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HLA Antigens as Immunogenetic Markers of Alcoholism and Alcoholic Liver Disease

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SHIGETA, Y., H. ISHII, S. TAKAGI, Y. YOSHITAKE, T. HIRANO, H. TAKATA, H. KOHNO AND M. TSUCHIYA. *HLA antigens as immunogenetic markers of alcoholism and alcoholic liver disease.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 89–94, 1980.—HLA antigens were studied among 94 chronic alcoholics. Concerning A and B-loci, there was no significant change of phenotype frequency (PF) in the HLA typing between the patients and controls (80 healthy subjects). However, there was a significant difference in the PF of CW3 between chronic alcoholics and controls (58.5% in alcoholics vs 30.0% in controls). The corrected p value was less than 0.05 with relative risk value being 3.29. HLA-DR loci were also detected in 26 patients, but there was no significant difference between the patients and controls. All alcoholics were subdivided according to the hepatic morphology, and the PF of HLA was examined. A significant high frequency of HLA CW3 was found in patients with hepatitis (64%) compared to controls (30%). There was also an increased PF of CW3 in the liver cirrhosis group (59% in cirrhosis group vs 30% in controls). In conclusion, chronic alcoholics have a significantly higher PF of HLA-CW3 as compared to controls. This characteristic feature becomes even more distinct in alcoholics with severe hepatic lesions.

HLA antigens Immunogenetic markers Alcoholism Alcoholic liver disease

CHRONIC alcoholism is thought to originate from a number of social, psychological and biological factors. Animal and human studies have elucidated considerable variation in psychological and behavioral responses to alcohol, which are predominantly biological in origin and relatively independent of environmental influence. An association of alcoholism with other characteristics known to be inherited appears to support a biological factor in the etiology of alcoholism. There have been several reports exploring such an association, including adoption studies and twin studies [4]. Furthermore, it is well known that prolonged and excessive intake of alcohol can result in various degrees of liver damage. Although the development of hepatic disease in alcoholics is dependent on the duration and amount of ethanol intake, only a relatively small portion (10 to 30% of alcoholics) become cirrhotics. However the explanation for the increased susceptibility of certain individuals to severe alcoholic liver disease is still not known. Recently it has been suggested that individual susceptibility to alcoholic liver damage might develop on an immunological basis, and a number of immunological abnormalities have been reported [10,11]. Moreover, there is a possibility that the development of alcoholic liver disease might have a genetic basis. Therefore, the present study was designed to clarify the pattern of HLA antigens and Major Histocompatibility Complex (MHC) among chronic alcoholics, especially in relation to the development and severity of hepatic lesion.

METHOD

Ninety-four chronic alcoholics were admitted to the division of internal medicine or psychiatry, National Kurihama Hospital, National Institure on Alcoholism. They were all admitted to the hospital for detoxification and rehabilitation. Their average daily alcohol intake was approximately 150 g, and the average period of alcohol intake was 21 years. Forty-two patients had a positive family history of alcoholism, including heavy drinking by their parents or siblings, and 41 patients were negative. The overall average age was 44 years, and the average age was 42 years in the group with alcoholic fatty liver (36 cases), and 48 years in the group with alcoholic hepatitis (33 cases) and/or liver cirrhosis (17 cases). Diagnosis of liver disease was made by needle biopsy or laparoscopy. Blood HBs antigens were all negative. For controls, blood was supplied from normal healthy subjects. Lymphocytes were isolated by Ficoll-hypaque gradient centrifugation within 3 hours of venipuncture. HLA typing was done by the standard microcytotoxicity assay according to

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TABLE 1

HLA PHENOTYPE FREQUENCIES IN CHRONIC ALCOHOLICS

Locus A	Controls (80 cases)		Chronic Alcoholics (94 cases)				
	No.	P.F. (%)	No.	P.F. (%)	χ^2	corrected *p*	R.R.
A1	0		0				
A2	33	41.3	47	50.0		n.s.	
A3	0		0				
Aw24	50	62.5	61	64.9		n.s.	
A26	17	21.3	29	30.9		n.s.	
A11	16	20.0	20	21.3		n.s.	
Aw19	7	8.8	8	8.5		n.s.	
Blank	37		23				

