J. Hyg., Camb. (1975), **74**, 123
Printed in Great Britain

Subtypes of hepatitis B antigen among patients and symptomless carriers

By HELEN T. GREEN and G. C. TURNER
Regional Public Health Laboratory, Fazakerley Hospital, Liverpool

(*Received* 11 *July* 1974)

SUMMARY

A study of the distribution of subtypes *ad* and *ay* among sera from hepatitis B antigen-positive subjects in North West England and North Wales revealed a marked contrast between symptomless carriers among whom *ad* predominated and patients with acute hepatitis the majority of whom were *ay*. Those with hepatitis associated with drug addiction or other forms of 'needle transmission' were almost all of subtype *ay*. On the other hand in cases of 'sporadic' hepatitis without evidence of parenteral exposure subtypes *ad* and *ay* are about equally distributed.

These findings are similar to those reported from other countries in Northern Europe and North America. Although geographical and social factors clearly affect the distribution of the two subtypes it is suggested that the virus of subtype *ay* may be more readily transmitted than subtype *ad* by parenteral routes involving small amounts of blood.

INTRODUCTION

Since the recognition of Australia or hepatitis B antigen (HB Ag) as a marker for the virus of serum hepatitis (hepatitis B), it has become apparent that infection of man with this virus is much more frequent than had been realized previously.

Infection may present as acute hepatitis including cases that follow blood transfusion or are associated with other means of parenteral transmission such as the drug addict's shared syringe. It is also responsible for some of the so-called 'sporadic cases' of acute hepatitis (Cossart & Vahrman, 1970; Prince *et al.* 1970). On the other hand, it may be encountered as chronic infection presenting either as chronic liver disease or as long-term symptomless carriage detected when blood donors are screened or in population surveys for HB antigen. The incidence of symptomless carriage varies from 0·1 % of the inhabitants of North West Europe or North America to 5 % or more of those in tropical or some Mediterranean countries.

HB Ag-positive sera can be identified by a variety of serological methods including immunodiffusion, immunoelectroosmophoresis (IEOP) and radio-immunoassay (RIA). All require the use of detector antisera which are either human HB antibody-positive sera, or sera from animals in which antibody has been raised by immunization with HB Ag. With some antisera immunological differences between HB Ag-positive sera can be demonstrated by the development of 'spurs' in immunodiffusion tests, representing reactions of partial identity between

the HB Ag-positive sera; parallel differences can be demonstrated by IEOP or RIA. These differences can be expressed in terms of the presence or absence of certain antigenic determinants including those designated *a*, *d*, *y*, *r*, *w* and *x* (Le Bouvier, 1971; Bancroft, Mundon & Russell, 1972).

Determinant *a* is common to all HB Ag-positive sera which with rare exceptions also possess either *d* or *y* but not both. Determinants *r* and *w* are also mutually exclusive but *r* is confined to East Asia. Determinant *x* is present in nearly all HB Ag-positive sera and is probably a host component. For purposes of classification and epidemiological investigation attention has been directed mainly to *d* and *y* and sera have been assigned to subtypes *ad*, *ay* and rarely *a* (Le Bouvier, 1971). It is generally considered that these subtypes represent varieties of hepatitis B virus and that *d* and *y* are the phenotypic expression of 2 distinct genotypes of the virus (Le Bouvier *et al.* 1972).

The global distribution of subtypes shows zones of dominance by one subtype to the almost total exclusion of the other (Mazzur, Burgert & Blumberg, 1974). Examples are the dominance of *ay* in the Middle East and *ad* in East Asia. In North West Europe and North America, however, both types are encountered and this provides an opportunity to study the relation between various forms of hepatitis B infection and the subtypes associated with them.

The investigation reported here was based on serum samples sent between 1969 and 1973 to the hepatitis reference centre, Liverpool Public Health Laboratory, from patients, hospital and other medical staff, and blood donors found to be HB Ag-positive by the Regional Blood Transfusion Service. During the period under review 255 HB Ag-positive subjects were identified, nearly all resident in West Lancashire, Merseyside or North Wales.

