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Immunoelectron Microscopic Localization of MHC Class 1 and 2 Antigens on Bile Duct Epithelial Cells in Patients with Primary Biliary Cirrhosis

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Abstract

We studied the distribution of class 1 and class 2 major histocompatibility complex (MHC) antigens on bile duct epithelial cells in liver from patients with primary biliary cirrhosis (PBC) by an immunohistochemical method using monoclonal antibodies to HLA-ABC products and HLA-D subregion products (HLA-DR, -DP, -DQ). By light microscopy, the expression of MHC class 1 antigens (HLA-ABC antigens) was enhanced in PBC compared with controls. While negligible staining of MHC class 2 antigens was detected on the bile duct in controls, de novo expression of MHC class 2 antigens, as well as the coexpression of HLA-DR, HLA-DQ, and HLA-DP antigens on the bile duct epithelial cells, was observed in PBC. By electron microscopy, HLA-ABC and HLA-DR antigens were present preferentially along the basolateral domain of the cell surface of the bile duct epithelial cells and on the membrane of the endoplasmic reticulum in the cytoplasm, suggesting that these MHC antigens are synthesized by the bile duct epithelial cells in PBC. The distribution of these MHC antigens on the basolateral surface of the bile duct epithelial cells, where they are easily accessible to immunocytes, supports the idea that MHC-restricted cytotoxic T lymphocytes are involved in the bile duct injury in PBC.

KEYWORDS: MHC class I antigens, MHC class 2 antigens, bile duct epithelial cell, primary biliary cirrhosis

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We studied the distribution of class 1 and class 2 major histocompatibility complex (MHC) antigens on bile duct epithelial cells in liver from patients with primary biliary cirrhosis (PBC) by an immunohistochemical method using monoclonal antibodies to HLA-ABC products and HLA-D subregion products (HLA-DR, -DP, -DQ). By light microscopy, the expression of MHC class 1 antigens (HLA-ABC antigens) was enhanced in PBC compared with controls. While negligible staining of MHC class 2 antigens was detected on the bile duct in controls, de novo expression of MHC class 2 antigens, as well as the coexpression of HLA-DR, HLA-DQ, and HLA-DP antigens on the bile duct epithelial cells, was observed in PBC. By electron microscopy, HLA-ABC and HLA-DR antigens were present preferentially along the basolateral domain of the cell surface of the bile duct epithelial cells and on the membrane of the endoplasmic reticulum in the cytoplasm, suggesting that these MHC antigens are synthesized by the bile duct epithelial cells in PBC. The distribution of these MHC antigens on the basolateral surface of the bile duct epithelial cells, where they are easily accessible to immunocytes, supports the idea that MHC-restricted cytotoxic T lymphocytes are involved in the bile duct injury in PBC.

Key words: MHC class I antigens, MHC class 2 antigens, bile duct epithelial cell, primary biliary cirrhosis

Primary biliary cirrhosis (PBC), which predominantly affects middle aged women, is characterized by the gradual inflammatory destruction of interlobular and septal bile duct cells, resulting in cirrhosis (1). Several immunological aberrations, including polyclonal increase in serum IgM, the presence of circulating antibody to mitochondria, increased immune complexes, long-term complement activation, and the clinical manifestations of various autoimmune disorders, indicate that immunological factors are important in the pathogenesis and progression of the disease (2). While studies attempting to analyze the phenotypes of circulating lymphocytes have yielded conflicting results (3, 4), immunohistochemical analyses of the hepatic lesions of PBC have revealed that the majority of mononuclear infiltrates around the bile ducts consisted of cytotoxic T cells (5, 6). Cytotoxic T lymphocytes were frequently observed in the intraepithelial space of the bile duct, in close contact with the bile duct epithelial cells (6), suggesting that immunological mechanisms mediated by cytotoxic T lymphocytes are involved in the damaging of bile duct cells.

A variable immune response mediated by T lymphocytes is closely linked to the major histocompatibility complex (MHC) expressed on target cells (7, 8), and expression of MHC gene products on bile duct cells seems important for T cell-mediated bile duct injury in PBC. In this study, we examined immunohistochemically the distribution of MHC class 1 and 2 antigens on bile duct epithelial cells in patients with PBC.