TABLE 2

HLA PHENOTYPE FREQUENCIES IN CHRONIC ALCOHOLICS

Locus B	Controls (80 cases)		Chronic Alcoholics (94 cases)				
	No.	P.F. (%)	No.	P.F. (%)	χ^2	corrected *p*	R.R.
B5	36	45.0	40	42.6		n.s.	
B7	8	10.0	18	19.1		n.s.	
B8	3	3.8	2	2.1		n.s.	
B12	14	17.5	11	11.7		n.s.	
B13	0		3	3.2		n.s.	
B15	14	17.5	22	23.4		n.s.	
Bw16	5	6.3	6	6.4		n.s.	
B17	2	2.5	2	2.1		n.s.	
Bw54	12	15.0	13	13.8		n.s.	
Bw55	0		5	5.3		n.s.	
B27	1	1.3	0			n.s.	
Bw35	8	10.0	9	9.6		n.s.	
B37	0		0				
B40							
Bw60	9	11.3	13	13.8		n.s.	
Bw61	17	21.3	22	23.4		n.s.	
Bw48	0		0				
Blank	31		22				

TABLE 3

HLA PHENOTYPE FREQUENCIES IN CHRONIC ALCOHOLICS

Locus C	Controls (80 cases)		Chronic Alcoholics (94 cases)				
	No.	P.F. (%)	No.	P.F. (%)	χ^2	corrected *p*	R.R.
Cw 3	24	30.0	55	58.5	14.12	<0.05	3.29
Cw 4	15	18.8	12	12.8		n.s.	
Blank	121		121				

TABLE 4

HLA PHENOTYPE FREQUENCIES IN CHRONIC ALCOHOLICS

Locus DR	Controls (51 cases)		Chronic Alcoholics (26 cases)				
	No.	P.F. (%)	No.	P.F. (%)	χ^2	corrected p	R.R.
DR1	10	19.6	3	11.5		n.s.	
DR2	20	39.2	13	44.8		n.s.	
DR3	0		0			n.s.	
DR4	17	33.3	9	34.9		n.s.	
DR5	4	7.8	2	7.7		n.s.	
DRW6	8	15.7	7	26.9		n.s.	
DR7	0		0			n.s.	
DRW8	2	3.9	2	7.7		n.s.	
DRW9	12	23.5	10	38.5		n.s.	
DRW10	1	2.0	0			n.s.	
8WDRW6Y	19	37.3	5	19.2		n.s.	
blank	9	17.0	7	24.1			

No significant association was found between alcoholism and healthy controls.

TABLE 5

HLA PHENOTYPE FREQUENCIES IN CHRONIC ALCOHOLICS IN RELATION TO FAMILY HISTORY

Locus A	Controls (80 cases)		With Family History (42 cases)		Without Family History (41 cases)	
	No.	P.F. (%)	No.	P.F. (%)	No.	P.F. (%)
A1	0		0		0	
A2	33	41.3	20	47.6	23	56.1
A3	0		0		0	
Aw24	50	62.5	31	73.8	25	61.0
A26	17	21.3	14	33.3	11	26.8
A11	16	20.0	7	16.7	9	22.0
Aw19	7	8.8	3	7.1	5	12.2
Blank	37	46.3	9	21.4	9	22.0

NIH. Japanese-originated HLA antisera were supplied by Bohsei Sangyo Co. Ltd., HLA antisera by Hoechst Co. Ltd., and HLA-DR antisera were obtained from Dr. P. Terasaki, UCLA.

RESULTS

In 80 controls, A2, AW24, A26 and A19 were frequently detected in the HLA A-loci, and their phenotype frequency was similar to the data on Japanese previously reported at the 7th International Work Shop of HLA (Table 1). There was no significant difference of phenotype frequency in the HLA antigens typing between 94 chronic alcoholics and controls. In regard to the B-loci HLA antigens, the results obtained from chronic alcoholics were not different from those of controls (Table 2). There was no significant difference in phenotype frequencies of B5, B8, BW54 and B40, which were reported to be found frequently in patients with chronic active hepatitis in Japan as well as in Western countries. As shown in Table 3, HLA C-loci were detected mainly with CW3 and CW4. There was a significant difference in the phenotype frequency of CW3 between chronic alcoholics and controls (58.5% in alcoholics versus 30.0% in controls). The corrected p value was less than 0.05, and the relative risk (RR) value was 3.29. HLA DR-loci were examined in 26 patients, but there was no significant difference between the patients and controls (Table 4).

When HLA phenotype frequencies were compared between the groups of positive and negative family histories of chronic alcoholism, it was also shown that there were no significant differences in the HLA A-loci or B-loci (Tables 5 and 6). However, the phenotype frequency of CW3 was significantly higher in the group of positive family history than controls (Table 7).

