

Hepatitis Be antigen and antibody in hepatitis B surface antigen positive blood donors

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SUMMARY In a study of 105 asymptomatic HBsAg positive blood donors, 9 (8.6%) were found to have HBeAg, 38 (36.2%) anti-HBe, and the remaining 58 (55.2%) neither marker detectable by gel diffusion. There was no correlation between HBeAg/anti-HBe status and HBsAg sub-types, Gm allotypes, the presence of anti-Gm, red cell antibodies, or rheumatoid factor. Rheumatoid factor activity could be removed from anti-HBe positive sera without removing anti-HBe activity, indicating that separate entities were involved.

HBeAg was found only in donors under the age of 30 ($P < 0.005$), while anti-HBe did not show an age-related trend. HBeAg was also found less commonly in donors of blood group A than in the total carrier population ($P < 0.05$), indicating an apparent protection in carriers of group A. The blood group distribution for the 105 HBsAg positive donors was similar to that of the general population.

The possibility that the host genetic make-up may influence the type of response to human hepatitis B infection has led to a search for allotypic markers linked to certain types of response. For example, evidence has been presented for (Arndt-Hanser *et al.*, 1974) and against (Rundle *et al.*, 1975) an association between specific ABO blood groups and persistent hepatitis B surface antigen (HBsAg) carriage. In multiply transfused thalassaemic patients, Blumberg *et al.* (1972) described an association between the development of anti-HBs (that is, successful virus elimination) and the presence of both anti-Gm antibodies and relative homozygosity with respect to Gm immunoglobulin markers. Hillis *et al.* (1977) have reported a positive association between certain locus B HLA types and HBs antigenaemia in dialysis patients. Finally, it has been proposed that a tendency to HBsAg carriage after hepatitis B virus (HBV) infection may be inherited as a simple autosomal recessive gene (Blumberg *et al.*, 1969), although convincing evidence against this hypothesis in its simplest form has been presented (Vyas, 1974; Mazzur, 1976).

Among chronic carriers of HBsAg, the additional presence of HBeAg (Magnius and Espmark, 1972) is

associated with a greater likelihood of infectivity and progressive liver damage. Early in acute hepatitis B infection HBeAg can usually be detected, to be replaced later by anti-HBe in a majority of cases (Frösner *et al.*, 1978; Miyakawa and Mayumi, 1978); the persistence of HBeAg indicates a more serious prognosis. Thus the presence of HBeAg or anti-HBe in HBsAg carriers allows an additional serological distinction between different clinical responses to HBV infection, which might show a clear relationship to the above allotypic markers.

In addition, evidence was presented by Neurath and Strick (1977) that HBeAg itself may be an idiotypic IgG molecule and anti-HBe an anti-idiotypic. However, more recent work (Takahashi *et al.*, 1978) has shown that HBeAg activity can exist in serum as a molecule smaller than IgG, as well as in association with IgG. Nevertheless, the possibility that the HBeAg/anti-HBe system might be associated with the Gm system, or with naturally occurring rheumatoid factor, warranted investigation.

In this paper we have examined the HBeAg/anti-HBe status of 105 asymptomatic HBsAg positive blood donors detected at routine screening and correlated our findings with age, ABO and Rhesus (D) blood groups, HBsAg subtype, Gm allotype, the presence or absence of rheumatoid factor, anti-Gm, and red cell antibodies.

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Methods

HBeAg was detected by reverse passive haemagglutination (RPHA, Wellcome) or radioimmunoassay (RIA, Abbott). Five of the 105 donors were positive by RIA but negative by RPHA. HBeAg and anti-HBe were detected by gel diffusion after three-fold concentration of test sera; all positive results gave a line of identity with a positive control reaction, and gave no precipitin lines with negative control sera of groups AB and O. Sera were tested for rheumatoid factor (RF) using different commercial kits by latex agglutination and a modified Waaler-Rose procedure (Searle, Hyland and Denver) in the two laboratories and discrepant results were re-tested. HBeAg subtyping was performed using solid phase radioimmunoassay and by radioimmunoprecipitation using monospecific antisera. G1m(a) and G1m(f) allotyping was performed by a passive haemagglutination inhibition technique (Vyas *et al.*, 1968) using red cells coated with anti-Rh of appropriate G1m allotype. The screening for anti-Gm was carried out using a panel of cells coated with various anti-Rh sera known to detect most anti-Gm antibodies (M. M. Izatt, personal communication).

