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Immunologic Reaction and Genetic Factors in Biliary Atresia

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Nakada, M., Nakada, K., Kawaguchi, F., Wakisaka, M., Kashimura, T., Yamate, N., Maeyama, S. and Uchikoshi, T. *Immunologic Reaction and Genetic Factors in Biliary Atresia.* Tohoku J. Exp. Med., 1997, **181** (1), 41-47 —— The characteristic histopathological features seen in the livers of patients with biliary atresia (BA) are very similar to those of primary biliary cirrhosis, which is an autoimmune disease. To clarify whether BA liver possesses an immunological response similar to that in primary biliary cirrhosis, we studied HLA-DR expression in liver tissue of BA patients, using a HLA-DR staining method, and determined the frequency of HLA types in BA patients and their families. HLA-DR was expressed by the bile duct epithelium in 11 of 16 liver specimens obtained from 13 BA patients. By contrast, HLA-DR was not expressed in liver specimens from 6 patients with congenital biliary dilatation. Among the HLA types seen in BA patients and their families, HLA-A33, -B44 and -DR6 were frequently expressed in blood. These results suggest that certain immunological factors and disease-susceptible genes might be involved in the etiology of BA. —— biliary atresia; immunologic reaction; HLA-DR; genetic factor; etiology

Biliary atresia (BA) often shows progressive morphological changes of the liver due to massive intrahepatic bile stagnation even after Kasai's operation. We have noticed that such features are very similar to those of a progressive cholestatic liver disease, namely, primary biliary cirrhosis (PBC) in adults. PBC is an autoimmune disease in which aberrant expression of human leukocyte antigen (HLA) class II by bile duct epithelial cells is observed (Phillips et al. 1993). It has been suggested that patients with autoimmune disease, including

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PBC, possess certain disease-susceptible genes.

To clarify whether the etiology of BA involves an immunologic reaction (Ohya et al. 1995), genetic factors, or both, we examined the expression of HLA class II antigens in the liver tissue of patients with BA and we also examined the HLA types of the BA patients and their families serologically.

Methods

Patients. Sixteen hepatic tissue samples obtained from 13 patients with BA were examined immunohistochemically, and were compared with 6 liver samples from children with congenital biliary dilatation (CBD) and 10 from adults with PBC. The clinical and histopathological characteristics of the 13 BA patients are summarized in Table 1; each patient had undergone Kasai's hepatic portoenterostomy (at an average age of 82 days) with the diagnosis of type III atresia, with 1 exception (patient 4). Eight patients had attained adequate biliary diversion and their serum bilirubin levels fell to within the normal range. Seven patients had suffered from frequent postoperative ascending cholangitis, 3 of whom died. Histopathologically, we classified the hepatic fibrosis of BA biopsy specimens into 4 grades: hepatic fibrosis (HF) 1~3, and cirrhosis (LC). No patient was classified as having HF1, in which liver tissue showed minimal change. Eight

Table 1. *Clinical characteristics of patients with biliary atresia*

No.	Sex	Date of initial operation (days)	Type of obstraction	Jaundice	Cholangitis	Histology	Status
1	M	97	IIIc1 μ	+	+	HF3	Dead
2	F	83	IIIa1 ν	−	−	HF3	Alive
3	F	76	IIIc1 ν	−	+	HF3	Alive
4	M	62	I cyst	−	−	LC	Alive
5	F	110	IIIb1 μ	−	−	HF2	Alive
6	F	64	IIIb1 ν	−	−	HF2	Alive
7	M	109	IIIb1 ν	+	−	HF3	Dead
8	F	64	IIIa1 μ	−	+	HF2	Alive
9	M	65	IIIb1 ν	−	+	HF2	Alive
10	F	106	IIIc1 ν	−	−	LC	Alive
11	F	68	IIIb1 ν	+	+	HF2	Alive
12	M	65	IIIb1 μ	+	+	HF3	Dead
13	M	62	IIIc1 ν	+	−	HF2	Alive

Sixteen liver samples were obtained from 13 BA patients, as those who underwent Kasai's procedure, listed in this table. All patients except one had an atresia type III. Eight patients had attained adequate biliary drainage. Eight samples were classified into HF2, which meant the hepatic fibrosis were moderate. Six sample into HF3, severe fibosis, and the rest 2 samples were LC.

samples were classified as HF2, in which liver showed moderate fibrosis and with focal portal-poratal and/or central fibrosis, and 6 samples were classified as HF3, in which the liver showed severe fibrosis and with a tendency of lobular disorganization. Another 2 specimens showed LC.

