PBC,[8] have been proposed and used in practice, interobserver variations in diagnosing bile duct lesions have not been focused on. The article by Zen et al.[5] provides us with several valuable items of information on bile duct lesions in chronic liver diseases. First, some bile duct lesions seen in AIH livers could not be differentiated from those in PBC even by special hepatopathologists. The differential diagnosis of AIH from stage 2 PBC may be impossible by histological examination if the portal inflammation and interface hepatitis are not accompanied by obvious diffuse hepatitis or ductopenia. For proper differentiation, analyses of the laboratory and serological manifestations and other pathological findings are required. Second, the

diagnostic rates of PBC were not significantly different between general pathologists and hepatopathologists. However, compared to general pathologists, the hepatopathologists were superior in the evaluation of mildly injured bile ducts in PBC.

In Japan, the number of pathologists is significantly lower than the numbers in Western countries. In most middle-sized hospitals, a single, general, pathologist tends to work in all diseases fields. These general pathologists have limited chances to observe liver biopsy specimens, especially those of relatively infrequent diseases such as PBC and AIH. Pathological assessments are usually influenced by diagnosticians' experience or their training system. Therefore, the assessment of mild bile duct injuries may be difficult for general pathologists in Japan.

A number of studies have suggested that the term, "PBC", is a misnomer, particularly in regard to "cirrhosis", which may cause serious confusion or misunderstanding in patients with this disease at an early stage. In some cases, problems arise because PBC patients lack serum AMA on standard indirect immunofluorescence or because features of both AIH and PBC coexist in various combinations in a single patient. Autoimmune cholangitis can be considered as being synonymous with AMA-negative PBC.[9] Recently, a new term, "primary cholangiohepatitis", was proposed as an alternative name for PBC, to cover the hepatitis form of PBC, or PBC overlapping with chronic active hepatitis, and also to correct the perceived misnomer "PBC".[10]

Masahiro Ito, M.D., and Hiromi Ishibashi, M.D.
Clinical Research Center, National Hospital Organization (NHO) Nagasaki Medical Center, 2-1001-1 Kubara, Omura 856-8562, Japan

Masahiro Ito, M.D., and Hiromi Ishibashi, M.D.
Department of Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

References

1. Portmann BC, Nakanuma Y. Diseases of the bile ducts. In: MacSween RNM, Burt AD, Portman BC, Ishak KG, Scheuer PJ, Anthody PP, editors. Pathology of the liver. 4th ed. London: Churchill Livingstone; 2001. p. 435–506.
2. Sato Y, Harada K, Sudo Y, Watanabe K, Nakahama T, Morimoto H, et al. Autoimmune hepatitis associated with bile duct injury resembling chronic non-suppurative destructive cholangitis. Pathol Int 2002;52:478–82.
3. Czaja AJ, Carpenter HA. Autoimmune hepatitis with incidental histologic features of bile duct injury. Hepatology 2001;34:659–65.
4. Scheuer PJ, Davies SE, Dhillon AP. Histological aspects of viral hepatitis. J Viral Hepat 1996;3:277–83.
5. Zen Y, Harada K, Sasaki M, Tsuneyama K, Matsui K, Haratake J, et al. Are bile duct lesions of primary biliary cirrhosis distinguishable from those of autoimmune hepatitis and chronic viral hepatitis? Interobserver histological agreement on trimmed bile ducts. J Gastroenterol 2005;40:164–70.
6. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–20.
7. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–74.
8. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histopathol 1978; 379:103–12.
9. Ben Ari Z, Dhillon AP, Sherlock S. Autoimmune cholangiopathy: part of the spectrum of autoimmune chronic active hepatitis. Hepatology 1993;18:10–5.
10. Nakanuma Y, Harada K. Primary cholangiohepatitis as an alternative name for primary biliary cirrhosis. Pathol Int 2003; 53:412–4.

3．獲得免疫の立場から

小森　敦正　中村　稔　石橋　大海

はじめに

原発性胆汁性肝硬変（primary biliary cirrhosis；PBC）に認められる慢性非化膿性破壊性胆管炎（CNSDC）は，変性・壊死胆管を取り囲むように浸潤するリンパ球の大多数がCD4及びCD8陽性T細胞であることから，小型肝内胆管に対する（細胞特異的自己免疫性）獲得免疫の存在を強く示唆する病理組織所見とみなされている．一方，同疾患の血清学的特徴である抗ミトコンドリア抗体（antimitochondrial antibody；AMA）の対応抗原は，ピルビン酸脱水素酵素複合体（pyruvate dehydrogenase complex；PDC）を含むミトコンドリア2-オキソ酸脱水素酵素複合体（2-oxo dehydrogenase complex；2-OADC）であることが同定されているが[1]，その発現は胆管細胞に限定されるものではない．このdiscrepancyにもかかわらず，PDC E2コンポーネント（PDC-E2）反応性CD4陽性T細胞がPBC患者の末梢単核球中で健常人に比し有意に増加し[2]，かつPBC患者肝内浸潤リンパ球では末梢血に比し100－150倍と高い頻度で存在していること[3]，さらにはPDC-E2抗原特異的細胞傷害性T細胞株のPBC特異的な誘導[4]が近年次々に報告され，ミトコンドリア抗原に対するトレランスの破綻，自己抗原反応性T・B細胞の活性化，クローン性増殖（clonal expansion）が，胆管に対する獲得免疫の形成，維持に果たす重要性はほぼ確立されたかのように思われる．

