Journal of Pediatric Gastroenterology and Nutrition
16:114–117 © 1993 Raven Press, Ltd., New York

Association Between HLA and Extrahepatic Biliary Atresia

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Summary: The etiopathogenesis of extrahepatic biliary atresia (EHBA) remains undefined. There are clinical and pathological suggestions supporting the idea that EHBA could consist of at least two forms: the congenital (embryonic or fetal) and the acquired (perinatal) types. To test the hypothesis that susceptibility to this disease would be influenced by host genetic factors, we studied the human leukocyte antigen (HLA) system in 55 patients with and without major extrahepatic congenital anomalies. We found, especially in those without associated malformations, a significantly higher frequency of HLA-B12, of haplotypes A9-B5 and A28-B35, and of their disequilibrium values, as compared with the 8th International Histocompatibility Workshop controls. This study suggests that immunogenetic factors may play a role in determining susceptibility to EHBA, and the different HLA frequencies in those with and without anomalies lend support to the hypothesis that biliary atresia may be an etiologically heterogeneous disorder. **Key Words:** Extrahepatic biliary atresia—Biliary atresia—HLA and disease.

Extrahepatic biliary atresia (EHBA), a rare disease [0.5–1.0/10,000 live births (1)] restricted to infancy, can be defined as a complete occlusion of part or all of the extrahepatic biliary tract. It is the main hepatic cause of death in early childhood. The etiology is unknown. Two clinically distinct groups of patients have been described: those who have EHBA as an isolated lesion, and those who have EHBA with several congenital anomalies. Although its pathogenesis remains undefined, two main possibilities are being considered: (a) It is a congenital structural anomaly; or (b) It is an inflammatory, sclerosing, acquired lesion. There are clinical, laboratory, and experimental support for both theories, and the possibility that EHBA is a heterogeneous condition should be seriously considered (2).

Despite the fact that infections may determine EHBA, only a minority of infected babies develop the disease, suggesting that host immune response may be important in determining biliary damage. Therefore, as a part of a more general clinical and epidemiological study of this disorder, we decided to test a sample of these patients for the HLA system. As is well known, this system has a fundamental role in the control of self and nonself recognition, in antibody production, lymphocyte proliferation, and T-cell effector functions (3).

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Manuscript received November 14, 1991; revisions received March 6, 1992, July 21, 1992; final revision accepted July 24, 1992.

SUBJECTS AND METHODS

Fifty-five patients who had EHBA and who attended King's College Hospital during a 12-month period were studied, using the opportunity that venepuncture had to be performed to obtain other data for management purposes. Their ages ranged from 2 to 126 (mean 28) months. Thirty-two were female, and 23 were male. Eight (15%) had major congenital anomalies: four had polysplenia syndrome, one had congenital hydronephrosis and hy-

droureter, 1 had asplenia with immobile cilia, one had pulmonary stenosis and preduodenal portal vein, and one had pulmonary stenosis and intestinal malrotation. All were sporadic cases and none were children of consanguineous marriages. Sixty-three laboratory workers (44 were male) were studied as controls. All patients and controls were whites of Northern European ancestry. HLA frequencies were also compared with those reported in the 8th International Histocompatibility Workshop, in which data for European whites were compiled from different series, tested in the cooperating laboratories (4).

HLA phenotypes were determined using a standard complement-dependent cytotoxicity assay for 29 class I (AB) and 9 class II (DR) antigens. B cells for DR typing were prepared from peripheral blood mononuclear cells using a goat anti-human F(ab')2 immunoglobulin G. When B-cell yield was low, or cell viability was <60%, DR typing was not performed.

Gene and haplotype frequencies, as well as linkage disequilibrium values, were calculated using the POPGEN computer program made available to the Immunology Unit of Porto Alegre's Clinical Hospital by Dr. H. Festenstein. The χ^2 test with Yates correction for small numbers was used to determine statistical significance. The probability values were multiplied by the number of antigens tested as a correction, necessary when multiple tests are done. Relative risks were calculated as described in (5).

