The epidemiological importance of 'ay' and 'ad' subtypes of the HB-Ag

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SUMMARY The distribution of the subtypes 'ay' and 'ad' has been assessed in 159 hepatitis B antigen (HB-Ag) positive subjects with a variety of associated hepatic conditions. A specific subtype could not be correlated with any particular hepatic pathology when the whole group was considered. However, the distribution of these two subtypes did vary according to the geographical origin of the subject or infection, or the presumed route of infection, so that there was an apparent association of one subtype with a certain disease state. Subtyping performed on 10 HB-Ag-positive households showed the subtype to be the same within nine, emphasizing the epidemiological rather than the pathological importance of the 'ay' and 'ad' subtypes of the HB-Ag.

It is now well established that various subtypes of the HB-Ag exist. Most HB-Ag positive sera contain a common antigenic determinant 'a'. Le Bouvier (1971) has described two mutually exclusive subtypes 'y' and 'd', which appear to be viral in origin. Several authors have commented upon the distribution of the 'ay' and 'ad' subtypes amongst the various hepatic conditions associated with the HB-Ag, some reporting association of a specific subtype with certain disease states (Nielsen and Le Bouvier, 1973), although others have noted a scattering of subtypes throughout the spectrum of liver diseases (Holland, Purcell, Smith, and Alter, 1972; Gordon, Berberian, Stevenson, and Redeker, 1972).

We have assessed the distribution of subtypes 'ay' and 'ad' amongst our HB-Ag positive subjects to determine whether these two subtypes are of pathological or epidemiological importance.

Patients Studied

DIAGNOSTIC GROUPS

A total of 159 HB-Ag positive subjects have been studied. Sixty-nine patients were diagnosed as having acute hepatitis on a clinical and biochemical basis. Seventeen subjects were thought to be healthy carriers of the HB-Ag, as they were persistently HB-Ag positive for six months or more without any clinical or biochemical evidence of liver disease. Forty-one patients had biopsy-proven chronic aggressive hepatitis, many of whom had superimposed cirrhosis. Another 27 patients with chronic persistent hepatitis were studied. However, three of

the subjects with chronic persistent hepatitis did not have liver biopsies performed (one refused, one left the country, and the third is a haemophiliac). Five subjects had biopsy-proven primary liver cell cancer.

GEOGRAPHICAL GROUPS

The country of birth was used to locate the geographical origin of each subject so that each was found to fall into one of four geographical groups. Seventy-eight came from northern Europe, 57 from the Mediterranean countries or thereabouts, eight from the Middle East, and the remaining 16 subjects came from a variety of countries not included in these regions, and they were therefore labelled the 'miscellaneous' group.

In the northern European group, 72 were from Great Britain, two from Switzerland, two from Norway, one from Belgium, and one from Austria. In the Mediterranean group 28 came from Greece, 10 from Italy, seven from Turkey, seven from Spain, two from Yugoslavia, two from Portugal, and one from Malta. In the Middle Eastern group three were from Persia, three from Egypt, one from Saudi Arabia, and one from Libya. The miscellaneous group included five subjects from Pakistan, three from the USA, two from the West Indies, two from Argentina, two from Australia, one from South Africa, and one from Bermuda.

HOUSEHOLD STUDIES

Amongst these 159 HB-Ag positive subjects there were 23 persons who came from 10 households where more than one member had been found to be HB-Ag

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positive. All but two of these households were resident in Great Britain at the time of the study but the geographical origin of the household members was varied. In only two households were all the HB-Ag positive members from Great Britain, in two other households British persons were found to be living with a Spaniard in one instance and a West Indian in another. The remaining six households originated from Turkey, Greece, Pakistan, Persia, Bermuda, and Spain. Three patients who presented with acute, type B, hepatitis were found to have sexual partners who were healthy carriers of the HB-Ag and in two of these households a relative of the carrier, living with them, was also found to be an HB-Ag carrier. One other patient (a male homosexual) was found to be living with another male HB-Ag carrier, but sexual contact was denied. Four other patients with acute hepatitis who came from three households, were found to have sexual partners who were persistently HB-Ag positive. These sexual contacts were thought to have chronic persistent hepatitis; in two this was proven by liver biopsy. The three other households included two *propositi* with chronic persistent hepatitis whose sexual partners also had HB-Ag-positive chronic persistent hepatitis and one *propositus* with chronic aggressive hepatitis whose sister had HB-Ag-positive chronic persistent hepatitis.

Method

Reference sera representing 'ay' and 'ad' antigen were standardized for us by Le Bouvier. Hepatitis B antibody (HB-Ab) was obtained from two multiply transfused patients. The subtype of each antiserum was determined by gel diffusion using the standardized HB-Ag sera. Each antiserum was then rendered monovalent by appropriate absorption (Gordon *et al.*, 1972).