METHODS

HB Ag-positive sera were identified initially by IEOP (crossover electrophoresis) (White, Lasheen, Baillie & Turner, 1971). Positive sera were subtyped by immunodiffusion using HB antibody-positive sera selected for this purpose because they gave spurs characteristic of partial identity reactions between precipitin lines with reference *ad* and *ay* HB Ag-positive sera. These reference sera were provided by Dr Y. E. Cossart, Virus Reference Laboratory, Colindale, London. The 2 antisera used for subtyping behaved as anti-*ad* and anti-*ay* respectively. The anti-*ad* serum was a commercial antiserum (Behringwerke anti-Au rabbit serum No. 2413C) that had been used in routine tests for the detection of HB Ag; it was the only batch of commercial antiserum tested by us that behaved in this way. The anti-*ay* serum was EK, a human serum (Skinhøj, 1973) from Dr P. Skinhøj, Bispebjerg Hospital, Copenhagen.

Dr G. Le Bouvier tested HB Ag-positive sera identified by us as *ad* and *ay* and confirmed that they behaved as his original *ad* and *ay* subtypes.

Sera were subtyped by testing in wells adjacent to standard *ad* and *ay* sera in immunodiffusion tests with anti-*ad* and anti-*ay* sera respectively. Those that were weakly positive were concentrated 2- to 8-fold by addition of calculated weights of a polyacrylamide hydrogel 'Lyphogel' (Gelman).

The strength of a precipitin reaction depends on the relative proportions of antigen and antibody. The sera under test varied greatly in the strength of reaction for HB Ag and consequently in the ease with which spurring could be demonstrated. In some instances spurs could only be detected after lyphogel concentration; on the other hand a few sera that were strongly positive for HB Ag had to be diluted. Because of weak reactions spurring could not be demonstrated with sera from about 7% of our subjects and these were classified as 'not typable'; although included in the tables they are excluded from totals in calculating percentages of subtypes *ad* and *ay*.

In two instances sera under test behaved as *ay* with the anti-*ad* serum and as *ad* with the anti-*ay* serum. In each case the spur was formed by the control *ad* or *ay* serum adjacent to the test serum which we concluded must possess neither *d* nor *y* determinant, i.e. it belonged to the uncommon subtype *a*.

RESULTS

Of the 255 subjects studied, 127 were patients with acute hepatitis and 125 were symptomless carriers of HB antigen or patients with chronic liver disease. Of the other 3, 2 were babies, whose mothers were HB Ag-positive at term, and who became HB Ag-positive (both *ay*) at about 2 months of age, and 1 was a medical laboratory technician with transient antigenaemia (not typed) but no evidence of hepatitis.

Acute hepatitis B (Table 1)

In all 127 cases antigenaemia was of limited duration and associated with typical clinical and biochemical features of acute hepatitis. Evidence of parenteral exposure to blood-borne infection was lacking in 24 (19%) although 6 of these were in high-risk occupations in medicine, dentistry or hospital work. The remainder of the 127 cases fell into 4 main categories consisting of those who became ill after:

(*a*) blood transfusion, the incubation period varying from 62 to 149 days,

(*b*) illicit injections of drugs or admitted social contact with drug addicts,

(*c*) other forms of parenteral exposure namely, injections abroad (2 patients), tattooing (2 patients), dental treatment (4 patients), accidental exposure to HB Ag-positive blood (3 hospital staff),

(*d*) contact with HB Ag-positive patients undergoing haemodialysis.

There were two distinct patterns of distribution of subtypes among these groups of patients. Subtypes *ad* and *ay* were found with about equal frequency in those without a history of parenteral exposure and in those whose infection was attributed to blood transfusion. On the other hand there was an overwhelming preponderance of subtype *ay* among those who had been exposed to infection through illicit use of intravenous drugs, social contact with drug addicts, tattooing or injections or accidents in medical or dental practice. All the haemodialysis-associated cases were of subtype *ay* but they were all from the same outbreak in Liverpool (Jones *et al.* 1967; Turner & White, 1969).