Materials and Methods

Tissues. Liver biopsy specimens were obtained from 7 patients with PBC during laparoscopic examination. The clinical background of the patients is summarized in Table 1. The diagnosis of PBC was made on the

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Table I Clinical, histological and serological data of 7 patients with primary biliary cirrhosis

Case no	Age	Sex	Itching/ jaundice	Histological stage[a]	AMA[b]	s-GPT (IU/l)	ALP (IU/l)	HBsAg	Anti-c1003
1	51	F	−	I	+	60	711	−	−
2	47	F	−	I	+	83	347	−	−
3	37	F	−	II	+	29	136	−	−
4	78	M	−	II	+	46	297	−	−
5	55	F	−	II	+	41	276	−	−
6	59	F	+	III	+	51	371	−	−
7	35	F	+	III	+	55	285	−	−

a: According to the staging system of Scheuer (22) *b:* Antibody to mitochondria

basis of accepted clinical, serological and histological criteria (5). The histological grading according to Scheuer's criteria (9) was: grade 1 (n = 2), grade 2 (n = 3), and grade 3 (n = 2). As controls, we used liver biopsies obtained from 7 patients with chronic hepatitis B (3 chronic persistent hepatitis and 4 chronic active hepatitis). Informed consent for the procedure was obtained from all patients. None of the patients studied had received immunosuppressive therapy before the biopsies were taken. One-half of each tissue specimen was fixed in Bouin's solution for routine histological examination, and the other half was fixed in periodate-lysine-paraformaldehyde fixative (10) for immunohistochemical staining.

Immunohistochemistry. For light microscopic studies, cryostat sections of liver specimens, pretreated with periodic acid and sodium borohydrate to inactivate endogenous tissue peroxidase (11), were incubated for 12 h at 4°C with mouse monoclonal antibodies to HLA-ABC for MHC class 1 antigens, or HLA-DR, HLA-DP, or HLA-DQ for MHC class 2 subregion products (Becton-Dickinson, Mountain View, CA, USA). The sections were then incubated for 4 h at 4°C with horseradish peroxidase-labeled Fab' fragments of rabbit antimouse immunoglobulins (HRP anti-mouse Ig, DAKOPATTS, Glostrup, Denmark) (12), followed by incubation for 10 min with 0.025 % diaminobenzidine solution containing 0.005 % hydrogen peroxide; the sections were then counterstained with methyl green, dehydrated, and mounted.

For immunoelectron microscopy, the sections were reacted with the monoclonal antibody and the HRP anti-mouse Ig in the same way as in the light microscopic studies. They were then postfixed with 2 % glutaraldehyde for 20 min and incubated sequentially with diaminobenzidine solution for 30 min and diaminobenzidine solution containing hydrogen peroxide for 10 min. The stained sections were osmicated, washed, dehydrated, and embedded in Epon-Araldite. Ultrathin sections were examined with a Hitachi H 700H electron microscope without additional staining.

Results

Light microscopic observation. In all the patients with PBC, HLA-ABC antigens were observed on the sinusoidal lining cells, on the infiltrating mononuclear cells, and on the bile duct epithelial cells (Table 2, Fig. 1A). Expression of HLA-ABC was also found on the surface of hepatocytes, especially in the periportal zone with piecemeal necrosis. In the control tissues of chronic hepatitis B, almost the same distribution of HLA-ABC antigens was observed, but the staining of the HLA-ABC antigens in the bile duct epithelial cells was more prominent in PBC than in chronic hepatitis B.

While negligible staining of MHC class 2 antigens was detected on the bile duct in chronic hepatitis B, HLA-DR

and HLA-DP antigen-positive bile duct epithelial cells were observed in all the patients with PBC, and HLA-DQ-positive bile duct cells were observed in 5 of the 7 patients (Table 2). These MHC class 2 antigens were positive in non-affected bile duct epithelial cells that had sparse infiltrates of lymphocytes, as well as in degenerating bile duct epithelial cells with dense infiltrates. In the analysis of serial sections, the coexpression of HLA-DR, HLA-DP, and HLA-DQ antigens was also observed in some bile duct epithelial cells in the PBC patients (Fig. 1B,C,D). MHC class 2 antigens were also observed on sinusoidal lining cells and infiltrating lymphocytes in both patients with PBC and chronic hepatitis B, but no expression on hepatocytes was detected in either PBC or chronic hepatitis B.

Table 2 Expression of major histocompatibility complex products on bile duct epitherial cells in primary biliary cirrhosis

	Number of bile ducts (Antigen-positive/Total)			
Case no	HLA-ABC	HLA-DR	HLA-DP	HLA-DQ
1	8/8	2/6	6/9	4/7
2	13/13	12/15	5/8	2/5
3	5/5	1/4	2/3	0/4
4	6/6	4/4	4/4	4/4
5	ND	1/1	1/1	1/2
6	8/8	5/5	5/7	5/5
7	1/1	1/2	1/1	0/1

ND: Not detected.

