Difference in the distribution of e antigen among different ethnic groups in a population of blood donors

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Sensitive techniques were used to detect e antigen and the corresponding antibody (anti-e) among 368 voluntary blood donors positive for hepatitis B surface antigen in the Montreal area and 310 people living in close contact with them. Neither e nor anti-e was found in the absence of markers of hepatitis B virus (HBV). Among the blood donors e antigen was detected in 23 and anti-e in 313, and 32 were negative for both markers. Of the 368 blood donors 330 were of French origin and 38 from other ethnic groups. The 23 e-positive subjects were unequally distributed among the ethnic groups: only 14 (4.2%) were recruited among the French group while 9 (23.7%) were recruited among other ethnic groups ($P < 0.001$). This difference among ethnic groups might be related to the vertical or horizontal mode of dissemination of HBV infection.

Des techniques sensibles ont été utilisées pour détecter l'antigène e et l'anticorps correspondant (anti-e) chez 368 donneurs de sang volontaires dans la région de Montréal trouvés positifs pour l'antigène de surface de l'hépatite de type B et chez 310 personnes vivant en contact étroit avec eux. Ni e ni anti-e n'a été retrouvé en l'absence de marqueurs du virus de l'hépatite B (HBV). Chez les donneurs de sang l'antigène e a été détecté chez 23 et l'anti-e chez 313, et 32 étaient négatifs pour les deux marqueurs. Des 368 donneurs de sang, 330 étaient d'origine française et 38 d'autres groupes ethniques. Les 23 sujets positifs pour l'antigène e étaient répartis inégalement selon l'ethnie: 14 (4.2%) seulement étaient recrutés dans la population d'origine française alors que 9 (23.7%) étaient recrutés dans les autres groupes ethniques ($P < 0.001$). Cette différence entre les groupes ethniques pourrait être reliée au mode de dissémination vertical ou horizontal de l'infection par l'HBV.

Magnius and Espmark[1,2] reported in 1972 on new antigen–antibody systems found in some sera positive for the surface antigen (HB_sAg) of hepatitis B virus (HBV). They designated the main system as e/anti-e. Despite the fact that this new antigen–antibody system was never found in HB_sAg-negative sera, they showed clearly that e and HB_sAg did not cross-react immunologically. They suggested that e antigen might be related to contagiosity, being found in highly contagious hemodialysed patients, while anti-e was found mainly in HB_sAg-positive asymptomatic carriers. Subsequent publications on small groups of healthy HB_sAg carriers have confirmed that anti-e was found frequently (30 to 82%) among carriers while e was absent or rare,[3-10] and when present was associated with evidence of chronic liver disease.[4,7,8] However, in two recent studies a high prevalence of e antigen was found in HB_sAg-positive asymptomatic carriers among the Eskimos[11] and Japanese.[12] It remains to be established if this represents a different distribution of e antigen among different ethnic groups or differences in the techniques or reactants used to detect it, or both. Indeed the proportion of HB_sAg carriers without either e or anti-e varies from 0[9] to 79%[10] in the published reports.

Sensitive techniques for the detection of e and anti-e were used to screen a population of HB_sAg-positive voluntary blood donors in the Montreal area and people living in close contact with them. This report shows that the distribution of e antigen varies according to ethnic origin among HB_sAg carriers.

Patients and methods

A total of 1317 serum samples from 368 voluntary blood donors found to be HB_sAg carriers by the Montreal branch of the Canadian Red Cross Society and referred to our group for medical evaluation and prospective study, and also 335 samples from 310 people living in close contact (relatives, house contacts or sexual partners), were tested for the presence of e and anti-e. Sera were preserved for up to 4 years at −20°C, but many of them had undergone many cycles of freezing and thawing.

HB_sAg was detected by counterimmunoelectrophoresis (CIEP).[13] Sera negative by CIEP were retested by radioimmunoassay (Ausria II-125, Abbott Laboratories, Chicago, IL). Anti-HB_s was detected by passive hemagglutination.[14] Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) concentrations were measured by standard techniques.

Testing for e and anti-e was done in hexagonal Ouchterlony wells (diameter, 3 mm, with a distance of 3 mm from edge to edge). The central well was filled with the standard e, two opposite wells with the standard anti-e and the four others with unknown sera. (This pattern allows one to detect both e and anti-e and offers the advantage of showing precipitin band bending in weak systems.[15]) Each serum sample was also tested for e by CIEP with premigration of the standard anti-e for 15 minutes before the cathodic well was filled with the serum to be tested.

The standard anti-e used in these tests came from an asymptomatic HB_sAg carrier and the standard e from an HB_sAg-positive patient who died with postnecrotic cirrhosis. The standard e contained both e_1 and e_2[16] and the standard anti-e detected both specificities (G. Le Bouvier: personal communication, 1976).