Table 1. *Subtypes* ad, ay *and* a *in patients with acute hepatitis B*

Probable mode of transmission	Number of patients	Number of serum samples: Subtype *ad*	Subtype *ay*	Subtype *a*	Not typable	Not available for typing
No history of parenteral exposure:						
Patients	18	6 (46%)	7 (54%)	0	0	5
Medical, dental, hospital staff	6	3 (50%)	3 (50%)	0	0	0
Blood transfusion	34	16 (52%)	14 (45%)	1 (3%)	3	0
Other parenteral exposure:						
Illicit drug use (injections admitted)	31	0	30 (100%)	0	1	0
Illicit drugs (soft drugs or social contact with addict admitted)	12	1 (8%)	11 (91%)	0	0	0
Injections, tattoos, dentistry, etc.	11	2 (20%)	8 (80%)	0	1	0
Associated with haemodialysis (one outbreak):						
Staff of unit	10	0	10 (100%)	0	0	0
Relatives at home	5	0	4 (100%)	0	1	0

In this and succeeding tables figures in parentheses are percentages of total number of serum samples that were subtypable.

Table 2. *Subtypes* ad, ay *and* a *in patients with acute hepatitis B after blood transfusion*

Place and time of transfusion	Number of patients	Number of serum samples: Subtype *ad*	Subtype *ay*	Subtype *a*	Not typable
In Liverpool region or North Wales					
Before 1972	12	7 (64%)	4 (36%)	0	1
In 1972 or 1973	11	6 (60%)	3 (30%)	1 (10%)	1
Elsewhere in Britain or abroad 1969 to 1973	11	3 (30%)	7 (70%)	0	1

The 34 cases of post-transfusion hepatitis fell into three groups (Table 2). The first two groups received their blood transfusions in the Liverpool region or North Wales, the area covered by this survey; 12 between 1967 and 1971 when donors were not screened for HB Ag and 11 in 1972 and 1973 when screening had been introduced and some HB Ag-positive donors had been identified and excluded. Among the two groups the majority of cases were of subtype *ad*, 64 and 60% respectively. The third group comprised those who had received blood elsewhere in Britain or abroad; of these 70% were of subtype *ay*.

Of 31 cases of post-transfusion hepatitis in which the subtype was known the incubation period could be determined with reasonable accuracy in 25. Of the 12 that were of subtype *ad* it was less than 80 days in 3 (25%) and greater than

Table 3. *Subtypes* ad, ay *and* a *in long-term carriers of hepatitis B antigen*

Type of subject	Number of subjects	Number of serum samples: Subtype *ad*	Subtype *ay*	Subtype *a*	Not typable	Not available for typing
Symptomless carriers:						
Blood donors 1972–3	95	62 (78 %)	17 (21 %)	1 (1 %)	13	2
Hospital staff	2	2	0	0	0	0
Hospital patients	8	5 (71 %)	2 (29 %)	0	1	0
Patients with chronic liver disease	10	5 (55 %)	4 (45 %)	0	0	1
Patients on haemodialysis (one outbreak)	10	0	10	0	0	0

80 days in 9 (75 %). For the 13 of subtype *ay* the corresponding figures were 5 (38 %) and 8 (62 %).

There was a fatal outcome in 6 of the 34 cases of post-transfusion hepatitis; of the fatal cases 3 were of subtype *ad*, 3 of subtype *ay*. There was thus no evidence of any great difference between the subtypes in relation to the incubation period of the infection with which they were associated or the severity of the disease.

Chronic hepatitis B infection (Table 3)

Subjects with chronic infection fell into 3 categories:

(*a*) symptomless carriers of HB antigen detected among blood donors screened in 1972 and 1973 and hospital staff,

(*b*) patients with chronic liver or other diseases,

(*c*) patients with chronic renal failure on maintenance haemodialysis.

Among the symptomless carriers, most of whom were volunteer blood donors detected by routine IEOP screening, there was a preponderance of subtype *ad*. Of 97 HB Ag-positive carriers, sera from 82 were subtyped; 64 (78 %) were *ad*, 17 (21 %) *ay* and 1 (1 %) *a*. Of 8 hospital patients with diseases other than those associated with the liver 7 were subtyped; 5 (71 %) were *ad* and 2 (29 %) were *ay*. Among 10 with chronic liver disease, however, the subtypes were more equally distributed; of 9 subtyped 5 (55 %) were *ad* and 4 (45 %) *ay*.

