*The Korean Journal of Pathology*
*2004; 38: 357-63*

# Differential Expression of Promyelocytic Leukemia Protein in Autoimmune Liver Diseases

**Hyun Jung Kim · Jung-Sun Kim[1] Yong Sang Lee[2] Young-Hwa Chung[2] · Han Joo Lee[2] Dong Jin Suh[2] · Chong Jai Kim[3] Eunsil Yu[1]**

Department of Pathology, University of Inje University of Medicine, Sanggye Paik Hospital, Seoul; [1]Department of Pathology and [2]Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul; [3]Department of Pathology, Seoul National University College of Medicine, Seoul, Korea

Received : July 20, 2004
Accepted : September 27, 2004

**Corresponding Author**
Eunsil Yu, M.D.
Department of Pathology, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-dong, Songpa-gu, Seoul 138-736, Korea
Tel: 02-3010-4552
Fax: 02-472-7898
E-mail: esyu@amc.seoul.kr

**Background :** Promyelocytic leukemia protein (PML) is a primary biliary cirrhosis (PBC)-specific autoantigen. Anti-PML antibody is analyzed using cultured cells with patient sera, however, PML expression has rarely been examined in liver tissues. **Methods :** In the present study, PML expression was examined immunohistochemically in paraffin embedded liver needle biopsy specimens obtained from 20 cases of PBC, 10 cases of autoimmune cholangitis, 36 cases of autoimmune hepatitis and from 5 cases of noninflammatory livers. **Results :** Variable PML immunopositivity was detected in the bile duct epithelial cells of 18 (90.0%) of 20 PBC cases and in all 10 cases (100.0%) of autoimmune cholangitis, whereas it was only present in 6 (16.7%) of 36 cases of autoimmune hepatitis ($p<0.001$). In contrast, hepatocyte PML immunopositivity was higher in autoimmune hepatitis (33/36 cases, 90.8%), than in PBC (10/20 cases, 50.0%) or autoimmune cholangitis (3/10 cases, 30.0%) ($p<0.05$). **Conclusions :** Our data indicate that the differential expression of PML is closely related to autoimmune liver diseases type, and suggest that the overexpression of PML protein in bile duct cells is associated with the development of autoantibodies in patients with PBC or autoimmune cholangitis. Furthermore, PML immunoreactivity may be useful for the diagnosis of autoimmune cholangitis and overlap syndrome.

**Key Words :** Autoimmune diseases; Hepatitis; Primary biliary cirrhosis; Nuclear proteins; PML protein, human

Primary biliary cirrhosis (PBC) is an autoimmune liver disease that selectively affects the small intrahepatic bile ducts and variably hepatocytes.[1] The etiology and pathogenesis of PBC are obscure, however, the presence of autoantibodies including antimitochondrial antibodies (AMAs) supports the autoimmune mechanism of PBC.[2] AMAs react with autoantigens in mitochondria, such as, the 2-oxoacid dehydrogenase complexes (2-OADC)[3] and the E2 component of pyruvate dehydrogenase complex (PDC-E2), the latter of which is aberrantly expressed in the small intrahepatic bile ducts in PBC.[4,5] In addition to AMAs, several antinuclear antibodies (ANAs) have been described in PBC patients,[2,6] and the associated nuclear antigens have been cloned and characterized. These include centromeric proteins,[7] proteins of the nuclear pore complex,[8] nuclear dot (ND) proteins (including Sp100) and promyelocytic leukemia protein (PML).[9,10] Sp100 was the first antigen identified as a target for PBC-specific anti-ND antibodies,[11] and PML is known to colocalize with Sp100 in NDs.[12] Assays for anti-Sp100 antibodies have been performed in various liver and rheumatic diseases.[13] In European patients, anti-Sp100 antibodies were detected in a group of patients with liver diseases, namely in PBC patients at a frequency of about 30%. They were also found to be present in the sera of patients with rheumatic autoimmune diseases, but with a frequency of $\leq$3%. Anti-PML antibody decorates a special type of nuclear body named ND10 or PODs.[6,8,10,14] NDs are distributed in a speckled pattern and on average 10 dots (about 0.3 to 1 $\mu$m in diameter) are present per nucleus, excluding nucleoli.[14] Anti-ND antibodies have been mostly detected by the ELISA using phage display,[15] however immunofluorescent staining patterns of anti-ND antibodies have not been analyzed in PBC patients. There-

fore, the immunostaining patterns of ANAs might be a diagnostic adjunct in autoimmune liver diseases.