本稿ではBiliary Cell Lineageを標的とした獲得免疫研究を，ミトコンドリア抗原に対する獲得免疫研究の面から，すなわち，PDC-E2ないしPDC-E2に分子相同性を有する分子の抗原エピトープの提示，PDC-E2抗原特異的T細胞の活性化，引き続く*in situ*胆管細胞障害の成立に至る免疫応答研究の進歩を中心にレビューするものである（Table）．

1．ミトコンドリア抗原に対するトレランスの破綻とAMAおよびPDC-E2反応性T細胞の誘導

PBC患者血清とウシあるいはブタ心筋から得られたミトコンドリア分画を用いてWestern blottingを行い，PDC-E2，PDC-E3結合蛋白（PDC-E3BP），PDC-E1 α，OGDCのE2コンポーネント（OGDC-E2），BCOADCのE2コンポーネント（BCOADC-E2）がAMAの対応抗原であることが明らかにされ，なかでも，PDC-E2に対する抗体が最も高頻度，高力価に検出されることから，PDC-E2がAMAの主要な対応抗原であることが明らかにされた．抗体エピトープは内側および外側リポ酸ドメインのリポ酸を結合したリジン（内側173K，外側47K）を中心とした領域にマップされている[5]．胆管に対する自己免疫標的抗原としてPDC-E2を想定したトレランス破綻開始機構としては，molecular mimicry（分子相同説）およびxenobiotics説が提唱されている．尿路感染症患者にPBCが高頻度に発症するとの疫学的データの存在，さらには，PBC患者血清中のAMAが大腸菌をはじめとした微生物のミトコンドリア蛋白と交差反応することから，AMAの出現と細菌感染との関連が指摘されてきた．しかしながら，患者血清中の細菌由来PDC-E2に対する抗体価の低さや，その出現が疾患後期からしか認められない点など，トレランス破綻の原因とするには不充分な点も指摘されていた．Selmiら[6]は最近，PDC-E2の内側リポ酸ドメインと最も高い相同性を有するアミノ酸配列をグラム陰性好気性菌*Novoshingobium aromaticivorans*の2-OADC蛋白内に見出し，実際にAMA陽性PBC患者血清が同蛋白と特異的に反応し，その力価も大腸菌由来PDC-E2に対するものより100－1000倍高いと報告している．同バクテリアは土壌や水源における常在菌であるが，興味深いことに，同菌由来16S ribosomal RNAがPBCならびに対照患者の約25%の糞便中にPCR法で検出されている[6]．

Shimodaらが樹立したPBC患者末梢血由来PDC-E2特異的CD4陽性T細胞クローンはHLA DR53（DRA1*0101/DRB4*0101）拘束性にPDC-E2 163－176（GDLLAEIETDKATI）と反応し，また同エピトープはBリンパ球エピトープとオーバーラップしてPDC-E2内側リポ酸結合ドメインに存在する．このヘルパーT細胞エピトープと分子相同性を有するペプチドの検

国立病院機構長崎医療センター・臨床研究センター

Table ミトコンドリア抗原に対する獲得免疫からみたPBCの発症と進展

Step 0：遺伝的素因	
＋自然免疫	
Step 1：常在菌・細菌感染，Xenobiotics による mimicry peptide を介した	
ミトコンドリア抗原反応性 T/B 細胞の priming	
＋自然免疫	
＋局所における炎症	
Step 2：ミトコンドリア抗原反応性 T/B 細胞の affinity maturation/クローン性増殖	
＋自然免疫	
Step 3：ミトコンドリア抗原反応性 T/B の epitope spreading	
＋自然免疫	
Step 4：胆管上皮細胞をターゲットとした免疫反応	⟶ cholangitis
＋自然免疫	
Step 5：胆管細胞障害	⟶ cholestasis
＋自然免疫	
Step 6：胆汁うっ滞	⟶ biliary cirrhosis
＋自然免疫	

索から，彼らは PDC-E2 163－176 反応性 T リンパ球を活性化し得る大腸菌など微生物由来のペプチドを多数同定しており[2]，慢性細菌感染症に伴う外来菌体成分に対する CD4 陽性 T 細胞のプライミングが PBC 発症の初期要因であろうと推察している．