RESULTS

The frequencies of HLA class I and II antigens in patients (whole sample and subgroup without congenital anomalies) are presented in Table 1, with the results for the two control series. A total of 11 antigens controlled by the A locus, 18 by the B locus, and 8 by the DR locus were compared with the controls. When due allowance is made for these multiple comparisons, only one difference remained significant, that involving B12. The frequency of this antigen in the total sample of patients was 44%, and in those without associated extrahepatic anomalies was 49%, whereas in the controls of the 8th Workshop it was only 23%. This gives a relative risk of 2.61 [probability corrected (pc) due to the multiple comparisons, 0.009] for all patients and of 3.23 (pc: 0.003) for those without extrahepatic congenital anomalies. It should also be emphasized that of the 24 patients with the antigen, 23 did not show associated anomalies. The frequencies in the two groups of patients were as follows. EHBA without associated anomalies: 23 × 100/47—49%; EHBA with associated anomalies: 1 × 100/8—12% (χ^2: 5.3; 1 *df;* $p < 0.05$). The haplotype comparisons are somewhat hindered because of the small number of carriers in each group of patients. In only two cases there are large deviations in the comparisons with the 8th Workshop prevalence and sufficient numbers among the patients: A9-B5 (frequency × 10^4: 344, linkage disequilibrium or delta × 10^4: 272, p: 0.04 in the total patient's sample; 417 and 343, p: 0.02 in those without associated anomalies; 91 and −10 in the controls); and A28-B35 (269 and 234, p: 0.02; 316 and 272, p: 0.02; and 50 and 13, respectively).

DISCUSSION

The focus of most discussion concerning the etiology of EHBA has been on factors extrinsic to the infant. Several viruses have been reported as associated with this condition, particularly reovirus 3. In relation to the latter, the serological investigations (using immunofluorescence and enzyme-linked immunosorbent assay methods), as well as the immunohistochemical staining in hepatobiliary tissue, have yielded conflicting results (6–10). Recently, however, the virus RNA was not detected by the very sensitive technique of polymerase chain reaction in tissues from 29 EHBA patients, providing further evidence against the etiologic role of this virus in this condition (11).

To explain the pathogenesis of EHBA, there are two main hypotheses: (a) the ductal plate theory, which implicates abnormal embryogenesis or a defect in the remodeling of the ductal plate (12); and (b) the unitarian theory, which proposes that EHBA, idiopathic neonatal hepatitis, and choledochal cyst are all manifestations of the same basic inflammatory process (13).

This disease can occur as an isolated lesion or in conjunction with several congenital anomalies. The majority of cases have no associated malformations, and are presumably caused by factors acting after organogenesis is complete. Whether these factors act late in gestation, at birth, or postnatally is unknown. The remaining 20–25% have congenital anomalies, but they are not a homogeneous group. Three etiopathogenic mechanisms may be involved in this category: chromosome abnormalities, disordered organogenesis due to environmental factors,

TABLE 1. *HLA-A, -B, and -DR antigen frequencies in biliary atresia patients and respective controls*