All serum samples were set up against 'y' and 'd' antisera and tested by counterimmunoelectrophoresis, as described by Holland *et al.* (1972).

Testing of statistical significance was in general done by standard χ^2 techniques except where the cell frequencies were very small when use was made of Fisher's exact method for solution of the 2×2 contingency table.

Results

The HB-Ag-positive samples from all 159 subjects could be subtyped either 'ay' or 'ad'. In no sample could both subtypes be detected. Twenty of the subjects found to be persistently HB-Ag positive were subtyped twice, the two serum samples tested being obtained generally six months apart; the subtype

was found to remain the same in each subject.

When the subtyping results from all 159 subjects were considered there was no significant dishomogeneity between 'ay': 'ad' proportions in the various diagnostic groups (as assessed by the $2 \times 5 \chi^2$ test) (table I).

Subtype	Hepatitis			Carriers	Primary Liver Cancer
	Acute	Chronic Aggressive	Chronic Persistent		
'ad'	32	13	12	10	2
'ay'	37	28	15	7	3

Table I Distribution of 'ay' and 'ad' subtypes in 159 HB-Ag-positive subjects

It can be seen from table I that patients with chronic aggressive hepatitis were commonly 'ay' in type. Although even when this group of patients were compared with all the other HB-Ag-positive subjects, there was still no significant difference in the distribution of the two subtypes. But further analysis of these 41 patients with chronic aggressive hepatitis showed 27 to be Mediterranean in origin.

We have therefore compared the subtype findings in the Mediterranean subjects with those from the other large geographical group, ie, northern Europeans. The ratio 'ay' to 'ad' was found to be significantly greater in the Mediterraneans ($2 \times 2 \chi^2$ test: $P < 0.005$) (table II).

Subtype	Northern European	Mediterranean
'ad'	45	18
'ay'	33	39 ($P < 0.005$)

Table II Distribution of 'ay' and 'ad' subtypes of the HB-Ag: northern European versus Mediterranean subjects

Differences in the distribution of the two subtypes were also seen when the results for each diagnostic group (acute hepatitis, chronic persistent hepatitis, chronic aggressive hepatitis, and carriers) from northern Europe and the Mediterranean were compared.

The two subtypes were nearly equally distributed amongst the patients with acute hepatitis from these two geographical areas (table III). However, if these patients are further subdivided where possible, into where and how they were thought to have contracted hepatitis, a difference in distribution of the two subtypes is seen.

Amongst the 51 northern Europeans with acute hepatitis, information regarding recent travel abroad was not obtained from 12. Of the remaining 39

Group	Hepatitis			Carriers	Primary Liver Cell Cancer
	Acute	Chronic Aggressive	Chronic Persistent		
N. European	24/27	6/2	5/3	9/1	1/0 ad/ay
Mediterranean	7/7	6/21	3/7	1/3	1/1 ad/ay

Table III Distribution of 'ay' and 'ad' subtypes of HB-Ag in different diagnostic groups: northern European and Mediterranean

patients, 35 were thought to have contracted hepatitis in Great Britain and four abroad—in Greece, Portugal, Norway, and Madagascar respectively. The distribution of the two subtypes still appeared to be fairly equally distributed (table IV). However, a history of likely parenteral exposure to the HB-Ag, most commonly as a result of being a drug addict or a friend of a drug addict, was obtained from 16 of the acute hepatitis patients from northern Europe; 15 were subtype 'ay', only one 'ad'.

Subject	Total No.	Great Britain		Abroad	
		ay	ad	ay	ad
N. European	(39) ¹	19	16	2	2
Mediterranean	(14)	3	4	4	3

Table IV Origin of infection with HB-Ag in n. European and Mediterranean subjects with acute type B hepatitis

¹No history available from 12, therefore no¹ included in tab¹e.

Of the 14 patients with acute hepatitis who originated from the Mediterranean, seven were thought to have contracted their illness in Great Britain. Four were 'ad' and three 'ay', but the latter three were all living in Mediterranean communities in this country, two of whom were known to have sexual partners who were carriers of the 'ay' subtype. The other seven Mediterranean patients had become ill in their country of origin; four were 'ay' and three 'ad'.

When the results from the patients with chronic persistent hepatitis from these two geographical areas were considered, there was no significant difference in the distribution of 'ay' and 'ad' subtypes.

However, the ratio 'ay' to 'ad' was found to be significantly greater in our Mediterranean than our northern European patients with chronic aggressive hepatitis ($2 \times 2 \chi^2$ test: $P < 0.05$).

Amongst the healthy carriers from these two geographical areas the ratio 'ay' to 'ad' was found to be significantly lower in the northern Europeans (Fisher's exact method $P = 0.041$).