Xenobiotics 説は，PDC-E2 ペプチドが外来性化学物質により直接修飾を受けることにより，同リポ残基と同等もしくはそれ以上の免疫原性を得ることが，AMA 誘導の原因となるという説である．Gershwin らは雌ウサギに PDC-E2 リポ残基類似 lipoyl mimetopes 結合 BSA を免疫すると，抗 lipoyl 化 PDC-E2 ペプチド抗体の誘導を経て，recombinant PDC-E2/OGDC-E2/BCOAD-E2 と反応可能な AMA が誘導されることを報告している[7]．肝臓は薬物代謝の主臓器であり，代謝産物による胆管上皮内蛋白由来ペプチドの化学修飾と，胆管上皮障害後に生ずる修飾ペプチドの遊離は想定され得る事象である．

2．ミトコンドリア抗原反応性リンパ球の活性化とクローン性増殖およびその維持

Molecular mimicry あるいは xenobiotics 理論を介した AMA 産生 B 細胞および PDC-E2 反応性 T 細胞の誘導は魅力的な仮説だが，Xenobiotics により AMA は誘導されるが胆管病変は生じないとされることから，hit & run となるには，少なくとも引き続く炎症局所もしくは所属リンパ節胚中心における naïve ミトコンドリア抗原の提示，T 細胞の clonal expansion および PDC-E2 分子内 epitope spreading が必要だと思われる．すなわち intact な遊離自己抗原決定基が，自己抗体を介して，もしくは直接抗原提示細胞(APC)である樹状細胞/貪食細胞に取り込まれ，抗原提示されるプロセスと場が要求される．Odin ら[8]は，ラット正常胆管上皮細胞(BEC)に *in vitro* でアポトーシスを誘導した際に，PDC-E2 抗原決定基は細胞内で caspase によって分解されずに AMA に対する反応性を保持していたが，胆管以外の細胞ではグルタチオンにより修飾され抗原性を失う現象を見出した．また Matsumura ら[9]は *in vitro* cleavage assay の結果，PDC-E2 蛋白は caspase-3,6,8 による分解によってもその抗原性を失わないが，granzyme B 処理では抗原エピトープの分解が生じると報告した．即ちアポトーシスに陥った胆管上皮細胞は，intact な PDC-E2 抗原の産生源になりうるという結論が両者に共通して導き出されている．自己抗体と BEC 由来自己抗原による免疫複合体が一旦生じると，Fc レセプター III を介した抗原・抗体複合体の取り込みによる樹状細胞の活性化と，MHC クラス I およびクラス II を介した自己抗原ペプチドの提示が持続し[10]，CD4 および CD8 陽性 T 細胞の活性化を経て慢性炎症の場が完成すると推定される．さらには PDC-E2 163－176 反応性 T リンパ球は，ミトコンドリア抗原である E3BP や OGDC-E2 由来のペプチドとも反応することが報告されており[11]，分子間(2-OADC 構成蛋白内)epitope spreading も自己反応性リンパ球の活性化を維持・増強させるメカニズムとして作動しうると考えられる．また最近，PBC における自己反応性 T 細胞が，末梢の免疫寛容状態を回避し自己免疫反応を維持する機構が Kamihira ら[12]によって提唱された．彼

らは，側副刺激分子非依存性と考えられるCD4陽性CD28陰性T細胞の頻度が，PBC患者の末梢血および肝内で健常者慢性肝炎患者に比し有意に高値であることを示し，さらに，PBC患者に存在する側副刺激分子に非依存性のPDC-E2 163－176ペプチド反応性CD4陽性T細胞クローンは，PDC-E2ペプチドをパルス刺激した側副刺激分子誘導シグナルを欠くBECを*in vitro*にて傷害した後もアナジーとならずに，プロフェショナルAPCの存在下で増殖可能であることを報告した．BEC自らがエフェクター細胞として振る舞う末梢での免疫寛容誘導機構からの逸脱も，自己抗原反応性リンパ球の維持に重要であると考えられる．

3．獲得免疫の誘導および維持における自然免疫の関与

小葉間胆管上皮に発現したPDC-E2由来ないしは未知の自己抗原ペプチド/MHC複合体を認識して，ヘルパーT細胞およびCTLによる細胞傷害カスケードが始動する前後では，自然免疫系のToll like receptoes (TLRs)によるAPCの活性化を経たトレランスの破綻やTh1優位の炎症環境の誘導が必要と思われる．実際にLPS(TLR4)，二重鎖RNA(TLR3)の刺激はType I IFNの産生とそのレセプターを介した細胞内シグナル伝達によって，APC上の側副刺激分子発現亢進をもたらすことが報告されている[13]．逆に実験的自己免疫性脳脊髄炎抵抗性マウスにおいては，CpG DNA(TLR 9)はIFNの誘導を介さずにトレランスを破綻させ，炎症を惹起する[14]．Jonesら[15]はウシPDCとTLR-9のリガンドであるCpGDNA含有オリゴヌクレオチドをSJL/Jマウスに同時に免疫することで，マウス脾臓由来T細胞の，自己PDCに対するTh1応答と胆管炎の誘導を報告した．Takiiらは，PBC門脈域および肝実質においてType I IFN mRNAの発現亢進がみられることをLaser captured microdissection法によって分取した標本を用いて明らかにしている(Takii et.al., manuscript in submission)．