The HB Ag-positive sera from patients on maintenance haemodialysis were all of subtype *ay*. All the patients were however in one haemodialysis unit (*vide supra*) and became long-term symptomless carriers after mild or inapparent infection.

Stability of subtypes

In patients with acute hepatitis, testing of serial samples of serum collected during convalescence showed a progressive weakening and loss of the HB Ag-positive reaction. In 22 cases where serial subtyping was done the subtype, *ay* in 17, *ad* in 5, was unchanged by the methods used in this investigation. In 4 patients who were long-term carriers of HB Ag, the subtype, *ad* in each case, was unchanged for 3, 11, 12 and 18 months respectively.

In epidemiologically-related cases of infection, subtypes were the same. Thus in two instances of post-transfusion hepatitis in which an HB Ag-positive donor was identified the subtype of donor and recipient was the same, *ay* in each instance. One case of acute HB Ag-positive hepatitis was a pregnant woman at full term; the baby developed antigenaemia at 2 months of age (Turner *et al.* 1971). Sera from mother and baby were both of subtype *ay*.

In an outbreak of hepatitis B infection in a haemodialysis unit, all 25 HB Ag-positive sera from patients, staff and relatives were of subtype *ay*. In 9 patients who became long-term carriers of HB Ag the subtype was unchanged for periods of up to 6 years.

DISCUSSION

In this study of 255 subjects in North West England and North Wales who were found to be hepatitis B antigen-positive, subtype *ad* accounted for over 70% of those with chronic or symptomless infection but subtype *ay* for the majority of cases of acute hepatitis. This pattern is similar to that reported from Canada (Perry & Chaudhury, 1973), Denmark (Nielsen, Le Bouvier and Copenhagen hepatitis acute program, 1973), Sweden (Iwarson, Magnius, Lindholm & Lundin, 1973) and U.S.A. (Holland, Purcell, Smith & Alter, 1972). It has been suggested that determinants *d* and *y* are phenotypic expressions of 2 genotypes of hepatitis B virus (Le Bouvier *et al.* 1972) but it is not clear whether the genotypes defined in this way differ in respect of communicability or tendency to cause persistent infection with or without active liver disease.

Use of the test for HB Ag has shed considerable light on the epidemiology of hepatitis B. It has shown that two groups of subjects are sources of infection. These are on the one hand the long-term carriers of HB Ag, the majority symptomless, and on the other hand cases of acute HB Ag-positive hepatitis who are infective during the antigenaemic phase of the incubation period and acute illness. There is evidence for at least 3 modes of spread from these sources through (i) blood transfusion, (ii) penetration of skin by instruments (and perhaps insects) contaminated with blood, and (iii) transmission by various 'non-parenteral' routes associated with the presence of HB Ag in saliva (Ward, Borchert, Wright & Kline, 1972), faeces (Sonnabend *et al.* 1972), urine (Apostolov *et al.* 1971), or semen (Heathcote, Cameron & Dane, 1974).

The most clearly defined mode of spread is transmission by blood transfusion, characterized by the intravenous administration at a known time of a relatively large amount of infective material from a symptomless carrier or rarely a donor incubating acute hepatitis. Theoretically it might be instructive to relate the subtype distribution in the donor population to that in patients with post-transfusion hepatitis but any attempt to do this is complicated by several factors. These include the fact that in the past many cases of post-transfusion hepatitis have not been reported or investigated; since screening for HB Ag began, the donors responsible for cases of post-transfusion hepatitis are those whose HB Ag-positive state was not detected by the routine method; and, as in our series, some patients with post-transfusion hepatitis may have been given their blood transfusions 2–3 months earlier in an area other than that from which the donor population

under review has been drawn. Moreover, as many as 30 % of patients given blood may possess immunity to HB Ag (Reed *et al.* 1974) and this may affect not only the number of cases of post-transfusion hepatitis but also the *ad*/*ay* subtype distribution among them. These limitations must be taken into account in assessing the significance of our finding that, although only 17 (21 %) of 80 HB Ag-positive donors were of subtype *ay* (Table 3), this subtype accounted for 36 and 40 % respectively of hepatitis in patients transfused in the region under review before and after the introduction of screening (Table 2). There is evidence that all donors are not equally infective and it has been claimed that only those with abnormal liver histology are infective (Reinicke *et al.* 1972). Among our patients with chronic liver disease (Table 3) there was a higher proportion (45 %) with subtype *ay*, than among symptomless carriers.