Furthermore all alcoholics were subdivided according to their hepatic morphology (fatty liver, alcoholic hepatitis and cirrhosis), and phenotype frequencies of HLA were exam-

TABLE 6

HLA PHENOTYPE FREQUENCIES IN CHRONIC ALCOHOLICS IN RELATION TO FAMILY HISTORY

Locus B	Controls (80 cases)		With Family History (42 cases)		Without Family History (41 cases)	
	No.	P.F. (%)	No.	P.F. (%	No.	P.F. (%)
B5	36	45.0	20	47.6	16	39.0
B7	8	10.0	7	16.7	9	22.0
B8	3	3.8	2	4.8	0	
B12	14	17.5	5	11.9	6	14.6
B13	0		0		2	4.9
B15	14	17.5	7	16.7	9	22.0
Bw16	5	6.3	1	2.4	4	9.8
B17	2	2.5	0		0	
Bw54	12	15.0	5	11.9	5	12.2
Bw55	0		4	9.5	1	2.4
B27	1	1.3	0		0	
Bw35	8	10.0	4	9.5	4	9.8
B37	0		0		0	
B40						
Bw60	9	11.3	6	14.3	7	17.1
Bw61	17	21.3	11	26.2	10	24.4
Bw48	0		0		0	
Blank	31	38.8	12	28.6	9	22.0

TABLE 7

HLA PHENOTYPE FREQUENICES IN CHRONIC ALCOHOLICS IN RELATION TO FAMILY HISTORY

Locus C	Controls (80 cases)		With Family History (42 cases)		Without Family History (41 cases)	
	No.	P. F. (%)	No.	P.F. (%)	No.	P.F. (%)
Cw 3	24	30.0	26	61.9*	21	51.2
Cw 4	15	18.8	2	4.8	7	17.1
Blank	121		56		54	

*: $\chi^2=11.59$, corrected $p<0.05$, R.R.=3.79.

TABLE 8

HLA PHENOTYPE FREQUENCIES IN VARIOUS ALCOHOLIC LIVER DAMAGES

Locus A	Controls (80 cases)		Fatty Liver (36 cases)		Hepatitis (33 cases)		Liver Cirrhosis (17 cases)	
	No.	P.F. (%)	No.	P.F. (%)	No.	P.F. (%)	No.	P.F. (%)
A1	0		0		0		0	
A2	33	41.3	21	58.3	13	39.4	9	52.9
A3	0		0		0		0	
Aw24	50	62.5	24	66.7	26	78.8	8	47.1
A26	17	21.3	8	22.2	10	30.3	7	41.2
A11	16	20.0	7	19.4	6	18.2	6	35.3
Aw19	7	8.8	6	16.7	1	3.0	0	
Blank	37	46.3	6	16.7	10	30.3	4	23.5

TABLE 9

HLA PHENOTYPE FREQUENCIES IN VARIOUS ALCOHOLIC LIVER DAMAGES

Locus B	Controls (80 cases)		Fatty Liver (36 cases)		Hepatitis (33 cases)		Liver Cirrhosis (17 cases)	
	No.	P.F. (%)	No.	P.F. (%)	No.	P.F. (%)	No.	P.F. (%)
B5	36	45.0	17	47.2	14	42.4	7	41.2
B7	8	10.0	7	19.4	6	18.2	3	17.6
B8	3	3.8	1	2.8	1	3.0	0	
B12	14	17.5	4	11.1	5	15.2	1	5.9
B13	0	0	0		2	6.1	0	
B15	14	17.5	9	25.0	6	18.2	4	23.5
Bw16	5	6.3	3	8.3	3	9.1	0	
B17	2	2.5	1	2.8	2	6.1	0	
Bw54	12	15.0	6	16.7	3	9.1	4	11.8
Bw55	0		3	8.3	1	3.0	1	5.9
B27	1	1.3	0		0		0	
Bw35	8	10.0	3	8.3	3	9.1	2	11.8
B37	0		0		0		0	
B40								
Bw60	9	11.3	5	13.9	4	12.1	4	23.5
Bw61	17	21.3	8	22.2	6	18.2	3	17.6
Bw48	0.		0		0		0	
Blank	31	38.8	7	19.4	8	24.2	5	29.4