Results

HBeAg was found in 9 (8.6%) sera and anti-HBe in 38 (36.2%), while 58 (55.2%) sera showed neither reaction. Blood group A was less common ($P < 0.05$) in HBeAg positive donors than in the total carrier population, which itself showed ABO and Rhesus(D) blood group frequencies similar to those of the overall regional population (Table 1).

Table 1 *Distribution of ABO and Rhesus(D) blood groups in relation to the HBeAg/anti-HBe status of 105 carriers of HBsAg*

Group	HBeAg	anti-HBe	Neither	Total
O	8	19	31	58
A	0	14	20	34
B	1	2	5	8
AB	0	3	2	5
Rh(D) positive	9	31	52	92
Rh(D) negative	0	7	6	13
Total	9	38	58	105

The percentage of carriers with HBeAg declined with age, whereas no age-specific trend was seen in anti-HBe frequency (Figure). All nine HBeAg positive carriers were less than 30 years of age, while 57 of the total group of 105 were aged less than 30 ($P < 0.005$). No age specific trend in blood group

distribution was present, indicating that age and blood group were independently correlated with HBeAg distribution.

No correlation was seen between the presence or absence of rheumatoid factor and the presence of HBeAg or anti-HBe (Table 2). Similar conclusions have been made in other studies of healthy blood donors (Tedder and Briggs, 1977; Furuta *et al.*, 1977), although an association between RF and anti-HBe was reported in one study (Kacaki *et al.*, 1977). To examine formally the possibility that anti-HBe and RF recognised the same determinants on IgG molecules and were functionally identical, RF activity was removed from RF positive, anti-HBe positive sera by caprylic acid treatment or absorption with 5 mg/ml of denatured (75°C for 15 min) pooled human gammaglobulin. Both treatments removed all detectable RF activity, while the reconcentrated treated sera contained anti-HBe in 2/2 sera after absorption with globulin, and in 1/3 sera after treatment with caprylic acid. These results provided further evidence that RF and anti-HBe activity were not related.

Table 2 *Distribution of rheumatoid factor(RF) in relation to HBeAg/anti-HBe status of 105 carriers of HBsAg*

	RF positive	RF negative	Total
HBeAg	0	9	9
Anti-HBe	4	34	38
Neither	6	52	58
Total	10	95	105

The G1m allotype frequencies among all HBsAg carriers were similar to those for the region as a whole, and no relationship was seen between G1m allotype and HBeAg/anti-HBe status or the presence of RF (Table 3). Similarly, the presence of anti-Gm or red cell antibodies was not correlated with HBeAg/anti-HBe status.

The HBsAg subtype composition of the sera is also shown in Table 3. No association existed between either major subtype and the presence of RF or HBeAg/anti-HBe status of the donors. In a further analysis, no effect of age was found on the prevalence of HBsAg subtype.

Finally, as only seven of the 105 HBsAg carriers were female it was not possible to correlate the above information in relation to the sexes.