Histological procedure. HLA-DR (class II) antigen was detected by immunohistochemical staining with a HLA-DR monoclonal antibody (CR 3/43, DAKO) by the labeled streptavidin biotin peroxidase method. Aberrant expression of HLA-DR in the bile duct epithelium of BA specimens was graded into 3 levels of staining: no staining, focally stained and extensive staining over the semicircular area of the bile canaliculi as shown in Fig. 1.

HLA antigen determination. HLA-A, -B and -C antigens (class I antigens) were measured serologically and HLA-DQ, -DR antigens (class II antigens) were detected by the microdloplet lymphocyte cytotoxicity test (Terasaki et al. 1978). Normal control values were taken from the proceedings of the 11th International Histocompatibility Workshop and Conference held in 1991 (Imanishi et al. 1991).

Statistical analysis. The values were analyzed by the chi-squared test and a p value of less than 0.05 was considered to indicate statistical significance.

Results

Expression of HLA-DR in bile duct epithelial cells. HLA-DR was expressed by the bile duct epithelium in 10 liver specimens obtained from patients with PBC seen as strongly positive staining in Fig. 2, whereas HLA-DR was not detected in 6 liver specimens from patients with CBD. In BA livers, HLA-DR was expressed in bile duct epithelial cells as well as in periportal and sinusoidal areas which is a similar pattern of expression to that seen in PBC or viral hepatitis. The bile duct epithelium of 11 out of the 16 biopsy specimens obtained from BA patients showed HLA-DR positive staining, including 5 specimens that were extensively stained, but the other 5 specimens lacked HLA-DR staining (Table 2). This aberrant expression of HLA-DR in bile duct epithelial cells of BA livers had no relation to the grade of hepatic fibrosis.

Class I and class II major histocompatibility complex antigen in BA patients and their families. The frequencies of HLA class I 31 and 33 at the A locus, 44 and 55 at the B locus and 3 at the C locus were significantly higher in BA patients and their families ($p<0.05$) than in the control subjects. The frequencies of the DR4 antigen (class II) was significantly lower than in the controls (Table 3).

Discussion

BA is considered to be an etiologically heterogeneous disorder (Mowat et al. 1994). In the hepatic tissues of BA patients, we often find degeneration of the bile duct epithelial cells with a marked inflammatory cell response, fibrotic change with considerable bile stagnation in the parenchyma (Dehner 1987), and a slow progression towards the terminal phase of liver tissue destruction. These his-