The subtyping results from the HB-Ag-positive subjects from the Middle East and from those placed in the miscellaneous group have not been included in this statistical analysis as the numbers were so small. All but one of the eight patients from the Middle East (one acute hepatitis, two chronic aggressive hepatitis, two chronic persistent hepatitis, one carrier, and two primary liver cell cancers) were 'ay' in type. Similarly, the 'ay' subtype predominated amongst the 16 HB-Ag-positive subjects placed in the miscellaneous group (11/16 'ay').

The results from the nine households where intra-household, non-parenteral transmission of the HB-Ag was suspected showed the subtype to be the same within each household. Five households were all 'ay' and four all 'ad' in type. In the tenth household where a male homosexual, who presented with acute hepatitis, was found to be living in the same house as a healthy carrier of the HB-Ag, but denied sexual contact with the carrier, the subtypes were different.

Discussion

Our results demonstrate that both subtypes 'ay' and 'ad' may be associated with all forms of HB-Ag-positive acute and chronic liver disease and in healthy carriers. However, the geographical origin of the HB-Ag-positive person, and possibly the route of infection with the HB-Ag may influence the subtype findings in any one area.

Although our figures suggest that subtype 'ay' is associated with chronic aggressive hepatitis in our Mediterranean patients, this same subtype was also seen more commonly amongst those Mediterranean patients with acute hepatitis who either acquired the infection in their country of origin or from other Mediterranean subjects living in Great Britain. This predominance of the 'ay' subtype was also seen amongst the HB-Ag-positive subjects with chronic persistent hepatitis and the healthy carriers from the Mediterranean. These findings are in accordance with those of a much larger survey of HB-Ag-positive subjects from Greece (Hadziyannis and Le Bouvier, 1972).

It would seem that subtype 'ad' predominates in northern Europe. This was clearly seen from the results of subtyping the healthy HB-Ag carriers from this area, and subtype 'ad' also predominated in the other diagnostic groups from northern Europe, apart from those subjects with acute hepatitis. However, if the subtyping results of those with acute hepatitis who gave a history of parenteral exposure were withdrawn, again subtype 'ad' predominated in the northern European subjects.

Other workers have found HB-Ag-positive cirrhosis to be exclusively 'ad' in type (Nielsen and

Le Bouvier, 1973) but it is likely that their patients came from one geographical area. It is noteworthy in the context of this Danish study that reference made to a preliminary report by Magnius states that subtyping of hepatitis serum collected in Stockholm in 1953 has shown subtype 'ad' to have predominated then, whereas subtype 'ay' now appears to predominate in the same geographical area (Iwarson, Magnius, Lindholm, and Lundin, 1973).

Therefore, it would seem that infection with a particular subtype of the hepatitis B virus is associated with epidemiological factors and cannot be related to any particular form of hepatic pathology.

The concordance of subtyping in our household studies and in those of others (Holland *et al*, 1972) emphasizes the epidemiological rather than the pathological importance of the 'ay' and 'ad' subtypes. In seven of the studies where non-parenteral, intrahousehold transmission of the hepatitis B virus was suspected, there was no familial relationship between the HB-Ag-positive persons.

In summary, both 'ay' and 'ad' subtypes seem to be distributed throughout the spectrum of liver diseases associated with the HB-Ag. However, a particular subtype may predominate within certain geographical areas at a given time, or when acquired by a particular route of infection, thereby account-

ing for the apparent association of a specific subtype with a certain disease state.

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References

- Gordon, I., Berberian, M., Stevenson, D., and Redeker, A. G. (1972). Distribution of hepatitis B antigenic determinants in different forms of viral hepatitis. *J. infect. Dis.*, **126**, 569-573.
- Hadziyannis, S., and Le Bouvier, G. L. (1972). Australia antigen subtypes in Greece (Greek). *Iatrike*, **22**, 453-457.
- Holland, P. V., Purcell, R. H., Smith, H., and Alter, H. J. (1972). Subtyping of hepatitis-associated antigen (HB Ag); simplified technique with counterelectrophoresis. *J. Immunol.*, **109**, 420-425.
- Iwarson, S., Magnius, L., Lindholm, A., and Lundin, P. (1973). Subtypes of hepatitis B antigen in blood donors and post-transfusion hepatitis; clinical and epidemiological aspects. *Brit. med. J.*, **1**, 84-87.
- Le Bouvier, G. L. (1971). The heterogeneity of Australia antigen. *J. infect. Dis.*, **123**, 671-675.
- Nielsen, J. O., Le Bouvier, G. L., and the Copenhagen hepatitis program (1973). Subtypes of Australia antigen among patients and healthy carriers in Copenhagen. *New Engl. J. Med.*, **288**, 1257-1261.