4．PBCの進行に関わる獲得免疫

核膜孔蛋白の一種であるgp210に対する自己抗体はPBCの診断に特異的なものであるが，PDC-E2 163－176反応性Tリンパ球はgp210由来ペプチドとも反応することが最近明らかとなり[16]，PDC-E2 163－176反応性Tリンパ球によって認識される自己抗原が，細胞内局在を超えて拡がっていく可能性が示唆されている．以前より抗gp210抗体陽性患者は病期の進んだPBC患者に多く，予後不良であるとの報告があったが，最近Nakamuraらはヒトrecombinant gp210のC末端ペプチドを用いたELISA法を確立し，抗gp210抗体値が持続陽性のPBC患者の予後が，持続陰性群もしくは抗体が陰性化した群に比べ有意に悪かったことを明らかにした[17]．この結果を裏づけるように，欧州でのprospective studyからも，抗核膜孔抗体の存在がPBCの予後因子となることが報告され[18]，核膜抗原に対するトレランスの破綻と，核膜抗原反応性リンパ球の誘導活性化，クローン性増殖とその維持は，症候性PBCの進行に関わる獲得免疫である可能性がクローズアップされている．

おわりに

PBCにおけるミトコンドリア抗原に対する獲得免疫研究の近年の進歩を詳説した．もうひとつの自己免疫性胆管疾患である原発性硬化性胆管炎(PSC)においては，胆管障害をもたらす自己抗原は未だ明らかになっていない．この領域でも今後の研究の発展が期待される．

文　献

1) Gershwin ME, Mackay IR, Sturgess A, et al. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 1987；138：3525—31
2) 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 disease. J Exp Med 1995；181：1835—45
3) Shimoda S, Van de Water J, Ansari A, et al. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. J Clin Invest 1998；102：1831—40
4) Kita H, Matsumura S, He XS, et al. Quantitative and functional analysis of PDC-E2 specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. J Clin Invest 2002；109：1231—40
5) Matsui M, Nakamura M, Ishibashi H, et al. Human monoclonal antibodies from a patient with primary biliary cirrhosis which recognize two distinct autoepitopes in the E2 component

of the pyruvate dehydrogenase complex. Hepatology 1993 ; 18 : 1069—77
6) Selmi C, Balkwill D, Invernizzi P. et al. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. Hepatology 2003 ; 38 : 1250—7
7) Amano K, Leung PSC, Xu Q, et al. Xenobiotic-induced loss of tolerance in rabbits to the mitochondrial autoantigen of primary biliary cirrhosis is reversible. J Immunol 2004 ; 172 : 6444—52
8) Odin JA, Huebert RC, Casciola-Rosen L, et al. Bcl-2-dependent oxidation of pyruvate dehydrogenase-E2, a primary biliary cirrhosis autoantigen, during apoptosis. J Clin Invest 2001 ; 108 : 223—32
9) Matsushima S, Van de Water J, Kita H, et al. Contribution to antimitochondrial antibody production: cleavage of pyruvate dehydrogenase complex-E2 by apoptosis-related proteases. Hepatology 2002 ; 35 : 14—22
10) Kita H, Lian ZX, Van de Water J, et al. Identification of HLA-A 2 restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. J Exp Med 2002 ; 195 : 113—23
11) Shigematsu H, Shimoda S, Nakamura M, et al. Implications for molecular mimicry and cross-recognition among mitochondrial autoantigens. Hepatology 2000 ; 32 : 901—9
12) Kamihira T, Shimoda S, Harada K, et al. Distinct costimulation dependent and independent autoreactive T-cell clones in primary biliary cirrhosis. Gastroenterology 2003 ; 125 : 1379—87
13) Hoebe K, Janssen EM, Kim SO, et al. Upregulation of costimulatory molecules induced by lipopolysaccharide and double-strandes RNA occurs by Trif-dependent and Trif-independent pathways. Nat Immunol 2003 ; 12 : 1223—9
14) Waldner H, Collins M, and Kuchroo VK. Activation of antigen-presenting cells by microbial products breaks self tolerance and induces autoimmune disease. J Clin Invest 2004 ; 113 : 990—7
15) Jones DEJ, Palmer JM, Burt AD, et al. Bacterial DNA as an adjuvant for the breakdown of immune self-tolerance to pyruvate dehydrogenase complex. Hepatology 2002 ; 36 : 679—86
16) Shimoda S, Nakamura M, Ishibashi H, et al. Molecular mimicry of mitochondrial and nuclear autoantigens in primary biliary cirrhosis. Gastroenterology 2003 ; 124 : 1915—25
17) Nakamura M, Shimizu Y, Takii Y, et al. Antibody titer to gp210-C terminal peptide as a clinical parameter for monitoring primary biliary cirrhosis. J. Hepatol (in press)
18) Invernizzi P, Wesierska-Gadek J, Battezzati PM, et al. Prognostic value of autoantibodies against proteins of nuclear pore complexes (anti-NPC) in early primary biliary cirrhosis (PBC). J Hepatol 40 (Suppl No 1) 2004 ; 159

