SUBDETERMINANTS \(d\) AND \(y\) OF HEPATITIS B ANTIGEN AS EPIDEMIOLOGIC MARKERS

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Mosley, J. W. (John Wesley Hospital, 2826 S. Hope St., Los Angeles, Calif. 90007), V. M. Edwards, J. E. Meihaus and A. G. Redeker. Subdeterminants \(d\) and \(y\) of hepatitis B antigen as epidemiologic markers. Am J Epidemiol 95: 529–535, 1972.—A simple Ouchterlony technique for the mutually exclusive \(d\) and \(y\) subdeterminants of hepatitis B antigen has been applied to selected specimens to investigate whether these antigenic subtypes are derived from the host or the strains of virus. Four \(y^+\) infections produced \(y^+\) disease in seven genetically related household contacts; likewise, three \(d^+\) and 13 \(y^+\) index cases gave rise to the consistent subtype in 16 secondary cases among genetically unrelated persons. Further, in a hemodialysis unit in which intra-unit transmission rather than transfusion was suspected as a source of hepatitis B antigen-positive cases, \(y^+\) infections were observed in all 23 dialysis patients, all 13 medical-paramedical persons, and all 5 household contacts with specimens available for typing. This result differed significantly from the frequency of \(y^+\) infection among other transfused patients (44%) and other medical-paramedical personnel (61%). It is concluded that the \(d\) and \(y\) subdeterminants are characteristic of the viral strain, and useful as indices of epidemiologic relationship.

epidemiologic methods; hemodialysis; hepatitis, homologous serum; hepatitis virus, homologous serum

Several groups of investigators have now demonstrated the occurrence of subdeterminants of hepatitis B antigen (HBAg, also called hepatitis-associated antigen, SH antigen, and Australian antigen), as well as corresponding subspecificities of antibody (HBAb). These subdeterminants are recognizable by several techniques: 1) divergences in positivity or in titers when HBAg and HBAb from various patients are compared (1–3); 2) precipitin lines of partial rather than complete identity in agar gel diffusion (AGD) systems (4–6); and 3) the production of monotypic HBAb by injection of laboratory animals with concentrated HBAg from selected patients (7–10).
Nomenclature for the subdeterminants is not yet standardized, a situation to be expected in view of the early stage at which work now stands. Le Bouvier (5) has identified four HBAg subdeterminants, and more may exist. His designations of a, x, d and y are widely used at present, and other terminologies to at least some extent have been reconciled with these.

The occurrence of subtypes of HBAg is not surprising to those investigators who feel that the 20 nm particle is either the type B hepatitis virus itself or a byproduct of its replication. Most if not all microbial agents display strain differences of at least some magnitude. It must be stated, however, that the fact of subdeterminants is also consistent with the view of London and associates (11) that the particle incorporates a variety of proteins immunologically characteristic of the host. The mere existence of HBAg subtypes, therefore, provides no immediate clue concerning their nature or general significance.

In addition to the theoretical considerations, there are practical reasons for deciding whether HBAg subtypes are determined by the type B hepatitis virus or the infected host. Microbial subtyping can provide evidence that cases are epidemiologically related, and sometimes permits one to distinguish among suspected sources of infection. Fortunately, the two hypotheses concerning subtypes can be investigated by relatively simple observations. If subtype is a host-determined characteristic, then genetically related persons experiencing HBAg-positive infections should have a much greater consistency of subspecificity than genetically unrelated persons. If subtype is a characteristic of the hepatitis B virus, then epidemiologically related cases in genetically unrelated persons should have just as much consistency of subspecificity.

Our investigation of this problem was facilitated by two circumstances. First, we have as HBAb reagent a human serum that permits rapid characterization of unknown HBAg-positive specimens as d+ or y+. Secondly, we have a large store of HBAg-positive sera from cases about which much epidemiologic information is also already available. These specimens have been collected for several years from routine admissions, and during a variety of special, often prospective investigations, at John Wesley County Hospital (JWCH) and the Los Angeles County-University of Southern California Medical Center (LAC-USC).

**Method of study**

**HBAg testing.** Since 1969, essentially all patients with suspected viral hepatitis at JWCH and LAC-USC have been tested for HBAg in the Clinical Laboratories, John Wesley County Hospital, under the supervision of Dr. Robert L. Peters. The techniques used were initially agar gel diffusion, and then counter-immunoelectrophoresis (CIEP). Preliminary concentration of serum with Lyphogel (12) provided much greater sensitivity by both methods. Patients included in the present study were identified as HBAg-positive by AGD or CIEP.