Autoimmune hepatitis and PBC have characteristic clinicopathologic features and are treated with different medications. However, nonspecific clinical features and limited biopsy specimens showing mild pathologic changes make definite diagnosis difficult in some cases. In addition, autoimmune cholangitis [16,17] and overlap syndrome of autoimmune hepatitis[18,19] have led to further confusion in the diagnosis and treatment of autoimmune liver diseases. Among the varied diagnostic criteria of autoimmune liver diseases, the pertinent autoantibody types are most helpful for differential diagnosis.

Although nuclear autoantigens have been characterized in autoimmune liver diseases, their immunologic responses against target proteins in liver tissues have not been clarified. Furthermore, the distribution of antigens has not been investigated in the liver tissues of autoimmune liver diseases. In the present study, PML expression was examined in liver biopsy specimens of autoimmune liver diseases, namely, PBC, autoimmune cholangitis, and autoimmune hepatitis. The results obtained were compared with those of normal liver and chronic viral hepatitis livers to elucidate the implication of PML expression in autoimmune liver diseases.

## MATERIALS AND METHODS

### Materials

All 100 cases of liver needle biopsies enrolled were selected from the files of the Department of Pathology, Asan Medical Center. They included 20 cases of PBC, 10 cases of autoimmune cholangitis, 36 cases of autoimmune hepatitis, 12 cases of chronic hepatitis B, and 12 cases of chronic hepatitis C. Autoimmune liver diseases were; PBC, autoimmune cholangitis, and autoimmune hepatitis according to clinical, laboratory and histopathologic characteristics. PBC was diagnosed when suggested by clinical findings, AMA was positive and histopathologic features were compatible with PBC.[1,20] Autoimmune cholangitis was diagnosed when AMA was negative, but the other clinicopathologic features were as those of PBC.[16-18] And, a diagnosis of autoimmune hepatitis was made according to the guidelines issued by the International Autoimmune Hepatitis Group.[21] Patients with chronic B viral hepatitis were positive for hepatitis B viral proteins and HBV DNA, but serologically negative for HCV and autoantibodies, and those with C viral hepatitis were positive for HCV RNA or anti-HCV antibody, but negative for any autoantibodies.

### Histopathology and immunohistochemistry

Liver needle biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Specimens were stained with hematoxylin and eosin or Masson's trichrome for light microscopic examinations. For immunohistochemistry, serial 4 $\mu$m sections were placed on poly-L-lysine coated slides. Sections were deparaffinized and rehydrated using xylene and ethanol. Slides were treated for antigen retrieval by boiling twice in a boiled citrate buffer pH 6.0 for 5 min, then cooled to room temperature over 20 min, kept in Tris buffer, and incubated with the primary antibody for the PML (Santa Cruz, SM-3, California, USA) at a 1:100 dilution for 1 h. Biotinylated secondary antibody was applied for 10 min, and after a wash in the buffer, the slides were incubated in streptavidin conjugated to horseradish peroxidase in Tris-HCl buffer for 10 min. Substrate-chromogen solution, which was prepared from DAKO DAB chromogen tablets, was then applied for 5 min and the slides were then counterstained in Meyer's hematoxylin solution for 5 min. Specimens were mounted using a non-aqueous, permanent mounting medium.

Histopathologic features were analyzed according to a previously described chronic hepatitis grading system.[22] PML expression was graded according to the sum of; its staining intensity in nucleoplasm and the number of NDs in hepatocytes and bile duct epithelial cells as: −, negative; +, mild; ++, moderate to strong.