Electron microscopic observation. In the PBC patients, electron-dense reaction products, indicating the ultrastructural sites of HLA-ABC antigens, were

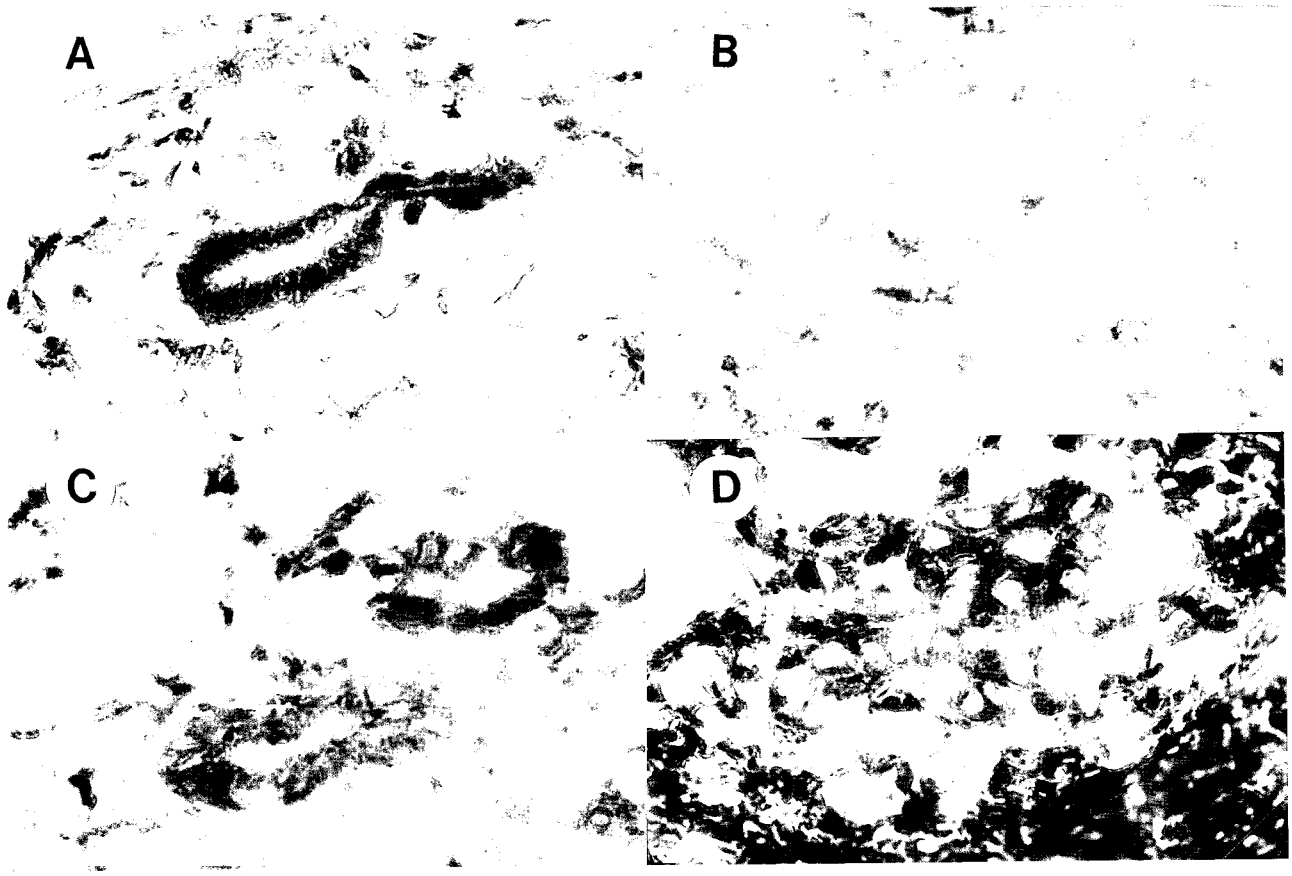

Fig. 1 Immunohistochemical staining of HLA-ABC, HLA- DR, HLA-DP, and HLA-DQ antigens in the bile duct epithelial cells of primary biliary cirrhosis (Case 4). Nuclei are counterstained with methylgreen. (**A**) Strong expression of HLA-ABC in the bile duct epithelial cells is observed. (**B, C, D**) In the analysis of serial sections, the coexpression of HLA-DR (**B**), HLA-DP (**C**), and HLA-DQ (**D**) antigens in the bile duct epithelial cells is observed. Inflammatory cells and stromal cells are also positive.