**HBAg subtyping.** In the Hepatic Epidemiology Laboratory, we have used a modified micro-Ouchterlony technique to determine identity or partial identity of an unknown with reagent HBAg-positive sera of known subtypes. Glass slides 1 x 3 inches were covered with 2 ml of 0.8 per cent agarose containing Tris buffer (0.01 M Tris, 0.01 M disodium-ethylendiaminetetraacetic acid, and 0.15 M sodium chloride) at pH 7.6. Wells were cut with a Behring punch in the pattern illustrated in figure 1. The reagent HBAb in each center well was derived in moderate quantity from a patient (J.P.) four days after he was heavily transfused for a gunshot wound. Without absorption it gave clear AGD reactions of identity or partial identity with reagent HBAg’s, the only one of 10 human and one commercially available sera to do so. Thus, it may be necessary to screen a number of specimens to find a source of comparably suitable HBAb as reagent.
Figure 1. Subtyping of unknown HBAg by comparison with reagent HBAg with known subdeterminants. Top: Specimen 1 (y+) shows identity with reagent a (well II) and reagent y + (a) (well III), and partial identity with d (well I). Bottom: Specimen 2 (d+) shows identity with reagent d (well I), and partial identity with reagents a (well II) and y + (a) (well III). The HBAb in all center wells is from patient J. P.

The reagent HBAg's were characterized by Dr. Irving Gordon and co-workers, Department of Microbiology, USC. Using adsorbed serum from selectively immunized rabbits (10), they characterized three HBAg-positive sera as containing d only (a not detected), a only (neither d nor y detected), and y with a small amount of a (y + (a)). Reagent a and reagent y + (a) showed reactions of identity with each other. Unknown specimens giving reactions of identity with reagent d and partial identity with reagents a and y + (a) have been called d+. Unknowns giving the reverse pattern have been called y+. These presumably correspond to Le Bouvier's subgroup D, and subgroups A and Y, respectively.

Reagent HBAg and unknown HBAg were each placed in two adjacent and one opposite wells to permit comparison of junctures between lines of the same sera with junctures of the reagent and unknown sera. Concentrations of reagent HBAg and HBAb in the typing of unknowns were semi-quantitatively adjusted according to the strength of the reaction observed on preliminary CIEP. Exact adjustments in unitage were not necessary because of the generally unequivocal reactions when patterns are compared. Weakly reactive unknowns usually could not be subtyped because of the faintness of the lines. Of the specimens covered in this report, our subtyping was confirmed for all 27 sera also tested by Gordon.

Cases. Persons with HBAg-positive hepatitis for whom results are reported were derived from several sources: 1) routine admissions to JWCH or LAC-USC for icteric disease diagnosed as viral hepatitis on the basis of characteristic symptoms, signs, and laboratory tests; 2) medical, nursing, laboratory, or other paramedical personnel at JWCH or LAC-USC who developed symptoms and findings compatible with viral hepatitis (all were seen by one of the au-
thors (A.G.R.) for primary care or in consultation; 3) a screening program for patients undergoing long-term hemodialysis for chronic renal disease in the Unit at LAC-USC.

Patients were defined as genetically related who fell within the following kinships: parent-child; sibling-sibling; and aunt-nephew. Cases classified as genetically unrelated include none known to have more distant but definite kinship; in many instances, the genetically unrelated pairs represented spouses or nonmarital sexual partners.

Cases were defined as epidemiologically related when the second had a known (accidental) percutaneous exposure to the first, or continued close personal contact with the first. Other criteria were incubation periods compatible with type B hepatitis, and the absence of any other likely source for the secondary infection. The difficulty of satisfying reliably the last criterion in persons who abuse drugs resulted in our excluding all instances in which the secondary case was suspected of illicit drug practices.

**RESULTS**

Our collection lacked subtypable specimens from genetically related, HBAg-positive pairs for whom a concomitant epidemiologic relationship could be excluded. We had, nevertheless, seven instances in four families of transmission to genetically related persons. Table 1 presents pertinent data concerning the index and secondary cases. All four index cases were $y^+$; all seven secondary cases were $y^+$. This result is consistent with subtype being either a host or viral characteristic; the former, however, would be strongly suggested if subtypes among epidemiologically related

| Table 1 |
|-------------------------|------------------------|------------------------|
| **Hepatitis B antigen subtypes in genetically and epidemiologically related cases** |
| **Family** | **Details** | **Subtype** | **Details** | **Subtype** |
| A | 30-year-old female with hemodialysis-associated chronic antigenemia that first appeared 5/20/70. | $y^+$ | 41-year-old sister in same household, onset icteric disease 5/20/71. | $y^+$ |
| | | | 6-year-old nephew in same household, onset icteric disease 6/12/71. | $y^+$ |
| | 23-year-old male with addiction-associated chronic antigenemia first observed 6/17/70. | $y^+$ | 4-year-old daughter in same household, onset icteric disease 4/18/70. | $y^+$ |
| | | | 2-year-old daughter in same household, observed to be HBAg-positive in 7/70. | $y^+$ |
| | 22-year-old male with addiction-associated icteric hepatitis, onset 7/13/71. | $y^+$ | 50-year-old mother who frequently took care of children, onset icteric hepatitis 10/21/70. | $y^+$ |
| | | | 21-year-old brother who was frequent visitor in household, shared drug equipment, onset icteric hepatitis 8/20/71. | $y^+$ |
| | 19-year-old female with addiction-associated hepatitis, onset 9/1/71. | $y^+$ | 20-year-old sister in same household, shared drug equipment, onset icteric hepatitis 11/15/71. | $y^+$ |
but genetically unrelated cases were less consistently the same.