## RESULTS

### Clinical features

The clinical features of patients with autoimmune liver diseases, including PBC, autoimmune cholangitis and autoimmune hepatitis are summarized in Table 1. No significant difference in age or sex was observed for the autoimmune liver diseases. Serum levels of GOT and GPT were increased in 19 of 20 cases of PBC, in 8 of 10 cases of autoimmune cholangitis, and in all cases of autoimmune hepatitis. Serum alkaline phosphatase and/or gamma glutamyltransferase levels were elevated in all cases of PBC and autoimmune cholangitis, and in 34 (94.4%) of 36 cases of autoimmune hepatitis. The pattern of hyperglobulinemia was variable: in cases of PBC, IgM and IgG levels were enhanced, whereas in autoimmune cholangitis IgG and IgA were enhanced. In autoim-

Table 1. Clinical summary of study cases with autoimmune liver diseases

| Diagnosis (No. of cases) | Age (years) | Sex (M:F) | Abnormal LFT (%) | | Hyperglobulinemia (%) | | | Pattern of ANA (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | GOT/GPT | AP/GT | IgG | IgM | IgA | Sp | Ho | Me |
| PBC (20) | 46.2 | 1:19 | 95.0 | 100.0 | 60.0 | 80.0 | 57.1 | 46.2 | 7.69 | 46.2 |
| AC (10) | 49.2 | 2:8 | 80.0 | 100.0 | 85.7 | 14.3 | 83.3 | 83.3 | 16.7 | 16.7 |
| AH (36) | 46.4 | 1:35 | 100.0 | 94.4 | 71.4 | 29.2 | 33.3 | 62.5 | 31.3 | 6.3 |

PBC, primary biliary cirrhosis; AC, autoimmune cholangitis; AH, autoimmune hepatitis; AP, alkaline phosphatase; GT, gamma glutamyl transferase; Sp, speckled; Ho, homogeneous; Me, membranous.

Table 2. PML immunoreactivity in autoimmune liver diseases and chronic viral hepatitis

| Diagnosis (No. of cases) | No. of cases (%) with PML immunoreactivity | | | | | |
|---|---|---|---|---|---|---|
| | Bile duct epithelial cells | | | Hepatocytes | | |
| | – | + | ++ | – | + | ++ |
| Primary biliary cirrhosis (20) | 2 (10.0) | 10 (50.0) | 8 (40.0) | 10 (50) | 10 (50.0) | 0 (0.0) |
| Autoimmune cholangitis (10) | 0 (0.0) | 9 (90.0) | 1 (10.0) | 7 (70.0) | 2 (20.0) | 1 (10.0) |
| Autoimmune hepatitis (36) | 30 (83.3) | 5 (13.9) | 1 (2.8) | 3 (8.3) | 17 (47.2) | 16 (44.5) |
| Chronic viral hepatitis (24) | 13 (54.2) | 11 (45.8) | 0 (0.0) | 7 (29.2) | 7 (29.2) | 10 (41.6) |

mune hepatitis, IgG levels were markedly increased. Significant ANA elevation was detected in 13 (68.4%) of 19 PBC cases examined, in 8 (80.0%) of 10 cases of autoimmune cholangitis, and in 34 (97.1%) of 36 cases of autoimmune hepatitis. In PBC cases, speckled and nuclear membrane patterns of ANA were detected in 6 (46.2%) of 13 cases, each, and a homogeneous nuclear staining pattern was observed in 1 (7.7%) of 13 cases. In cases of autoimmune cholangitis, a speckled pattern was predominantly detected (7/8, 87.5%), a homogenous pattern in 2 (25.0%) cases, and a nuclear membrane pattern in 1 (12.5%) case. In 34 cases of autoimmune hepatitis, a speckled pattern was observed in 22 (64.7%) cases, a homogeneous pattern in 14 (41.2%) cases, and a nuclear membrane pattern in 2 (5.9%) cases. In the other 2 cases of autoimmune hepatitis, anti-smooth muscle antibody was positive.

## Light microscopic features and immunohistochemical analysis

In cases of PBC, bile duct damage with or without granulomas (stage 1) was present in 1 case, a variable degree of bile duct loss and ductular proliferation (stages 2, 3) in 17 cases (Fig. 2A), and cirrhosis (stage 4) in 2 cases. In contrast to moderate to severe portal activity, lobular activity was minimal in 10 cases, mild in 9 cases, and moderate in 1 case. In autoimmune cholangitis, portal activity was minimal in 2 cases, mild in one, moderate in 4, and severe in 2 cases. The degree of fibrosis was variable ranging from portal fibrosis in 2 cases, periportal fibrosis in 4 cases, septal fibrosis in 2 cases, and cirrhosis in 2 cases. In autoimmune hepatitis, portal activity was minimal in 6 cases, mild in10 cases, moderate in 17 cases, and severe in 3 cases. Lobular activity was minimal in 2 cases, mild in 14 cases, moderate in 8 cases and severe in 12 cases. The degree of fibrosis was portal in 7 cases, periportal in 21 cases, and septal in 8 cases.