TABLE 10

HLA PHENOTYPE FREQUENCIES IN VARIOUS ALCOHOLIC LIVER DAMAGES

Locus C	Controls (80 cases)		Fatty Liver (36 cases)		Hepatitis (33 cases)		Liver Cirrhosis (17 cases)	
	No.	P.F. (%)	No.	P.F. (%)	No.	P.F. (%)	No.	P.F (%)
Cw 3	24	30.0	17	47.2	21	63.6*	10	58.8
Cw 4	15	18.8	3	8.3	4	12.1	5	29.4
Blank	121		52		41		19	

*: $\chi^2=11.03$, corrected $p<0.05$, R.R.=4.08.

ined. Concerning HLA A-loci in the cirrhotic group, as shown in Table 8, there was a tendency to increased phenotype frequencies of A2, A26 and A11, and a tendency toward a decreased frequency of AW24. However, these changes were not statistically significant. For B-loci, there was no significant difference of phenotype frequencies (Table 9). On the other hand, in C-loci (Table 10) the phenotype frequency of CW3 in the hepatitis group was 64%, compared to 30% in controls, and this difference was significant (corrected $p<0.05$, and relative risk value=4.08). There was also an increased phenotype frequency of CW3 in the liver cirrhosis group, although it was not statistically significant.

DISCUSSION

There appear to be individual differences in man with regard to behavioral tolerance and metabolism of alcohol. Whereas this biological variation is presumably genetic in origin, there is no direct evidence that inborn biological factors contribute to the pathogenesis of alcoholism in man. There have been several approaches for attempting to determine whether genetic factors play a role in alcoholism; namely adoption studies, twin studies and genetic marker studies [4]. In the present study, the possibility that individual susceptibility to the development of alcoholism as well as alcoholic liver disease might have a genetic basis, was investigated by determining the distribution of histocompatibility antigens.

The first MHC study in liver disease, irrespective of alcoholism was reported in 1972 by Vermylen *et al.* [12], who showed high phenotype frequencies of HLA A3 and AW19 in HBs Ag carriers. Subsequently, Mackey and Morris [5] have reported that in patients with active chronic hepatitis, HLA A1 and B8 were found to be more prevalent.

As far as alcoholic liver disease is concerned, HLA B8 was reported to be more prevalent in patients with cirrhosis

than in controls [1,7]. However, Bell *et al.* [2] studied HLA antigens in 41 patients with alcoholic liver cirrhosis, and reported that the phenotype frequency of HLA BW40 was three times higher in the cirrhotic group compared with controls, and there was no correlation with HLA B8. In Japan, a population study of HLA B-loci among healthy subjects revealed that the phenotype frequency of B8 is very low (ca. 2 to 3%), and in the present study, the phenotype frequency of B8 was also found to be low, both in alcoholics and in the controls. Furthermore there was no significant difference in phenotype frequency of BW40 between the patients and controls.

Recently, Meléndez [6] reported that the group of alcoholics with cirrhosis showed a significantly higher frequency of HLA B13 than normal subjects, whereas the frequency of HLA B13 was similar to normal subjects in alcoholics without cirrhosis. Association of BW40 in Norway (Bell *et al.* [2]) and B13 in Chile (Meléndez *et al.* [6]) might be explained by a linkage disequilibrium between genes of the B-loci [9]. In Japan, a haplotype of BW40-CW3 was reported to be frequently encountered (Haplotype Frequency: 106.0 per 1000 [8]). Therefore, it is interesting to see if there is a linkage with BW40-CW3 across the racial difference of HLA. However, our present study failed to show a significant difference in BW40 or B13 between patients and controls. Gluud *et al.* [3] in Denmark reported that no significant differences in the phenotype frequencies of HLA B8, B40 and other HLA-A, -B and -C phenotypes were found among patients with histologically verified alcoholic cirrhosis, compared with normal controls, when expressed by corrected p values. They emphasized, however, that these findings do not rule out the possibility that alcoholic cirrhosis might be associated with other HLA factors, such as HLA-D/DR antigens which control immune responses. In that respect, we also analysed DR antigens among a limited number of alcoholic patients, and found no statistically significant difference in DR antigens when compared with controls. Further study of DR antigens is in progress with a larger number of patients.

Although there were no specific HLA antigens in A-loci and B-loci prevailing among alcoholics, the phenotype frequency of CW3 was significantly higher in chronic alcoholics, especially in the groups of patients with hepatitis and cirrhosis. However, it should be borne in mind that the study of HLA C-loci is still somewhat controversial, and the disease association with the HLA C-loci remains to be further elucidated.

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