各・論

特集

原発性胆汁性肝硬変の診断と治療の最前線

石橋大海　国立病院機構長崎医療センター臨床研究センター長

SUMMARY

原発性胆汁性肝硬変（Primary biliary cirrhosis：PBC）は慢性進行性の肝内胆汁うっ滞性疾患である．胆汁うっ滞に伴い肝実質細胞の破壊と線維化を生じ，究極的には肝硬変から肝不全に至る．自己抗体である抗ミトコンドリア抗体（AMA）が陽性化することが特徴である．ウルソデオキシコール酸（UDCA）が第一選択治療薬であるが，最近ベザフィブラートなどフィブラート系薬物の有効性が確認され，試みられている．

I　症　候

❶ 症　状

症状は，①胆汁うっ滞に基づく症状，②肝障害・肝硬変に伴う症状，③合併したほかの自己免疫性疾患に伴う症状，に分けて考えることができる．初発症状として胆汁うっ滞に基づく皮膚瘙痒感が特徴的であるが，多くの症例では無症状である．進行すると倦怠感を生じる．

表1　PBCにおける自己免疫性疾患の合併（記載のあった3325例中）

Sjögren症候群	480例	(14.4％)
橋本病	193例	(5.8％)
リウマチ性関節炎	149例	(4.5％)
Raynaud現象	105例	(3.2％)
強皮症	59例	(1.8％)
潰瘍性大腸炎	10例	(0.3％)

（平成9年度「難治性の肝疾患に関する研究」班報告書5））

❷ 身体所見

症候期では，瘙痒感に伴う掻き疵や高脂血症に伴う眼瞼黄色腫がみられる．肝臓は腫大していることが多い．進行期には，黄疸とともに肝硬変に伴う身体所見が現れる．

❸ 合併症

慢性甲状腺炎，シェーグレン症候群など，**ほかの自己免疫性疾患・膠原病を合併しやすく（表1）**，それらの疾患を診療中に肝機能異常の存在からたまたま本疾患が診断されることも多い．そのため，合併した自己免疫性疾患の病態・症状が表面に出ていることも多い．**門脈圧亢進症としての食道静脈瘤破裂による突然の出血に気をつける必要がある**．肝細胞癌合併の頻度は高くはないが，PBC に**合併する悪性腫瘍の4分の1は肝細胞癌**である．

Ⅱ 診断・診断基準

❶ 診　断

PBC は以下の特徴を有する．

a．胆道系酵素（ALP，γ-GTP）優位の肝機能異常を呈する慢性の胆汁うっ滞性疾患である．

b．原則としてウイルスマーカーが陰性，かつ原因となるような薬剤の服用もない．

c．画像などにより閉塞性黄疸などほかの疾患が除外されている

d．血清中に抗ミトコンドリア抗体（Anti-mitochondrial antibody：AMA）あるいは抗ミトコンドリア M2 抗体が陽性である．

慢性胆汁うっ滞で，画像で閉塞性黄疸が除外され，AMA が陽性化であれば，PBC の可能性は高い．肝生検にて特徴的な病理学的所見が得られれば，診断確定できる．

❷ 診断基準

厚生省「難治性の肝炎」調査研究班（1992 年）による基準（**表2**）が用いられており，次のいず

表2　原発性胆汁性肝硬変（PBC）診断基準

概　念

中年以後の女性に好発し，皮膚瘙痒感で初発することが多い．黄疸は出現後は消退することなく漸増することが多く，門脈圧亢進症状が高頻度に出現する．なお，皮膚瘙痒感，黄疸など肝障害にもとづく自覚症状を欠く場合があり，無症候性（asymptomatic）PBC とよび，無症候性のまま数年以上経過する場合がある．

1．検査所見

黄疸の有無にかかわらず，血沈の促進，血清中の胆道系酵素（ALP など），総コレステロール，IgM の上昇を認める．抗糸粒体抗体（AMA）または抗 pyruvate dehydrogenase（PDH）抗体が高頻度に陽性で，高力価を示す．

2．組織学的所見

肝組織では中等大小葉間胆管ないし隔壁胆管に慢性非化膿性破壊性胆管炎（chronic non-suppurative destructive cholangitis；CNSDC）あるいは胆管消失を認める．連続切片による検索で診断率は向上する．