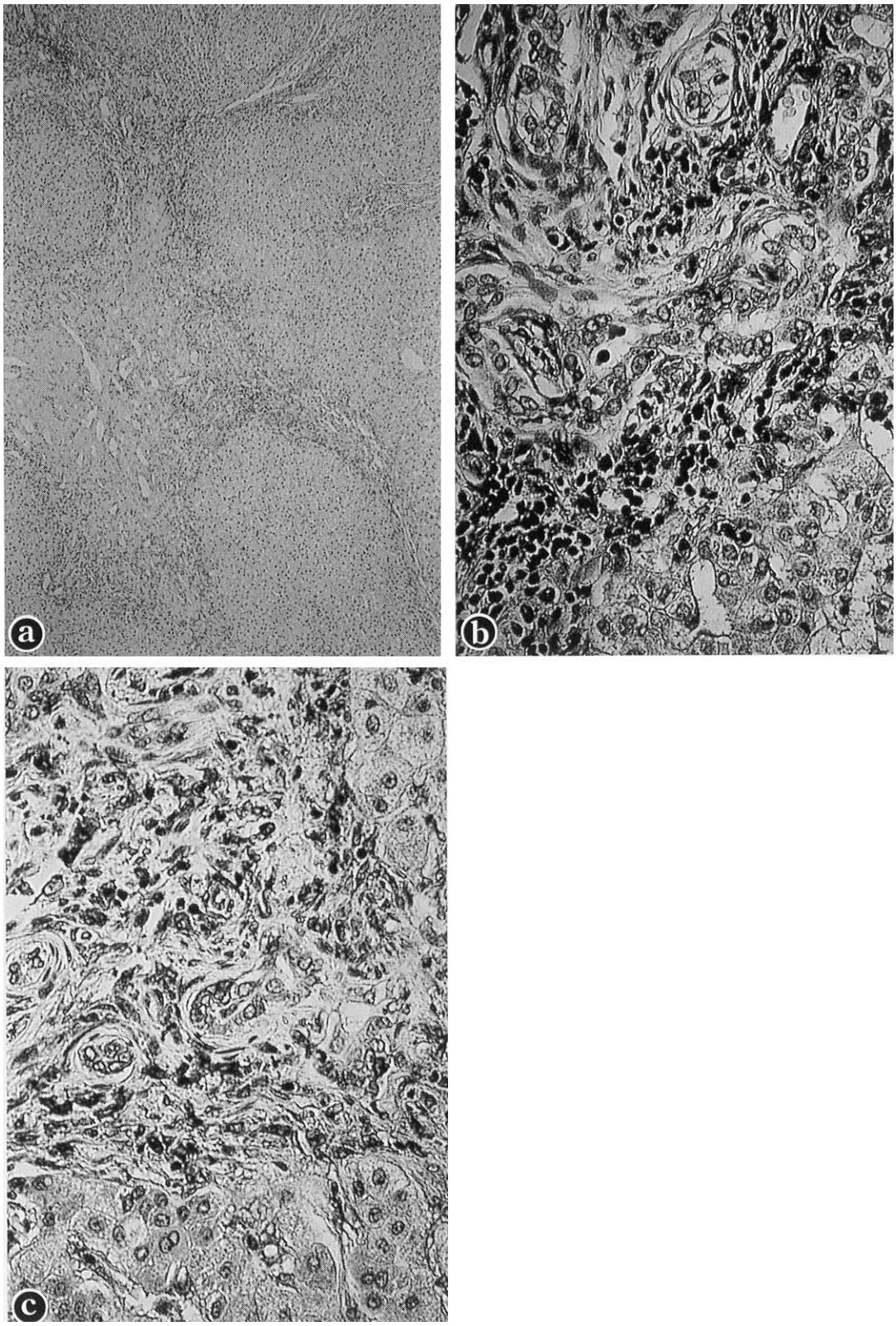

Fig. 1. Examples for immunological staining results of HLA-DR.
(a) Negative (×20), (b) focal (×400), and (c) extensive staining of HLA-DR in bile duct epithelial cells.

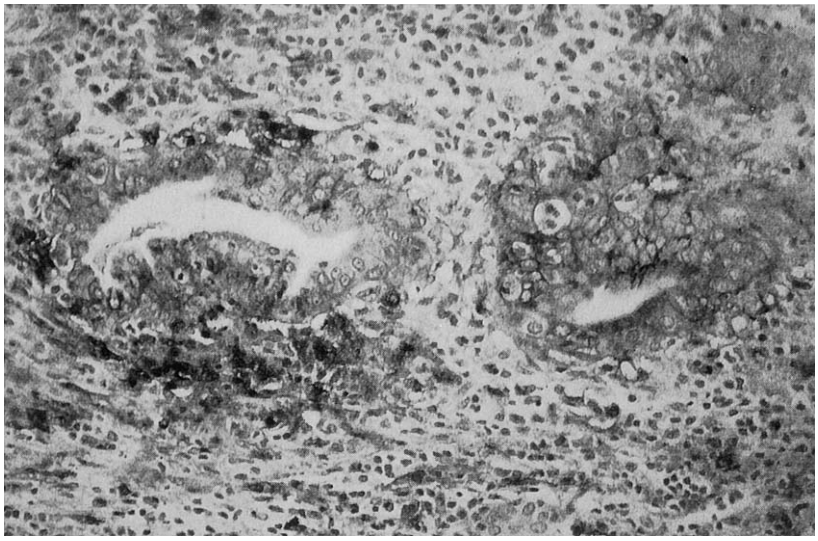

Fig. 2. HLA-DR staining of the liver with primary biliary cirrhosis.
Marked expression of HLA-DR in primary biliary cirrhosis was shown by the extensive staining in bile duct epithelial cells.