PML immunoreactivity results are summarized in Table 2. Normal hepatocytes and bile duct epithelial cells were negative for PML, whereas sinusoidal endothelial cells and Kupffer cells were strongly positive for PML, and showed diffuse nucleoplasmic or multiple nuclear dot staining patterns (Fig. 1).

In 18 (90.0%) of 20 PBC cases, intralobular bile duct epithelial cells were variably positive for PML, whereas hepatocytes were negative in 10 cases (50.0%) and weakly positive in 10 (50.0%) PBC cases (Fig. 2B). All (100%) 10 cases of autoimmune cholangitis revealed mild to moderate immunopositivity for PML in interlobular bile duct epithelial cells, whereas liver cells were negative for PML in 7 (70%) cases of autoimmune cholangitis (Fig. 3). PML expression in bile duct epithelail cells was not detectable in 13 (54.2%) cases and weak in 11 (45.8%) of 24 cases of chronic viral hepatitis.

In 30 (83.3%) of 36 autoimmune hepatitis cases, PML immunoreactivity was not detected in biliary epithelial cells, and was weak to moderately positive in 6 (16.7%). The PML expression in hepatocytes was closely correlated with the degree of inflammatory reaction and hepatocyte damage. Thirty-three (91.7%) of 36 cases of autoimmune hepatitis were variably positive for the PML in hepatocytes (Fig. 4). In viral hepatitis cases, 17 (70.8%) of 24 were positive for PML in hepatocyte. But, no significant difference was observed in PML expression between autoimmune

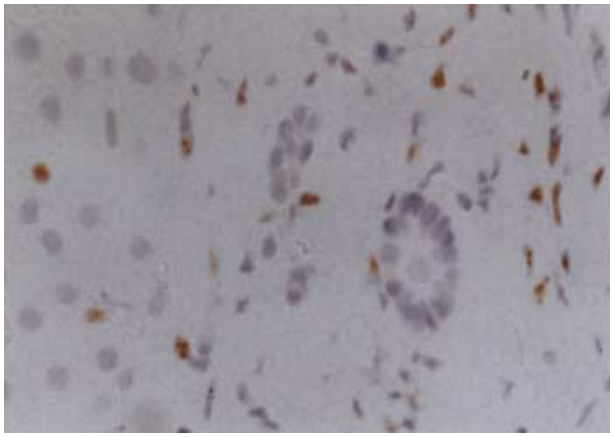

Fig. 1. Expression of the promyleocytic leukemia antibody in normal liver. Kupffer cells are strongly positive for PML. Hepatocytes and bile duct epithelial cells are negative for the PML.

hepatitis and chronic viral hepatitis, and no difference in PML expression was evident between B and C viral hepatitis.

PML expression in bile duct epithelial cells in PBC and in autoimmune cholangitis was significantly elevated compared with that in autoimmune hepatitis ($p<0.001$). In contrast, PML expression in hepatocytes was significantly higher in autoimmune hepatitis than in either PBC or autoimmune cholangitis ($p<0.05$), but no significant difference in hepatocyte PML immunoreactivity was observed between autoimmune hepatitis and chronic viral hepatitis.

