Distribution of HLA histocompatibility antigens, ABO blood groups and Rh antigens in alcoholic liver disease

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SUMMARY The distribution of 16 antigens of the HLA-A and 15 antigens of the HLA-B series of HLA system, the blood groups ABO, and Rh antigens were studied in 40 alcoholics with cirrhosis, 18 alcoholics without cirrhosis, and in normal control subjects. The group of alcoholics with cirrhosis showed a significantly high frequency of HLA-B13 (corrected $P < 0.01$) when compared with normal subjects, while the frequency of HLA-B13 was similar to normal in alcoholics without cirrhosis. On the basis of these findings, it seems that the carriers of HLA-B13 are more susceptible to liver damage caused by alcohol. Both groups of alcoholics and the normal controls had a similar distribution of ABO blood groups and Rh antigens.

Many alcoholics do not develop hepatic disease no matter how long and how much alcohol they have ingested (Insunza *et al.*, 1969). This seems to indicate a different individual resistance and/or susceptibility to hepatic damage related to diet, genetic background, or other possible factors. An important role has been assigned to the human leucocyte antigens (HLA) system in the susceptibility of the host to certain diseases, so that this system could well be a genetic marker in this respect (NIH, 1975; Sasazuki *et al.*, 1977).

On the other hand, an association between the blood groups of the ABO system and different diseases has also been reported (Doll *et al.*, 1960; Kingsbury, 1971).

In the present study, we examined the relationship between antigens of the HLA system and blood groups of the ABO system and Rh antigens and the heavy drinker's susceptibility to chronic liver damage.

Methods

HLA, blood groups, and Rh antigens were assayed in 58 subjects who were drinking or had been drinking more than the equivalent of 100 g ethanol daily for over 10 years. These 58 subjects were divided

into two groups: (1) 18 alcoholics without hepatic damage, in 15 of whom percutaneous liver biopsy was either normal or showed only minimal, non-specific changes, and three others who refused biopsy but had no clinical or laboratory (including serum bilirubin and transaminases and bromosulphalein excretion) evidence of liver diseases, and (2) 40 alcoholics with well-documented cirrhosis demonstrated by percutaneous liver biopsy.

HLA antigens were compared between these two groups and with 186 normal subjects of the general population of Santiago. The distribution of ABO blood groups and Rh antigens was compared between both groups of alcoholics and with 7711 normal controls. The HLA antigens analysis was performed by the microlymphocytotoxicity technique (Terasaki and McClelland, 1964; Mittal *et al.*, 1968) on lymphocytes separated by the Ficoll-Hypaque gradient according to English and Burton (1974). Sixteen antigens of the HLA-A series and 15 of the HLA-B series were analysed using antisera kindly supplied by the National Institute of Health, Bethesda, MA, USA.

In order to avoid false results due to the well-known cross-reactivity between HLA-B13 and HLA-BW40, highly specific antisera were used: for B13 Neugent NIH number: 00252 and for BW40 Vereen 74 NIH number: 01148 (*Catalog of Tissue Typing Antisera, NIH, 1976-77*). Blood groups and

HLA antigens in alcoholic cirrhosis

Table 1 Frequency of HLA antigens in alcoholics with and without cirrhosis and normal controls

Antigens	Frequency (%)		
	Controls n:186	Alcoholics	
		With cirrhosis n:40	Without cirrhosis n:18
HLA-A1	22.0	15.0	27.7
A2	48.9	47.5	38.8
A3	5.4	7.5	5.5
A9	20.4	27.5	16.6
A10	8.6	12.5	0.0
A11	5.4	5.0	0.0
A28	8.6	10.0	0.0
A29	0.0	0.0	0.0
AW19	1.1	0.0	0.0
AW23	0.5	0.0	0.0
AW25	0.5	0.0	0.0
AW26	1.1	0.0	0.0
AW30	1.6	2.5	0.0
AW31	0.0	0.0	0.0
AW32	0.0	0.0	0.0
AW33	0.0	0.0	0.0
HLA-B5	25.3	17.0	33.3
B7	15.1	15.0	0.0
B8	11.3	20.0	11.1
B12	17.7	15.0	11.1
B13	6.9	27.5*	11.1
B14	9.7	7.5	0.0
B18	14.5	10.0	0.0
B27	5.9	15.0	0.0
BW15	3.2	0.0	0.0
BW16	16.1	5.0	0.0
BW17	6.5	5.0	22.2
BW21	6.5	5.0	5.5
BW22	3.8	2.5	11.1
BW35	5.4	7.5	5.5
BW40	4.3	0.0	5.5

*Corrected P, compared with controls < 0.01.

Table 2 Frequency of blood group ABO and Rh antigen in alcoholics with and without cirrhosis and normal controls

Blood group	Frequency (%)		
	Controls n:7.711	Alcoholics	
		With cirrhosis n:40	Without cirrhosis n:18
AB	2.1	2.9	0.0
A	28.4	34.3	22.2
B	9.1	8.6	22.2
O	60.3	54.3	55.5
Rh (+)	94.6	98.6	83.3
Rh (-)	5.2	2.9	16.6

None of the differences was significant.

Rh antigens were identified using standard methods. For the statistical calculations the χ^2 test with Yates's correction was used. In the HLA evaluation P values were corrected for the number of specificities studied multiplying by 31 (Svejgaard *et al.*, 1974).

Results

The frequency of HLA-B13 was remarkably higher among alcoholics with cirrhosis. In fact, HLA-B13 had a frequency of 27.5% in the cirrhosis patients, 11.1% in the alcoholics with normal livers, and of 7% in the controls. Corrected P value was <0.01 when the frequency of this antigen was compared in alcoholics with cirrhosis and controls. Comparison between both groups of alcoholics and between controls and alcoholics without liver damage was not significant. There was no other significant difference in the HLA distribution between these three groups of subjects (Table 1).