TABLE 2. *Aberrant expression of HLA-DR antigens in bile duct epithelium*

		Number	Intensity of HLA-DR expression		
			−	+	++
BA	HF2	8	4	2	2
	HF3	6	0	2	4
	LC	2	1	1	0
	Subtotal	16	5	5	6
CBD	HF1	3	3	0	0
	HF2	1	1	0	0
	HF3	1	1	0	0
	LC	1	1	0	0
	Subtotal	6	6	0	0

BA, biliary atresia; CBD, congenital biliary dilatation.
HLA-DR was not expressed in 6 specimens of liver from CBD. The bile duct epitherium of 11 out of 16 biopsy specimens obtained from BA showed positive, including strongly positive in 5.

tological findings are similar to the changes seen in PBC, which is one of the characteristic autoimmune diseases (James et al. 1983; Onishi et al. 1994; Mella et al. 1995). It has been suggested that the chronic cholestasis seen in PBC may cause in part an aberrant expression of HLA-DR by bile duct epithelial cells (Phillips et al. 1993) and it has been shown that more activated T-lymphocytes exacerbates immunologically mediated cytotoxicity (Kaplan 1990; Diu et al. 1993).

Therefore we studied the etiology of BA from the aspect of immunologic and genetic considerations using immunological staining methods and blood analysis.

TABLE 3. *Analysis of HLA types in biliary atresia and their families*

HLA	BA	N	HLA	BA	N	HLA	BA	N	HLA	BA	N	HLA	BA	N
A2	16.7	24.3	B35	8.3	8.1	Cw1	20.0	11.8	DR2	11.8		DQ1	30.0	45.6
A11	16.7	10.4	B39	8.3	4.5	Cw3	60.0	39.0	DR4	5.9	22.8	DQ3	30.0	18.8
A24	25.0	35.1	B44	16.7	7.4	Cw7	20.0	15.3	DR6	23.5		DQ4	10.0	14.9
A26	8.3	10.9	B51	16.7	9.3				DR9	17.6	13.0	DQ6	20.0	
A31	16.7	8.0	B52	8.3	10.7				DR52	23.5		DQ7	10.0	15.2
A33	16.7	7.0	B54	8.3	6.3				DR53	17.6				
			B55	8.3	2.9									
			B61	16.7	10.7									
			B62	8.3	8.3									

BA, biliary atresia ($n=14$); N, normal subjects ($n=1024$).

The results of blood samples examination for the BA patients and their families. Frequency of HLA calss 1 antigens of 31 and 33 at A locus, 44 and 55 at B locus and 3 at Cw locus were significantuly higher than control subjects. However, DR4 was lower than the controls ($p<0.05$).

In this study, we detected the expression of HLA-DR in bile duct epithelial cells of the liver tissue from patient with PBC, which has been reported in previous investigations (Broome et al. 1993; Yasoshima et al. 1995). Moreover, we observed aberrant expression of HLA-DR in bile duct epithelial cells in 64.2% of the BA liver samples examined. By contrast, the liver specimens from patients with CBD did not show HLA-DR expression by bile duct epithelial cells. These results suggest that the etiology of BA is different to that of CBD, and that the immunological response observed in BA might participate in the destruction of intrahepatic bile canaliculi.

It is generally thought that BA is not a hereditary disorder (Moore and Hyman 1985). However, previous investigators have reported a certain level of familial occurrence of BA (Ksrrer and Raffensperger 1990; Silvenia et al. 1991) and/or association of other anomalies (Gautier 1979), although a low frequencies. In addition, HLA-B12, an infectious disease-susceptible gene, has been identified in BA patients (Mowat 1994), which suggests that BA might involve some disease-susceptible genes (Ito et al. 1994).

The results of our serological study indicates that disease-relating genes for BA may be susceptible by HLA class I status, in people susceptible to BA.

In conclusion, our result support the hypothesis that BA is an etiologically heterogeneous disorder. However, the most important factor for intrahepatic progressive change in BA may be an immunologic reaction induced by perinatal infection (such as neonatal hepatitis), environmental factors or some toxic agents. Moreover, some genetic factors might change the susceptibility of individuals to these factors.

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