## DISCUSSION

In the present study, we did not detect PML immunopositivi-

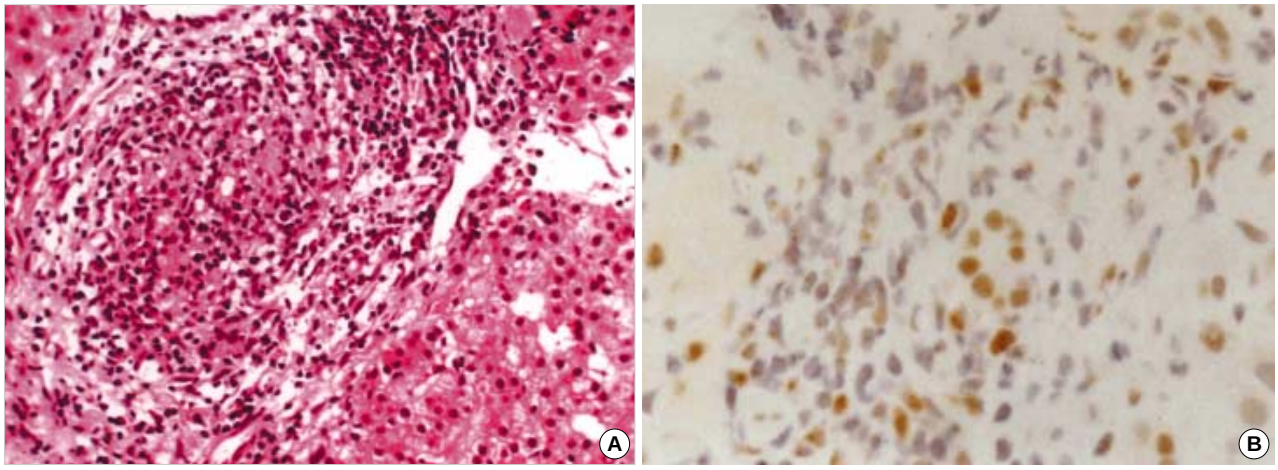


Fig. 2. Expression of the PML in a representative case of PBC. (A) Stage II PBC shows partial loss of bile ducts with granuloma formation. (B) Damaged bile duct epithelial cell are diffusely positive, but the hepatocytes are weakly positive for the PML, only at periportal area.

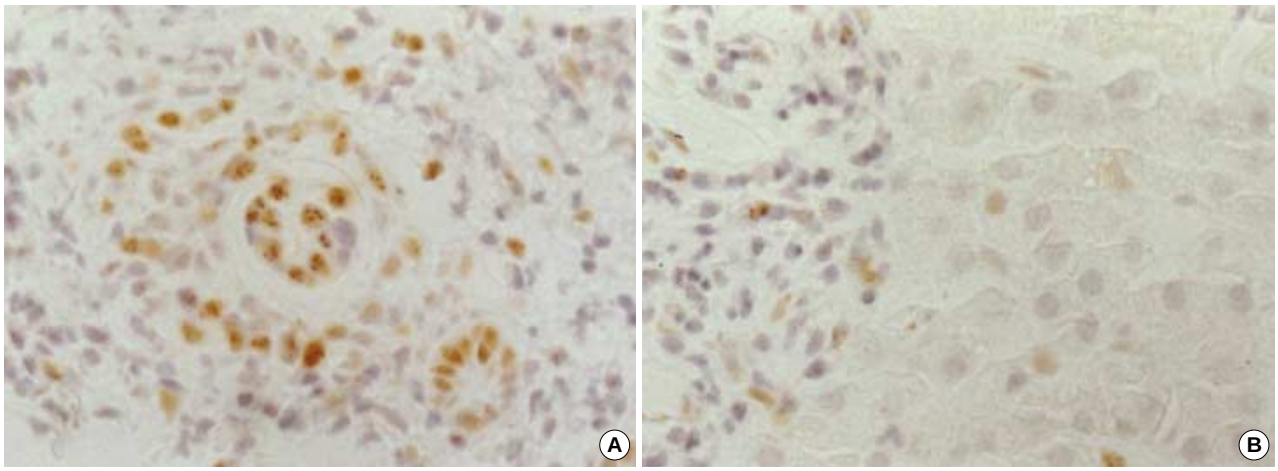


Fig. 3. Expression of the PML in a representative case of autoimmune cholangitis. (A) Bile duct epithelial cells show dot shaped PML nuclear bodies in addition to the diffuse nucleoplasmic positivity for the PML. (B) Hepatocyte nuclei are negative for the PML in the same case.