HLA antigen	Atresia				Laboratory controls[a]		8th Workshop controls	
	Total sample		Without anomalies					
	No.	%	No.	%	No.	%	No.	%
HLA-A	55		47		63		2,648	
1	16	29	14	30	25	40	728	27
2	32	58	26	55	32	59	1,199	45
3	12	22	9	19	13	21	580	22
9	10	18	9	19	7	12	601	23
10	1	2	1	2	9	14	288	11
11	7	13	7	15	5	8	304	11
19(29)	10	18	7	15	5	8	196	7
(30, 31)	4	7	3	6	5	8	267	10
(32)	0	0	0	0	2	3	233	9
28	10	18	9	19	4	6	203	8
HLA-B	55		47		63		2,652	
5	8	14	6	13	8	13	446	17
7	13	24	10	21	15	24	445	17
8	10	18	9	19	14	22	416	16
12	24[b]	44[b]	23[b]	49[b]	15[b]	24[b]	607[b]	23[b]
13	4	7	2	4	3	5	148	6
14	2	4	2	4	0	0	154	6
15	12	22	10	21	13	21	302	11
16(38)	1	2	1	2	2	3	132	5
(39)	1	2	1	2	3	5	108	4
17	4	7	3	6	5	8	223	8
18	3	5	3	6	2	3	297	11
21	0	0	0	0	3	5	185	7
22	1	2	0	0	3	5	145	5
27	4	7	3	6	7	12	204	8
35	4	7	4	8	7	12	483	18
37	1	2	1	2	1	2	79	3
40	7	13	6	13	12	19	323	12
HLA-DR	28		21		47		2,499	
1	6	21	5	24	5	11	332	13
2	7	25	3	14	12	25	627	25
3	10	36	8	38	14	30	509	20
4	8	28	7	33	15	32	457	18
5	6	21	6	29	7	15	487	19
6	1	4	0	0	9	19	107	4
7	10	36	7	38	12	25	585	23
9	2	7	2	9	3	6	55	2

[a] Liver Unit, King's College Hospital.
[b] Difference between patients and the 8th Workshop controls significant at the 1% level, after due correction for the multiple comparisons.

or disruptive events occurring late in gestation (2). The observation of a high past history of fetal losses among mothers of children with EHBA plus associated congenital anomalies lends further evidence to the suggestion that this disease can occur due to factors present in utero and not only to agents acting in the perinatal period (14).

McKusick (15) suggested that some cases of EHBA could be caused by a pair of autosomal-recessive genes, but the evidence in favor of this view is scanty, and the absence of familial cases or consanguineous parents in our series is also against it. Studies in twin pairs generally yielded discordant results (16).

A role for the host immune response in determining liver damage has been demonstrated using a mouse model; only athymic neonatal mice develop a biliary atresia-like lesion (17). Because the genes of the major histocompatibility complex are essential components of most humoral and cellular responses, we have studied the distribution of one of its components, the HLA antigens in patients with EHBA. Generally the diseases found to be associated with HLA present the following characteris-

tics. (a) They are not inherited in a simple way. (b) Their etiology sometimes is not defined and may involve environmental factors. (c) They are of an immunologic nature. (d) They evolve in a chronic way. (e) They may be heterogeneous, only a subgroup of them showing association. Selected examples are celiac disease, insulin-dependent diabetes mellitus, and rheumatoid arthritis (3,5). The HLA system includes a large genetic region, with at least three clusters of closely linked homologous genes (3). Therefore, associations may be found not only with products of one locus, but with those of two or three, that could in such cases show stronger linkage ties. This is why in the investigation of disease associations not only the isolated antigens, but combinations of them should be tested.

The present study suggests that immunogenetic factors may play a role in determining susceptibility to EHBA. We have found a significant departure in the frequencies of HLA B12 and haplotypes A9-B5 and A28-B35 in patients, especially in those without associated extrahepatic malformations. Population stratification cannot explain the findings, because both patients and controls were whites of Northern European ancestry. No other report about HLA typing in EHBA patients could be located by us. Lasky et al. (18), in a study of mononuclear cells in the liver and blood of 15 individuals with EHBA, compared with a group of sclerosing cholangitis patients, mentioned that both groups were HLA typed, but did not give the results in the biliary atresia cases.

How could the elevated frequency of a HLA antigen increase the risk of EHBA? One possibility might be that individuals with B12 or the other antigenic arrangements would have less efficient cell membrane receptors for pathogens and/or a poor response to an exogenous insult. However, the observation of different HLA frequencies in patients with and without anomalies lends support to the hypothesis that biliary atresia may be an etiologically heterogeneous disorder, a concept that should be borne in mind in future studies.

Acknowledgment: This research was made possible by a fellowship received by T.R.S. from the Brazilian Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES). The authors thank Dr. H. Festenstein, Immunology Department, London Hospital Medical College for the POPGEN program used to calculate gene and haplotype frequencies and linkage disequilibrium values.

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