Among patients in whom acute HB Ag-positive infection was associated with illicit use of drugs or social contact with drug addicts there was an overwhelming preponderance of subtype *ay*; 41 (98 %) of 42 infections were of this subtype. Skinhøj (1973) with similar findings in Denmark suggested that the explanation might lie in the frequent visits by drug addicts to countries of the Eastern Mediterranean area where subtype *ay* is dominant. Geographical and social factors may also have been responsible for our finding that among patients with other forms of parenteral exposure, 8 (80 %) were of subtype *ay* (Table 1). Two of these patients developed hepatitis, *ay* in each case, after injections in Nigeria; subtype *ay* is dominant in Africa (Mazzur *et al.* 1974). Of 3 hospital staff with hepatitis, 2, both *ay*, had taken part in the investigation of HB Ag-positive infection associated with illicit injections.

An alternative explanation for the predominance of subtype *ay* among patients with a history of parenteral exposure other than by blood transfusion might be that the virus of subtype *ay* is more readily communicable than *ad* by routes involving very small amounts of blood.

An outbreak of HB Ag-positive infection in a haemodialysis unit is presumably initiated by a single HB Ag-positive transfusion with subsequent patient-to-patient and patient-to-staff cross-infection in which the vehicle is small amounts of infected blood. In nearly all outbreaks in haemodialysis units from which subtype findings have been reported, a single subtype has been responsible, usually *ay*, as in the outbreak included in this investigation.

Some haemodialysis-associated outbreaks have however been caused by subtype *ad* (Skinhøj, 1973; Mosley, Edwards, Meihaus & Redeker, 1972). Doubt may also be cast on the suggestion that small-dose transmission favours subtype *ay* by our finding that among patients with HB Ag-positive hepatitis without a history of parenteral exposure to infection, subtypes were about equally distributed between *ad* (46 %) and *ay* (54 %). If, however, the vehicle for the infective agent in such cases was saliva, faeces, urine or semen rather than blood there may have been a different pattern of subtype communicability.

It is clear that some of our groups are too small for firm conclusions to be drawn from our findings but we suggest that extended observations on these lines may contribute useful information about the epidemiology of hepatitis B.

We thank members of staff of many pathology laboratories who referred blood samples to us for investigation and their clinical colleagues for information about patients; Dr D. A. Lehane and the staff of Liverpool Regional Blood Transfusion Service for many samples from donors and much helpful cooperation; Dr Y. E. Cossart, Dr G. Le Bouvier and Dr P. Skinhøj for the help described in the text and to Miss Margaret Fenwick, Mr Henry Nwaonu and Mrs Susan Jones for technical assistance.

REFERENCES

APOSTOLOV, K., BAUER, D. J., SELWAY, J. W. T., FOX, R. A., DUDLEY, F. J. & SHERLOCK, S. (1971). Australia antigen in urine. *Lancet* i, 1274.

BANCROFT, W. H., MUNDON, F. K. & RUSSELL, P. K. (1972). Detection of additional antigenic determinants of hepatitis B antigen. *Journal of Immunology* **109**, 842.

COSSART, Y. E. & VAHRMAN, J. (1970). Studies of Australia-SH antigen in sporadic viral hepatitis in London. *British Medical Journal* i, 403.

HEATHCOTE, J., CAMERON, C. H. & DANE, D. S. (1974). Hepatitis-B antigen in saliva and semen. *Lancet* i, 71.

HOLLAND, P. V., PURCELL, R. H., SMITH, H. & ALTER, H. J. (1972). Subtyping of hepatitis-associated antigen (HBAg); simplified technique with counterelectrophoresis. *Journal of Immunology* **109**, 420.