Discussion

Radioimmunoassay increased the anti-HBe rate from 35.9% (by gel diffusion) to 88.5% in a study of

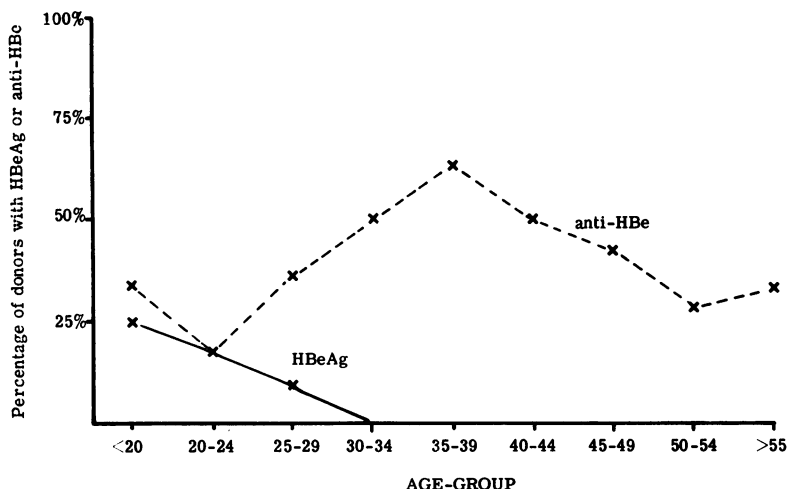


Figure Distribution of HBeAg and anti-HBe among HBsAg carriers with respect to age.

Table 3 Distribution of various markers with respect to HBeAg/anti-HBe and presence of rheumatoid factor

	HBeAg	anti-HBe	Negative for HBeAg and anti-HBe	RF	Total
<i>Glm allotype</i>					
Glm a+f-	1	5	9	1	15
Glm a+f+	4	13	26	6	43
Glm a-f+	4	20	23	3	47
<i>Antibody reactivity</i>					
Anti-Glm	2	10	8	2	20
Anti-RBC	2	6	12	2	20
Neither	5	22	38	6	65
<i>HBsAg subtype</i>					
ad	3	20	25	4	48
ay	3	16	28	5	47
untyped	3	2	5	1	10
Total	9	38	58	10	105

156 HBsAg carriers in West Germany (Biswas *et al.*, 1978), whereas the HBeAg rate was increased only from 2.6% to 3.6% with the more sensitive assay. It is therefore possible that the nine HBeAg positive donors detected by gel diffusion in our study represent nearly all those who would be detectable by a more sensitive assay, and that the majority of donors above with neither HBeAg nor anti-HBe detectable would prove to have low levels of anti-HBe.

In Japanese HBsAg carriers, the HBeAg rate declined with age from 76.9% in those aged 0-9 years to 9.5% in those aged 50 or above, while a reciprocal increase in anti-HBe rate with age was seen (Miyakawa and Mayumi, 1978). However, vertical transmission appears to play a much greater role in maintaining infection in Japan than in western Europe, introducing additional age-related factors. In a French study of 329 HBsAg carriers, no effect of age

on the HBeAg rate was seen, while the anti-HBe rate declined with age (Courouce-Pauty and Plancon, 1978). As factors such as age of acquisition of infection, prevalence of various subtypes, varying transmission routes, and possible differences in life expectancy for those carriers who are HBeAg positive could conceivably influence HBeAg/anti-HBe prevalence data, we cannot conclude from our age-related data that seroconversion from HBeAg to anti-HBe over a number of years commonly occurs in the natural history of the carrier state. In contrast, several workers have described seroconversion from HBeAg to anti-HBe positivity during most acute HBV infections (Frösner *et al.*, 1978; Miyakawa and Mayumi, 1978).

An association between blood group A and a lack of an HBeAg response has not to our knowledge been previously reported. We found no association be-

tween HBeAg/anti-HBe status and Gm allotype or the presence of anti-Gm, red cell antibodies or rheumatoid factor. In contrast to Courouce-Pauty and Plançon (1978), we did not find a higher prevalence of HBeAg or anti-HBe in *ad* infections compared to *ay* infections. We were not able to compare *adw* and *adr* subtypes, which were found to be significantly different with respect to HBeAg/anti-HBe in the French work. We were, however, able to show that subtype distribution was not related to age in our population, a correlation that was not examined in the French study.

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