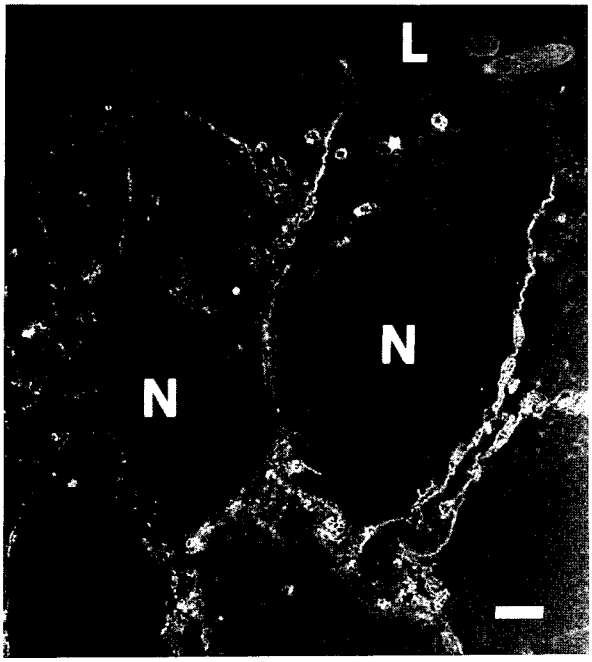

Fig. 2 Immunoelectron microscopic localization of HLA-ABC antigens in the bile duct epithelial cells of primary biliary cirrhosis (Case 3). Electron-dense reaction products, indicating ultrastructural sites of HLA-ABC antigens are present on the basolateral plasma membranes of the bile duct epithelial cells, while amounts are negligible on the apical surfaces. In the cytoplasm, the antigens are present on the membrane of the endoplasmic reticulum. (**L**: lumen of the bile duct, **N**: nucleus) (Bar = 1 μm)

present on the basolateral plasma membrane of the bile duct epithelial cells, but the amounts were negligible on the apical surface (Fig. 2). In the cytoplasm, HLA-ABC antigens were located on the membrane of the endoplasmic reticulum (Fig. 2). HLA-DR antigens were also expressed on the cell surface, preferentially on the basolateral domain, in the bile duct epithelial cells of patients with PBC (Fig. 3A). In some bile duct epithelial cells in patients with PBC, the membrane of the endoplasmic reticulum was positive for HLA-DR antigens (Fig. 3B) suggesting the synthesis of MHC class 2 antigens on these cells.

Discussion

We immunohistochemically demonstrated the enhanced expression of HLA-ABC antigens (MHC class 1 antigens) and the de novo expression of HLA-DR, -DP, and -DQ antigens (MHC class 2 antigens) on bile duct epithelial cells in patients with PBC. Various kinds of cytokines produced by infiltrating mononuclear cells are known to induce the expression of these MHC antigens. Enhanced expression of MHC class 1 antigens was induced on hepatocytes and on bile duct epithelial cells after exposure to interferon (IFN)-α, -β and- γ (13, 14). The de novo expression of class 2 antigens was induced