IWARSON, S., MAGNIUS, L., LINDHOLM, A. & LUNDIN, P. (1973). Subtypes of hepatitis B antigen in blood donors and post-transfusion hepatitis: clinical and epidemiological aspects. *British Medical Journal* i, 84.

JONES, P. O., GOLDSMITH, H. J., WRIGHT, F. K., ROBERTS, C. & WATSON, D. C. (1967). Viral hepatitis. A staff hazard in dialysis units. *Lancet* i, 835.

LE BOUVIER, G. L. (1971). The heterogeneity of Australia antigen. *Journal of Infectious Diseases* **123**, 671.

LE BOUVIER, G. L., MCCOLLUM, R. W., HIERHOLZER, W. J., IRWIN, G. R., KRUGMAN, S. & GILES, J. P. (1972). Subtypes of Australia antigen and Hepatitis-B virus. *Journal of the American Medical Association* **222**, 928.

MAZZUR, S., BURGERT, S. & BLUMBERG, B. S. (1974). Geographical distribution of Australia antigen determinants, d, y and w. *Nature, London* **247**, 38.

MOSLEY, J. W., EDWARDS, V. M., MEIHAUS, J. E. & REDEKER, A. G. (1972). Subdeterminants d and y of hepatitis B antigen as epidemiologic markers. *American Journal of Epidemiology* **95**, 529.

NIELSEN, J. O., LE BOUVIER, G. L. & Copenhagen hepatitis acute program (1973). Subtypes of Australia antigen among patients and healthy carriers in Copenhagen. *New England Journal of Medicine* **288**, 1257.

PERRY, E. & CHAUDHURY, R. K. (1973). Hepatitis-B antigen: distribution of ad and ay subtypes in blood donors and hepatitis patients. *Canadian Medical Association Journal* **109**, 857.

PRINCE, A. M., HARGROVE, R. L., SZMUNESS, W., CHERUBIN, C. E., FONTANA, V. J. & JEFFRIES, G. H. (1970). Immunologic distinction between infectious and serum hepatitis. *New England Journal of Medicine* **282**, 987.

REED, W. D., MITCHELL, C. G., EDDELSTON, A. L. W. F., LEE, W. M., WILLIAMS, R. & ZUCKERMAN, A. J. (1974). Exposure and immunity to hepatitis-B virus in a liver unit. *Lancet* i, 581.

REINICKE, V., DYBKJAER, E., POULSEN, H., BANKE, O., LYLLOF, K. & NORDENFELT, E. (1972). A study of Australia-antigen-positive blood donors and their recipients with special reference to liver histology. *New England Journal of Medicine* **286**, 867.

SKINHØJ, P. (1973). Hepatitis-associated antigen, subtypes D and Y. An epidemiological and clinical evaluation *Scandinavian Journal of Infectious Diseases* **5**, 85.

SONNABEND, W., KISTLER, G. S., KRECH, U., JUNG, M. & ÜBERSCHLAG, M. L. (1972). Immunologischer und elektronenmikroskopischer Nachweis von Australia-antigen (hepatitis-B antigen) in Fäzes-und-Urinproben von Patienten mit Virus hepatitis B. *Schweizerische Medizinische Wochenschrift* **102**, 1053.

TURNER, G. C., FIELD, A. M., LASHEEN, R. M., TODD, R. McL., WHITE, G. B. B. & PORTER, A. A. (1971). SH (Australia) antigen in early life. *Archives of Disease in Childhood* **46**, 616.

TURNER, G. C. & WHITE, G. B. B. (1969). SH antigen in haemodialysis-associated hepatitis. *Lancet* ii, 121.

WARD, R., BORCHERT, P., WRIGHT, A. & KLINE, E. (1972). Hepatitis B antigen in saliva and mouth washings. *Lancet* ii, 726.

WHITE, G. B. B., LASHEEN, R. M., BAILLIE, M. B. & TURNER, G. C. (1971). Comparison of three serological methods for the detection of hepatitis-associated antigen. *Journal of Clinical Pathology* **24**, 8.