Subtypes of Hepatitis B Surface Antigen (HBsAg) in Blood Donors in Kumamoto, Japan

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Several antigenic determinants are present on the surface of hepatitis B antigen (HBsAg) [2]: a commonly shared determinant, a [6], and two pairs of mutually exclusive determinants, d/y [5] and w/r [1], which yield four possible antigenic combinations, adw, adr, ayw and ayr. The last subtype has been found very rarely [1, 3, personal communication from Le Bouvier, G. L., Bancroft, W. H., and Holland, P. V.]. Since little is known concerning the distribution of the subtypes of HBsAg in Japan [4, 7], we have investigated the incidence of these four subtypes in HBsAg-positive plasmas in Kumamoto, located at the southern Japan.

One hundred and thirty-two HBsAg-positive plasmas obtained from healthy blood donors during 1972 and 1973, as determined by a conventional immunoelectrosyneresis procedure, were examined for subtypes. The positive rate of HBsAg in plasma was 1.4% when about 30,000 blood donors in Kumamoto were tested. Subtyping of HBsAg in the plasma was carried out by a micro-Ouchterlony technique described by Bancroft et al [1]. Subtypes of the test specimens were determined by precipitin lines of complete or partial (spur) identity formed with reference antigens. The reference antigens and antisera were as follows: antigen sera, V801-001-027 (ad) and V802-001-027 (ay); and guinea pig antisera, V801-501-058 (anti-ad) and V802-501-558 (anti-ay). They were supplied from Dr. R.J. Byrne of the Research Resource Branch of the National Institute of Allergy and Infectious Diseases, Md., U.S.A. Antigen sera, EA 47 (ayw) and EH 17 (adr); and rabbit antisera, R604 (anti-adr), R607 (anti-adw) and R563 (anti-ayw) were supplied from Dr. G.R. Irwin, Water Reed Army Institute for Research, Washington D. C., U.S.A. Subtyping of several plasmas or sera available in this laboratory was carried out against the reference reagents mentioned above, and those were used later as the references for subtyping of test specimens; the antigens were H-30 (adr), RC-142 (adw), RC-277 (ayr) and TaHC-80 (ay), and the antisera were rabbit 2441D/2360A (anti-aw) serum purchased from Boehlinger-Werke AG., Marburg, Germany, and goat No. 4 (anti-adr) serum supplied from Dr. S. Matsuda, Takeda Chemical Industries Ltd., Osaka, Japan.

The results of analysis on the antigenic subtyping of HBsAg in 132 plasmas are summarized in Tables 1 and 2. The d determinant was detected in 98%, but the y determinant was found in only 2%. The r and w determinants were found in 95% and 5%, respectively. The incidence of adr, adw and ayr subtypes were 93%, 5% and 2%, respectively, but no ayw subtype was detectable. The determinants of d, y, r and w in several representative specimens were further confirmed by adsorption tests [1].

The prevalence of the four subtypes of HBsAg in Kumamoto presented here resembles that in Thailand reported by Bancroft et al [1], although they tested only 23 specimens positive for HBsAg. However, it is different from that in U.S.A. where ayw,

| No. tested | 132 |
| No. of plasmas with the indicated determinant:
| d/y | 129/3 |
| r/w | 125/7 |

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Table 2. Incidence of subtypes in 132 HBsAg-positive plasmas in Kumamoto

<table>
<thead>
<tr>
<th>No. tested</th>
<th>No. of HBsAg with the indicated subtype</th>
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<tr>
<td>132</td>
<td>122 (93%) 7 (5%) 3 (2%) 0</td>
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$adr$ and $adw$ were detected in 81%, 3% and 16% in 280 specimens tested. Ishida et al [4] reported a result of subtyping of 166 specimens collected from the northern to southern parts of Japan. The $d$ was found in almost all but two specimens (possibly the $y$ subtype) in Nagasaki, the southern part of Japan. A percentage of $w$ subtype in total specimens was 30%, which is coincident with the result reported by Mazzur et al [7]. However, when specimens from the northern Japan alone were totaled, $w$ subtype was much higher in incidence (62%) than in other areas of Japan. The results of the present studies as well as the above-cited previous results suggest that the distribution of $w/r$ subtypes is markedly different from one area to another within Japan and that $y$ subtype can be found more frequently in the southern areas than in other areas of Japan. It should also be emphasized that subtype $ayr$, which is known to be very rare [3], was found in three out of 132 HBsAg-positive plasmas in Kumamoto.

ADDENDUM

After the manuscript was submitted for publication, we found that Dodd et al* had published a report on the incidence of $d/y$ subtypes of HBsAg in a large number of bloods in U.S.A. The $ad/ay$ ratio was 5.8 when 984 positive specimens were analyzed. (*Dodd, R. Y., Holland R. V., Ni, L.Y., Smith, H. M., and Greenwalt, J. J. 1973. Hepatitis B antigen: regional variation in incidence and subtype ratio in the American Red Cross donor population. Amer. J. Epidemiol. 97: 111–115).

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REFERENCES