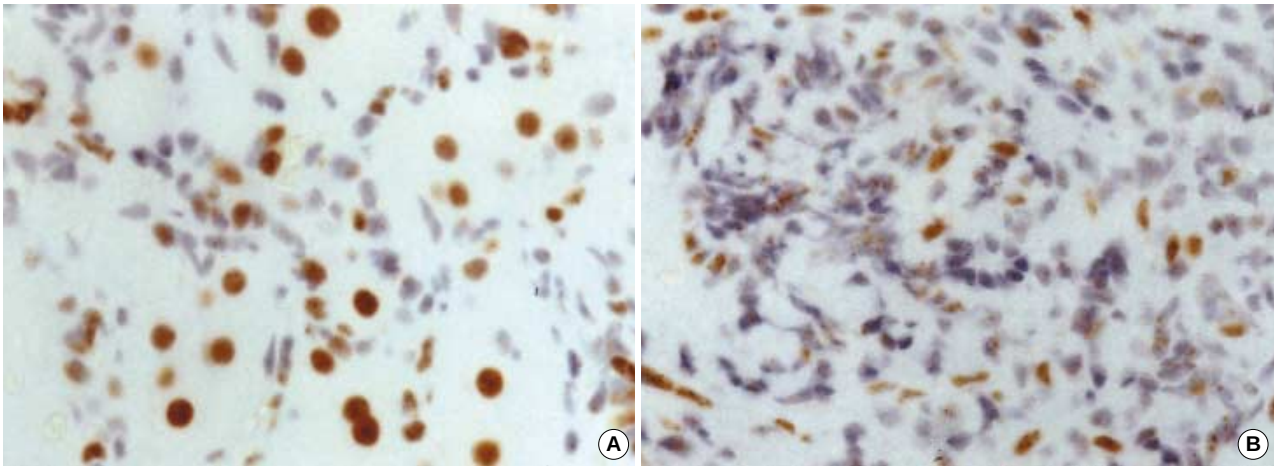


Fig. 4. Expression of the PML in a representative case of autoimmune hepatitis. (A) Ballooned hepatocytes are diffusely and strongly positive for the PML. (B) In the same case, bile ducts are intact and negative for the PML, while endothelial cells are strongly positive for the PML.

ty in the bile duct epithelial cells of formaline-fixed control liver tissue using monoclonal antibody PG-M3. Moreover, because PML is a well-characterized target protein in the autoimmune sera of PBC patients, analyses for PML in autoimmune sera by immunofluorescent stainng or immunoblotting have been extensively performed.[9,12] However, the distribution of PML in liver tissues is less well charaterized.[23-25] Terris et al.[25] using polyclonal anti-PML antibody, detected massive nuclear protein accumulation in portal tracts and lobular parenchyma in the presence of inflammatory liver diseases as compared with weak immunostaining in normal livers. In addition, the overexpression of PML was similar in hepatocytes and in biliary cells in non-viral inflammatory liver diseases like PBC and autoimmune or toxic hepatitis. Weak positive PML staining has been described in a few bile duct epithelial cells on paraffin sections and two to three dots were observed in liver cell nuclei on frozen sections using a polyclonal antibody.[23] However, the monoclonal anti-PML antibody used in this study detects only the 67KD PML isoform. Thus, antibody type may explain the differences in PML expression observed in the present and previous studies.

The patterns of PML expression observed in the present study differed according to inflammatory liver disease type. Furthermore, PML expressions differed significantly in different cell types in hepatitis and autoimmune cholangiopathy, including PBC, although degrees of portal activity were similar. These findings suggest that PML expression can be increased regardless of the degree of inflammatory cell infiltration in patients with PBC and autoimmune cholangitis, thus the PML overexpression may not be a secondary phenomenon against inflammatory cytokines, but may be specifically induced in the bile ducts of PBC and autoimmune cholangitis cases. However, the factors that provoke PML expression have not been identified.

In cases of viral and autoimmune hepatitis, PML overexpression was much more intense in hepatocytes near portal tract interfaces, which indicates that portal inflammatory cells induce PML expression in hepatocytes. PML overexpression may be induced by the interferon produced by inflammatory cells in inflammatory liver diseases or by direct viral stimulation. This assumption is supported by the finding that interferon greatly enhances the PML synthesis as well as that of Sp100 protein in PBC patients[26] and in persistent viral hepatitis cases.[27]

High PML immunopositivity in the bile duct epithelial cells of PBC cases may be associated with the high prevalence of anti-PML antibodies in PBC patients, although no the immunoassay using patient sera was performed in the present study. Anti-PML antibodies were detected simultaneously with anti-Sp100 antibodies in 86% of PBC patients, indicating the specificity of anti-PML antibodies in PBC.[11]

In contrast to autoimmune hepatitis cases, PBC and autoimmune cholangitis cases displayed a similar immunoreactive pattern for PML. Autoimmune cholangitis is histopathologically similar, but differs immunologically from PBC in some respects i.e., the absence of AMA, lower serum IgM and aspartate aminotransferase, a more frequent and higher SMA titer and a significantly higher frequency of ANAs.[28] In our series, cases of autoimmune cholangitis and PBC showed similar immunoreactivity for PML. This result supports that bile duct damage is a common pathogenetic mechanism of PBC and autoimmune cholangitis. However, ANA immunoreactivity patterns using patients' sera differed for PBC and autoimmune cholangitis: the frequency of a speckled pattern was much higher in autoimmune cholangitis. These nuclear speckles represent PML nuclear bodies, in which

PML is a major component.[29] Thus, a higher incidence of a speckled ANA pattern is closely related with a higher frequency of anti-PML antibody in autoimmune cholangitis.