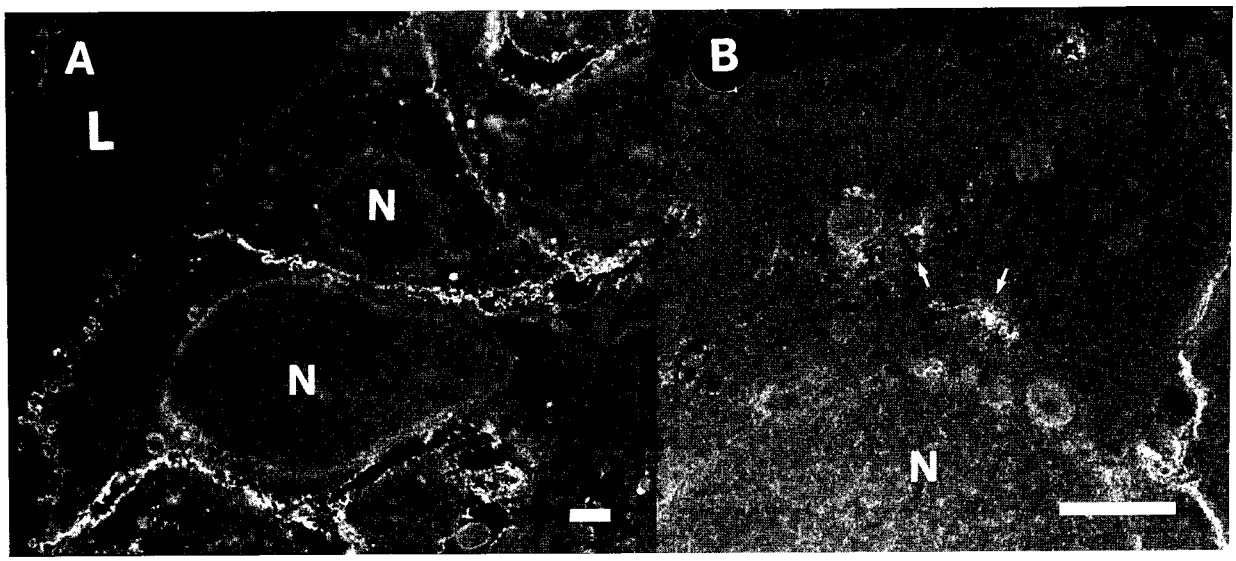

Fig. 3 Immunoelectron microscopic localization of HLA-DR antigens in the bile duct epithelial cells of primary biliary cirrhosis (Case 3). (**A**) HLA-DR antigens are expressed on the cell surfaces of the bile duct epithelial cells, preferentially in the basolateral domain. (**B**) In the cytoplasm, the membrane of the endoplasmic reticulum is positive for HLA-DR antigens (arrows). (**L**: lumen of the bile duct, **N**: nucleus) (Bar = 1 μm)

by IFN-γ and tumor necrosis factor α (15-17). Thus, it is possible that the enhanced expression of MHC class 1 antigens and the aberrant expression of class 2 antigens on bile duct epithelial cells observed here could have been induced by various cytokines liberated from the infiltrating mononuclear cells.

Our immunoelectron microscopic study also revealed that both the MHC class 1 and 2 antigens were preferentially distributed on the basolateral domain of the surfaces of the bile duct epithelial cells. We have previously shown that a majority of lymphocytes infiltrating the bile duct in chronic nonsuppurative destructive cholangitis, which is a characteristic feature of the bile duct injury in PBC, were CD8 + cytotoxic T lymphocytes (6). The enhanced HLA-ABC expression on the basolateral surface of the bile duct epithelial cells, where lymphocytes are accessible, may render these cells more susceptible to attack by CD8-positive cytotoxic T cells in PBC. In accordance with our findings, Nakanuma and Yoshida (18) reported, in an immunoelectron microscopic study that $\beta 2$ microglobulin, a variant chain of HLA-ABC antigens, was also detected linearly along the lateral surface of bile duct epithelial cells. Our observations of the enhanced expression of HLA-ABC antigens in PBC are also compatible with the reports of Ballardini *et al.* (19) and Van den Oord *et al.* (14).

The expression of MHC class 2 products is normally restricted to specialized cells, such as macrophages, B lymphocytes, endothelial cells, and dendritic reticular cells, which have the function of antigen presentation to T cells (20, 21). Indeed, we found that the bile duct epithelial cells in chronic hepatitis B did not display MHC class 2 antigens and other studies have demonstrated the occasional expression of HLA-DR antigen without the coexpression of HLA-DQ and HLA-DP in the bile duct in non-A non-B hepatitis and autoimmune hepatitis (14, 22). In PBC, in contrast, in our analysis of serial sections we detected HLA-DR antigens on bile duct epithelial cells, and the coexpression of HLA-DR, HLA-DP, HLA-DQ antigens as well. Although the de novo expression of MHC class 2 antigens in the bile duct of PBC has been reported by others (14, 19, 22), we further demonstrated, by immunoelectron microscopy, that HLA-DR was present on the basolateral surfaces of the bile duct epithelial cells and that it was also detectable on the membrane of the endoplasmic reticulum in the cytoplasm, suggesting that bile duct epithelial cells in PBC carry out the de novo synthesis of this antigen. These findings suggest that bile duct epithelial cells that coexpress HLA-DR and HLA-DQ antigens in PBC may preferentially present target antigen (s) in PBC and/or induce of MHC class 2 antigen-restricted cytotoxic T lymphocytes.

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