We observed 16 instances of epidemiologically related infection in genetically unrelated, HBAg-positive pairs for whom subtypable specimens were still available. There were no additional pairs for whom an “epidemiologically related” classification was changed after the subtyping result became available. Table 2 examines the consistency of subtype in the index and secondary cases in a 2 × 2 contingency table, and shows complete agreement in epidemiologically related cases despite the persons being genetically unrelated. The likelihood of this degree of association by chance alone is 0.3 per cent (Fisher exact test). We interpret these data as being most consistent with subtype as a viral characteristic.

Analysis of hemodialysis-associated infections. Information from a national survey has suggested that most cases of hemodialysis-associated hepatitis are related to transfusion rather than spread within the units themselves (13). For the Unit at LAC-USC, however, Egoz and co-workers (14) found evidence that intra-unit transmission was occurring. In particular, the interval from admission to onset indicated that infection occurred relatively soon after dialysis was started (within seven months), in a manner that would not be expected for randomly infective units of blood. Therefore, in these infections already thought to be related, study of subtype provided a further test of its epidemiologic consistency and usefulness.

### Table 3
*Comparison of hepatitis B antigen subtypes in transfusion-associated nondialysis patients with transfused hemodialysis patients*

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Transfusion-associated hepatitis nondialysis patients</th>
<th>Hemodialysis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>d+</td>
<td>18</td>
<td>56</td>
</tr>
<tr>
<td>y+</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100</td>
</tr>
</tbody>
</table>

*χ² = 16.8, n = 1, p < .001.

Table 3 compares the frequency of d+ and y+ infections in 32 nondialysis patients developing hepatitis within 23 to 173 days after transfusion of blood, and its frequency in 23 hemodialysis patients transiently or chronically HBAg-positive. The latter group received an average of 19 units of blood while on center dialysis. A highly significant difference (p < .001) by χ² testing is consistent with the previous conclusion, arrived at independently of the subtyping data, that transmission was occurring within the Unit.

As a further index of subtypic consistency, two other groups of available cases related to the Hemodialysis Unit were subtyped: 1) medical, nursing, and other personnel working in the Unit who acquired disease either while working in the Unit or within a six-month period thereafter; and 2) icteric household contacts of dialysis patients.

Table 4 compares frequency of subtypes among medical-paramedical personnel who worked on the Unit with those who did not. The complete absence of d+ infections in hemodialysis staff is statistically significant when compared with d+ cases among 28 other occupationally related infections. This again demonstrates the consistency of subtype in nongenetically related individuals.

Finally, HBAg-positive sera were available for five icteric cases among household contacts of hemodialysis patients. Two were
y+ infections, already included in table 1, in a sister and nephew of a hemodialysis patient; one year earlier, the same hemodialysis patient's husband had had overt HBAg-positive hepatitis that was y+. In addition, subtypable specimens were available for two spouses who acquired icteric hepatitis after their husband and wife, respectively, were observed to be HBAg-positive in the Unit; both secondary household cases were also y+. These two pairs are included in table 2.

Thus, all hemodialysis patients were y+, as were epidemiologically related cases in staff and household contacts. Except for the sister and nephew listed in table 1, none of these persons had any known genetic relationship.

**DISCUSSION**

Although specimens were not available from epidemiologically unrelated cases in genetically related persons, any correlation of their subtypes could not be better than that in epidemiologically related, genetically unrelated persons (table 2). In view of the known epidemiologic relationship, by the strict criteria given, of five of seven secondary cases, and the strong possibility of an epidemiologic relationship in the two pairs of siblings who shared equipment with each other as well as other drug users, the correlation in table 1 is also consistent with this conclusion. The possibility that the d and y subdeterminants are characteristic of the host rather than the viral strain seems, therefore, remote.

Regardless of this theoretical consideration, the consistency of subtype in epidemiologically related cases has practical consequences. That it can provide evidence for epidemiologic relatedness of cases is amply demonstrated by application to the hemodialysis-associated infections in patients, staff members of the Unit, and household contacts. The possibility that hemodialysis patients are capable only of y+ infections is excluded by our finding of one d+ infection among seven HBAg-positive patients at two other hemodialysis units in Los Angeles, and by Le Bouvier's characterization of all sera from six HBAg-positive hemodialysis patients in Iowa as d+ (15). We conclude, therefore that y+ infection was initially introduced through transfusion of blood, and then perpetuated by patient-to-patient transmission. Such transmission was probably indirect rather than direct in view of the low risk to household members other than spouses, but this aspect remains to be fully documented.

The characterization of strain with respect to the d and y subdeterminants offers a technique for determining consistency of cases with respect to source of infection. They appear, therefore, to be epidemiologic markers of great usefulness.

**REFERENCES**

15. Le Bouvier GL: Personal communication, 1971