During the late stage of autoimmune cholangitis, which is associated with destruction of bile ducts and ductular proliferation, the expression of PML was significantly increased in proliferating ductules and in damaged bile ducts. PML overexpression in newly bile ductules may be related with cellular proliferation and inflammatory response. In contrast, PML overexpression in normal appearing or damaged bile ducts during the early stage of autoimmune cholangitis, can be explained by either antigenic expression due to autoimmune response or to the effects of cytokine production by portal inflammatory cells. Polyclonality of immune response to Sp100 argues for the direct participation of Sp100 in the autoimmunization process and in the maintenance of the immune response, i.e., an antigen-driven mechanism. A further indication of an antigen-driven process is provided by the frequent co-occurrence of autoantibodies against sp100 and PML, which suggests that these proteins, like small nucleo-ribonulceoprotein particles and mitochondrial multienzyme complexes, participate in the autoimmune event in the form of a highly structured protein complex. Molecular mimicry as a triggering mechanism, however, cannot be excluded.[1] PML has a close structural similarity with the viral EBNA-5 protein in NDs, and therefore, Sp100 and EBV proteins are at least transiently, components of NDs.

In summary, this study indicates that the differential overexpressions of PML in hepatocytes and bile duct epithelial cells are related to autoimmune liver disease type and that the detection of PML in interlobular bile duct epithelial cells is helpful for the diagnosis of AMA-negative PBC (autoimmune cholangitis) and overlap syndrome of autoimmune liver diseases.

## ACKNOWLEDGEMENT

The authors thank for Yoon-Jung Kim for her technical assistance with the immunohistochemical stainings.

## REFERENCES

1. Gershwin ME, Ansari AA, Mackay IR, *et al*. Primary biliary cirrhosis: an orchestrated immune response against epithelial cells. Immunol Rev 2000; 174: 210-25.
2. Mackay IR, Whittingham S, Fida S, *et al*. The peculiar autoimmunity of primary biliary cirrhosis. Immunol Rev 2000; 174: 226-37.
3. Gershwin ME, Mackay IR, Sturgess A, Coppel PL. Identification and specificity of a cDNA encoding the 70kD mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 1987; 138: 3525-31.
4. Joplin R, Lindsay JG, Hübscher SG, *et al*. Distribution of dihydrolipoamide acetyltransferase (E2) in the liver and portal lymph nodes of patients with primary biliary cirrhosis: an immunohistochemical study. Hepatology 1991; 14: 442-7.
5. Joplin R, Lindsay JG, Johnson GD, Strain A, Neuberger J. Membrane dihydrolipoamide acetyltransferase (E2) on human biliary epithelial cells in primary biliary cirrhosis. Lancet 1992; 339: 93-4.
6. Mackay IR, Rowley MJ, Whittingham S. Nuclear antibodies in primary biliary cirrhosis. In: Meyer sum Buschenfelde K-H, Hoofnagle J, Manns M, eds. Immunology and liver. Falk Symposium 70. Dordrecht: Kluwer; 1993; 379-409.
7. Parveen S, Morshed SA, Nishioka M. High prevalence of antibodies to recombinant CENP-B in primary biliary cirrhosis: nuclear immunofluorescence patterns and ELISA reactivities. J Gastroenterol Hepatol 1995; 10: 438-45.
8. Courvalin JC, Worman HJ. Nuclear and envelope protein autoantibodies in primary biliary cirrhosis. Semin Liver Dis 1997; 17: 79-90.
9. Sternsdorf T, Guldner HH, Szostecki C, Grotzinger T, Will H. Two nuclear dot-associated protein, PML and Sp100, are often co-autoimmunogenic in patients with primary biliary cirrhosis. Scand J Immunol 1995; 42: 257-68.
10. Szostecki C, Guldner HH, Will H. Autoantibodies against "nuclear dots" in primary biliary cirrhosis. Semin Liver Dis 1997; 17: 71-8.
11. Szostecki C, Guldner HH, Netter HJ, Will H. Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. J Immunol 1990; 145: 4338-47.
12. Züchner D, Sternsdorf T, Szostecki C, Heathcote EJ, Cauch-Dudek K, Will H. Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp100 and promyelocytic leukemia protein in a large cohort of patients with primary biliary cirrhosis. Hepatology 1997; 26: 1123-30.
13. Muratori P, Muratori L, Cassani F, *et al*. Anti-multiple nuclear dots (anti-MND) and anti-SP100 antibodies in hepatic and rheumatological disorders. Clin Exp immunol 2002; 127: 172-5.
14. Maul GG, Negorev D, Bell P, Ishov AM. Review: properties and assembly mechanisms of ND10, PML bodies, or PODs. J Struct Biol 2000; 129: 278-87.
15. Ikuno N, Scealy M, Davies JM, *et al*. A comparative study of antibody expressions in primary biliary cirrhosis and autoimmune cholangitis using phage display. Hepatology 2001; 34: 478-86.
16. Goodman ZD, McNally PR, Davis DR, Ishak KG. Autoimmune