3．合併症

高脂血症が持続する場合に皮膚黄色腫を伴う．
Sjögren 症候群，関節リウマチ，慢性甲状腺炎などの自己免疫性疾患を合併することがある．

4．鑑　別

慢性薬剤起因性肝内胆汁うっ滞，肝内型原発性硬化性胆管炎，成人性肝内胆管減少症など．

【診　断】

次のいずれか1つに該当するものを PBC と診断する．

1）組織学的に CNSDC を認め，検査所見が PBC として矛盾しないもの．AMA または抗 PDH 抗体が陰性例もまれに存在する
2）AMA または抗 PDH 抗体が陽性で，組織学的には CNSDC を認めないか，PBC に矛盾しない（compatible）組織像を示すもの
3）組織学的検索の機会はないが，AMA または抗 PDH 抗体が陽性で，しかも臨床像および経過から PBC と考えられるもの

（厚生省「難治性の肝炎」調査研究班 1992 年）

れか1つに該当するものをPBCと診断する.

a. 組織学的にCNSDCを認め，検査所見がPBCとして矛盾しないもの．AMAまたは抗ミトコンドリアM2抗体が陰性例もまれに存在する.

b. AMAまたは抗ミトコンドリアM2抗体が陽性で，組織学的にはCNSDCを認めないか，PBCに矛盾しない（compatible）組織像を示すもの.

c. 組織学的検索の機会はないが，AMAまたは抗PDH抗体が陽性で，しかも臨床像および経過からPBCと考えられるもの.

❸ 鑑別診断

CNSDCに類似した胆管障害像は，原発性硬化性胆管炎（とくに肝内型），慢性薬剤起因性肝内胆汁うっ滞，成人性肝内胆管減少症，移植片対宿主病（GVHD），肝移植拒絶反応，サルコイドーシスとともに，自己免疫性肝炎でも認められる．C型肝炎，自己免疫性肝炎でも胆管障害は生じるが，原則として破壊性変化ではない.

Ⅲ 分類

❶ 臨床病期分類

PBCは自覚症状を認めない無症候性PBC（asymptomatic PBC；a-PBC）と，皮膚瘙痒感あるいは黄疸を伴う症候性PBC（symptomatic PBC；s-PBC）に分類され，症候性PBCはさらに皮膚瘙痒感のみを伴い黄疸を認めない$_{s1}$-PBCと，血清総ビリルビン値が2.0mg/dL以上の黄疸を認める$_{s2}$-PBCに細分されている（表3）.

❷ 組織学的分類

組織学的に4期に分けられている.

Ⅰ期：多彩な胆管病変（florid bile duct lesions）

Ⅱ期：細胆管増生（ductular proliferation）

Ⅲ期：瘢痕（線維性隔壁と架橋形成）（scarring（septal fibrosis and bridging））

Ⅳ期：肝硬変（cirrhosis）

表3 原発性胆汁性肝硬変（PBC）の臨床病期

無症候性PBC（aPBC）：皮膚瘙痒感，黄疸など肝障害に基づく自覚症状を欠く	
症候性PBC（sPBC）	
s1PBC	皮膚瘙痒感のみを伴う
s2PBC	黄疸を伴う（総ビリルビン値 2.0mg/dL以上）

❸ 特殊な病態

a. 早期PBC（early PBC）

血清生化学値の異常が出現する以前からAMAは陽性を呈し，肝組織の病理学的変化も始まっていることが観察されており，そのような時期のPBCは早期PBCと称されている[1].

b. 自己免疫性胆管炎（Autoimmune cholangitis：AIC）

臨床的にはPBCの像を呈しながらAMAは陰性であるがANAが高力価を呈する病態に対し自己免疫性胆管炎の名称が提唱された[2]．AIHかPBCか，あるいは独立した疾患か議論が続いたが，基本的にはPBCであると考えられている.

c. AIH・PBCオーバーラップ症候群 AIH・PBC overlap syndrome

PBCとAIHの両方の病像と検査所見を呈する病態はAIH-PBCオーバーラップ症候群と呼ばれている[3]．病理学的には，PBCに特徴的な肝組織所見に加え，活動性の肝炎像がみられる．本病態が単にPBCの亜型であるのか，それぞれ独立し

た疾患であるPBCとAIHが合併したものか，あるいはAIH，PBCから独立した疾患であるのか，結論は得られていない．

Ⅳ 治療

① 生活指導

初期から肝硬変期まで同じ病名で称されるが**実際に肝硬変期に至っている患者は少ない**．いたずらに不安を抱かぬように指導する．無症候期は生活上の規制はないが，進行性の疾患であるので定期的な受診が大切である．症候期では骨折に注意し，骨粗鬆症を予防し，アミノ酸代謝の改善を図るため散歩など適度の運動を行う．肝不全期では，ほかの原因による肝不全と同様に塩分の制限を行い，肝不全の程度により蛋白摂取の制限を行う．