The distribution of blood groups of the ABO system and Rh antigen was similar in all the groups analysed (Table 2).

Discussion

Analysis of the association between HLA antigens and various liver diseases reveals some disagreement between different authors (Mackay and Morris, 1972; Bailey *et al.*, 1976; Galbraith *et al.*, 1976; Scott *et al.*, 1977; Simon *et al.*, 1977; Bell and Nordhagen, 1978). Bailey *et al.* (1976) reported an abnormally high frequency of HLA-B8 ($P = 0.025$) in alcoholics with cirrhosis but without correcting P; had they done this correction for the specificities studied, the difference would have not been statistically significant.

Our results suggest that the carrier of HLA-B13 antigens may have a higher susceptibility to hepatic damage caused by ethanol, as it was found nearly four times as often ($P < 0.01$) in alcoholics with cirrhosis as in normal subjects. The fact that the distribution was statistically non-significant between both groups of alcoholics could well be because both groups were too small to yield statistical significance.

The fact that HLA-B13 was not increased in the other reports (Scott *et al.*, 1977; Simon *et al.*, 1977) could well be attributable to ethnic difference. Rotter *et al.* (1977) found that the frequency of B5 differed between black and white males with duodenal ulcer, suggesting that differences in an HLA antigen association with one particular disease may be due to racial differences.

It is most interesting that recent studies coming from Norway have shown an association between HLA-BW40 and alcoholic cirrhosis (Bell and Nordhagen, 1978). The fact that the susceptibility to alcoholic liver damage is associated in Europe with BW40 and with B13 in Chile, if a cross-reactivity is avoided, could well be explained through a linkage disequilibrium between genes of B loci

(Schaller and Omenn, 1976). The ABO blood groups and the Rh antigen had a similar distribution in the groups studied. This is consistent with other studies (Bailey *et al.*, 1976).

References

- Bailey, R. J., Krasner, N., Eddleston, A. L. W. F., Williams, R., Tee, D. E. H., Doniach, D., Kennedy, L. A., and Batchelor, J. R. (1976). Histocompatibility antigens, autoantibodies, and immunoglobulins in alcoholic liver disease. *British Medical Journal*, **2**, 727-729.
- Bell, H., and Nordhagen, R. (1978). Association between HLA-BW40 and alcoholic liver disease with cirrhosis. *British Medical Journal*, **1**, 822.
- Doll, R., Swynnerton, B. F., and Newell, A. C. (1960). Observations on blood group distribution in peptic ulcer and gastric cancer. *Gut*, **1**, 31-35.
- English, D., and Anderson, B. R. (1974). Single-step separation of red blood cells, granulocytes and mononuclear leukocytes in discontinuous density gradients of Ficoll-Hypaque. *Journal of Immunological Methodology*, **5**, 249-253.
- Galbraith, R. M., Eddleston, A. L. W. F., Williams, R., Webster, A. D. B., Pattison, J., Doniach, D., Kennedy, L. A., and Batchelor, J. R. (1976). Enhanced antibody responses in active chronic hepatitis: relation to HLA-B8 and HLA-B12 and porto-systemic shunting. *Lancet*, **1**, 930-934.
- Insunza, I., Iturriaga, H., Ugarte, G., and Altschiller, H. (1969). Estudio clínico patológico del daño hepático en pacientes alcohólicos. *Revista Medica Chile*, **97**, 765-772.
- Kingsbury, K. J. (1971). Relation of ABO blood-groups to atherosclerosis. *Lancet*, **1**, 199-202.
- Mackay, I. R., and Morris, P. J. (1972). Association of autoimmune active chronic hepatitis with HLA-A1,8. *Lancet*, **2**, 793-795.
- Mittal, K. K., Mickey, M. R., Singal, D. P., and Terasaki, P. I. (1968). Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation*, **6**, 913-927.
- Rotter, J. I., Rimoin, D. L., Gursky, J. M., Terasaki, P., and Sturdevant, R. A. L. (1977). HLA-B5 associated with duodenal ulcer. *Gastroenterology*, **73**, 438-440.
- Sasazuki, T., McDevitt, H. D., and Gurnet, F. C. (1977). The association between genes in the major histocompatibility complex and disease susceptibility. *Annual Review of Medicine*, **28**, 425-452.
- Schaller, J. G., and Omenn, G. S. (1976). The histocompatibility system and human disease. *Journal of Pediatrics*, **88**, 913-925.
- Scott, B. B., Rajah, S. M., and Losowsky, M. S. (1977). Histocompatibility antigens in chronic liver disease. *Gastroenterology*, **72**, 122-125.
- Simon, M., Bourel, M., Genetet, B., Fauchet, R., Edan, G., and Brissot, P. (1977). Idiopathic hemochromatosis and iron overload in alcoholic liver disease: differentiation by HLA Phenotype. *Gastroenterology*, **73**, 655-658.
- Svejgaard, A., Jersild, C., Nielsen, L. S., and Bodmer, W. F. (1974). HL-A antigens and disease: statistical and genetical consideration. *Tissue Antigens*, **4**, 95-105.
- Terasaki, P. I., and McClelland, J. D. (1964). Microdroplet assay of human serum cytotoxins. *Nature*, **204**, 998-1000.
- Transplantation and Immunology Research (1975). *Reported HL-A Antigens in Disease*. National Institutes of Health. April. Washington, DC.