cholangitis: a variant of primary biliary cirrhosis. Clinicopathologic and serologic correlations in 200 cases. Dig Dis Sci 1995; 40: 1232-42.
17. Kinoshita H, Omagari K, Whittingham S, *et al*. Autoimmune cholangitis and primary biliary cirrhosis-an autoimmune enigma. Liver 1999; 19: 122-8.
18. Czaja AJ. Frequency and nature of the variant syndrome of autoimmune liver diseases. Hepatology 1998; 28: 360-5.
19. Lohse AW, zum Büschenfelde KH, Franz B, Kanzler S, Gerken G, Dienes HP. Characterization of the overlap syndrome of primary biliary cirrhosis (PBC) and autoimmune hepatitis:evidence for it being a hepatitis from of PBC in genetically susceptible individuals. Hepatology 1999; 29: 1078-84.
20. Nakanuma Y, Tsuneyama K, Gershwin ME, Yasoshima M. Pathology and immunopathology of primary biliary cirrhosis with emphasis on bile duct lesions: recent progress. Semin Liver Dis 1995; 15: 313-28.
21. Alvarez F, Berg PA, Bianchi FB, *et al*. International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999; 31: 929-38.
22. Park YN, Kim HG, Chon CY, *et al*. Histological grading and staging of chronic hepatitis standardized guideline proposed by the Korean study group for the pathology of digestive disease. Korean J Pathol 1999; 33: 337-46.
23. Cho Y, Lee I, Maul GG, Yu E. A novel nuclear substructure, ND10: Distribution in normal and neoplastic human tissues. Int J Mol Med 1998; 1: 717-24.
24. Gambacorta M, Flenghi L, Fagioli M, *et al*. Heterogeneous nuclear expression of the promyelocytic leukemia (PML) protein in normal and neoplastic human tissues. Am J Pathol 1996; 149: 2023-35.
25. Terris B, Baldin V, Dubois S, *et al*. PML nuclear bodies are general targets for inflammation and cell proliferation. Cancer Res 1995; 55: 1590-7.
26. Guldner HH, Szostecki C, Grotzinger T, Will H. IFN enhance expression of Sp100, an antoantigen in primary biliary cirrhosis. J Immunol 1992; 149: 4067-73.
27. Brasch K, Ochs RL. Nuclear bodies (NBs): A newly "Rediscovered" organelle. Exp Cell Res 1992; 202: 211-23.
28. Michielleti P, Wanless IR, Katz A, *et al*. Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. Gut 1994; 35: 260-5.
29. Negorev D, Maul GG. Cellular proteins localized at and interacting within ND10/PML nuclear bodies/POPs suggest functions of a nuclear depot. Oncogene 2001; 20: 7234-42.
30. Szekely L, Pokrovskaja K, Kiang WQ, de The H, Ringertz N, Klein G. The Epstein-Barr virus-encoded nuclear antigen EBNA-5 accumulates in PML-containing bodies. J Virol 1996; 70: 2562-8.