② 治療ガイドライン

根治的治療法はまだ確立していないため，病期・病態に応じた対症療法が基本となる．初期から中期では，ウルソデオキシコール酸（UDCA）600mgを経口的に投与する．UDCAは利胆作用のほか免疫抑制作用も認められており，副作用も少なく現在では第一選択薬である．症候期になると自覚症状（瘙痒感）の改善，骨粗鬆症の予防のための治療が必要となる．また，門脈圧亢進をきたしやすく**突然食道静脈瘤の破裂をきたすことがあるので**，肝硬変に至る前の時期であっても留意が必要である．肝細胞癌の合併はまれであるが，硬変期に至ると発生率が高まる．定期的な画像診断が必要である．黄疸期になると進行性で予後不良である．進行期になると内科的治療の限界となり肝移植のよい適応となる．血清総ビリルビン値が5.0～6.0mg/dL以上になると肝移植を考慮し，移植専門医へコンサルトを行う．

③ 治療の実際

a．無症候性PBCの薬物治療

3～4ヵ月に1度肝機能を測定し胆道系酵素の上昇傾向がみられる症例に対してはUDCAの投与を行う．

b．特殊な病態に対する治療

トランスアミナーゼ，IgG値が高いAIH・PBCオーバーラップ症候群に対しては初期の炎症を抑えるために副腎皮質ホルモン薬（プレドニゾロン）が有効である．AMA陰性でANA陽性の自己免疫性胆管炎（AIC）も初期は副腎皮質ホルモン薬が有効である．いずれの場合も漸減し，維持療法の段階ではUDCAに切り替える．

c．合併症に対する治療

症例によっては皮膚瘙痒感は頑固である．抗ヒスタミン剤あるいはコレスチラミンを投与する．一部の症例にはリファンピシンも有効である．高脂血症に対しては，PBCへの治療効果もみられることから近年ベザフィブラートが用いられている．胆汁うっ滞に伴うビタミンD欠乏による**骨粗鬆症およびそれに伴う骨折は進行PBC例では深刻な問題である**．予防として乳酸カルシウム，ビタミンK_2製剤，合成ビタミンD_3製剤の投与を行う．ビタミンK欠乏による出血傾向に対してはビタミンK_2製剤投与を行う．食道静脈瘤，肝細胞癌，腹水，肝性脳症が生じたら，それぞれに対する治療を行う．合併しやすいほかの自己免疫疾患に対してもそれぞれの治療を行う．

④ 薬 物

a. ウルソデオキシコール酸 (Ursodeoxycholic acid : UDCA)

UDCAの治療効果が国内外において評価され，第一選択薬として広く使用されており，長期使用は肝移植までの期間を延長する[4,5]．肝細胞保護作用，利胆作用のほかIL-1，IL-2の産生抑制作用，免疫グロブリン産生抑制作用およびMHCクラスⅠ，クラスⅡの発現抑制作用などの免疫調節作用が認められている．これら免疫抑制効果の発現機序として，グルココルチコイド非存在下であってもUDCAによりグルココルチコイド・レセプター（GR）が核内に移行してDNAに結合し，遺伝子転写機構を調節する機序が報告されている．

b. 副腎皮質ステロイド剤

免疫抑制効果を期待して使用されたが，PBC症例の多くは中年女性であり，さらに胆汁うっ滞による脂溶性ビタミンの吸収不良のために骨粗鬆症が出現しやすいこともあり，使用は一般的ではない．しかし，AIH・PBC overlap症候群に対しては本剤の使用を検討する．AICも副腎皮質ステロイド薬が効果を示すことが多い．いずれの場合も漸減し，維持療法の段階ではUDCAに切り替えることが推奨されている．

c. フィブラート Fibrate

高脂血症薬の一つであるフィブラート（ベザフィブラート，フェノフィブラート）が胆道系酵素の低下作用を有する新たなPBC治療薬として注目されている[6]．フィブラートの作用機序は，肝細胞毛細胆管側に存在するMDR3-P糖蛋白の発現の増加をもたらすことによりリン脂質の胆汁中への分泌を促進することである．胆管に排泄されたリン脂質により疎水性胆汁酸のミセル化が促進し非活化することにより胆管上皮細胞障害が軽減することが推察されている．PPARαを介して炎症を鎮静化することによりMDR3-P糖蛋白の発現を増加する可能性も推察されている[6]．

d. 抗原特異的免疫抑制療法

これまで試みられてきた免疫抑制療法はいずれも非特異的な免疫抑制療法である．中には副作用が多いものもあり，しかも重篤なものも含まれる．今後は特異的に免疫反応を抑えることができるような薬剤の開発が望まれる．その候補としては，抗HLA抗体，抗接着分子療法，抗サイトカイン療法など免疫反応分子をアンタゴナイズするような療法がある．また，抗原ペプチドを用いたペプチドワクチンやペプチド療法なども期待される．

⑤ 肝移植

胆汁うっ滞性肝硬変へと進展した場合はもはや進展を抑えることができなくなり，肝移植が唯一の治療となる．脳死肝移植適応委員会のPBCの移植適応指針では，日本肝移植適応研究会モデルの6ヵ月後の死亡確率が50％以上で移植適応ありとされる．

PBCに対する肝移植成績は，米国UNOSの脳死肝移植の成績では3年生存率は81％，5年で79％，7年で74％と，肝疾患の中では一番優れている．脳死移植が少ないわが国ではすでに生体部分肝移植が定着している．

V 予後および経過

aPBCはaPBCにとどまる限り予後は大変よいが，約10～40％（5年間で約25％）はsPBCへ移行する（**図1**）[7]．黄疸期（S2-PBC）になると進行性で予後不良である．予後予測式はMayoモデ

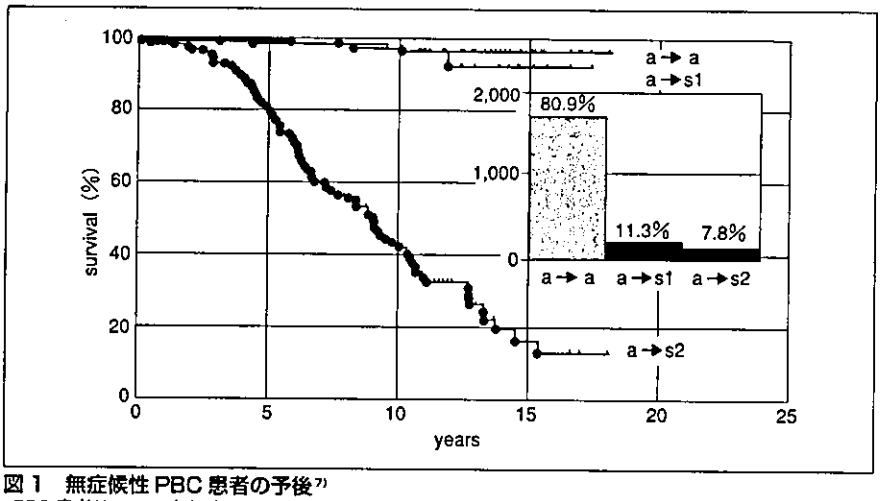

図1　無症候性PBC患者の予後[7]
aPBC患者はaPBCもしくは$_{a1}$-PBCにとどまる限り予後はよいが，$_{a2}$-PBCに移行すると予後は悪くなる．

表4　PBCの肝移植適応基準

Logistic回帰モデルによる6ヵ月後の予想死亡率（Death Rate；DR）

DR（death rate）＝1/（1＋$e^{-\lambda}$）

$\lambda = -4.333 + 1.2739 \times \log_e(\text{T.Bil}) + 4.4880 \times \log_e(\text{GOT/GPT})$

DRが50％以上の場合に肝移植適応があると判断される

（日本肝移植適応研究会，1991）

ルや日本肝移植適応研究会のモデル（表4）が用いられている．生存予測に関する独立因子としては，Mayoモデルでは年齢，ビリルビン，アルブミン，プロトロンビン時間，および浮腫が，一方，日本肝移植適応研究会では，ビリルビンとGOT/GPTがあげられているが，いずれにおいても**最も重要な因子は血清総ビリルビンである**．血清総ビリルビン値が2.0mg/dLを超えると，5年生存率は50％をきる．死因は，肝不全と食道静脈瘤の破裂による消化管出血である．

（参考文献）

1) Metcalf JV, et al：Natural history of early primary biliary cirrhosis. Lancet, 348：1399-402, 1996.
2) Sherlock S：Ludwig Symposium on biliary disorders. Autoimmune cholangitis：a unique entity? Mayo Clin Proc, 73：184-190, 1998.
3) Lohse AW, et al：Characterization of the overlap syndrome of primary biliary cirrhosis（PBC）and autoimmune hepatitis：evidence for it being a hepatitic form of PBC in genetically susceptible individuals. Hepatology, 29：1078-1084, 1999.
4) Poupon R, et al：Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet, 1：834-836, 1987.
5) Lindor KD, et al：Ursodeoxycholic acid for primary biliary cirrhosis. Lancet, 355：657-658, 2000.
6) Iwasaki S, et al：Bezafibrate may have a beneficial effect in precirrhotic primary biliary cirrhosis. Hepatol Res, 16：12-18, 1999.
7) Hirohara J, et al：Epidemiology and prognosis of primary biliary cirrhosis in Japan. In：Autoimmune Liver Disease-It's Recent Advances（Nishioka M, Watanabe S, Arima K, eds）, pp111-122, Elsevier Science BV, Tokyo. 2000.