Results

Among the 368 voluntary blood donors found to be HB_sAg carriers in the Montreal area, 336 (91.3%) were found to be positive for the e system (23 e-positive and 313 anti-e-positive) while in 32 (8.7%) neither the antigen

Table I—Serum positivity for e and anti-e among carriers of hepatitis B surface antigen (HB_sAg) and close contacts

Group	No. of subjects	No. (and %) of subjects and positivity/negativity		
		e +ve	anti-e +ve	e and anti-e negative
HB_sAg carriers	368	23* (6.3)	313 (85.0)	32 (8.7)
Contacts of carriers	310			
HB_sAg +ve	24	0	20 (83.3)	4 (16.7)
HB_sAg -ve	286	0	2† (0.7)	284 (99.3)

*Positivity detectable only by counterimmunoelectrophoresis in 7 subjects.
†Both also positive for anti-HB_s.

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nor the antibody could be detected (Table I). Among the 310 people living in close contact with these carriers e antigen was never detected, but anti-e was detected in 20 of the 24 contacts found to be HB_sAg-positive and in 2 of the remaining 286 HB_sAg-negative subjects, in both of whom anti-HB_s thus coexisted with anti-e.

Among the 23 e-positive subjects, 16 were detected by both the Ouchterlony technique and CIEP, but 7 of them were detectable by CIEP only. Ninety-two sera, collected over a period of 4 years, were available from these 23 subjects and e antigen was detected in 73 (79.3%) of these sera. In 15 of the 23 subjects (65.2%), e antigen was detected in all the serum samples available; in the 8 others, there was no consistent pattern of distribution of positive and negative sera according to the time of collection; if only the first available sample had been tested, antigen would have been detected in 22 of the 23 subjects. In two subjects both e and anti-e were detected: in one of them all four samples contained the antigen while the antibody was detected in three; in the other subject the antibody was present in all five samples available and the antigen in the first two samples only.

Among the 368 HB_sAg carriers, transaminase values were found to be elevated (defined as one serum sample with a value for SGOT or SGPT, or both, exceeding 60 U [Karmen] per ml) in 11 of 23 (47.8%) subjects positive for e, 21 of 313 (6.7%) positive for anti-e and 2 of 32 (6.3%) negative for e and anti-e.

Analysis of ethnic origin of these carriers referred to us by the Canadian Red Cross Society shows that 330 were of French origin (328 born in Canada) and 38 of other ethnic groups (8 born in Canada); e antigen was detected in 14 (4.2%) subjects of French origin and in 9 (23.7%) of other ethnic groups: the difference is statistically significant ($P < 0.001$). The breakdown of these other ethnic groups is given in Table II. This table shows also the relation between e-positivity and high transaminase values: the cumulated data for the first three groups reveal that 12 of the 19 e-positive subjects also had high transaminase values. This relation was not observed in the e-positive Asiatics ($P < 0.05$).

Among the 23 e-positive subjects, the sera of 5 showed two precipitin lines by CIEP, and the sera of 18, one line. Results with one e-positive serum sample of a subject of the latter group that gave a reaction of identity with the most anodic line of the standard antigen is shown in Fig. 1. All the 13 subjects' samples tested according to the same scheme showed a line of identity with this anodic line; only one precipitin line was demonstrated for 11, including the 3 Asiatics. Other precipitin lines, when present, were always weaker.

Discussion

As reported by others,[1-3,6,7,11] markers of the e system were never encountered without evidence of previous or present infection by HBV. Among 286 people living in close contact with HB_sAg carriers, e antigen was never encountered and anti-e was found in 2 persons, both also positive for anti-HB_s: this confirms the recent report of Maynard and colleagues,[11] who also found anti-e and anti-HB_s coexisting in 2 subjects.

Markers of the e system among HB_sAg carriers have been sought by many workers,[1-12] but on small groups and with wide variations in the reported results. In fact, the cumulated number of subjects included in these 12 reports is 384; in the present study, 368 subjects were included. The cumulated number of subjects negative for both e and anti-e in these same reports is 160 (41.7%), while it is 32 (8.7%) in our series. Many factors, not mutually exclusive, might explain these variations. First, our own data (Table I) show that of the HB_sAg-positive contacts of carriers 4 of 24 (16.7%) were negative for both e and anti-e; this might be due to the small number of subjects in this group, but the most obvious explanation appears to be that a mean of 3.6 sera were studied from each HB_sAg carrier, while the mean was 1.1 for the contacts. Second, the reactants used as standard e and anti-e vary from one laboratory to the other and it appears that the ones we used were strong (V. Edwards: personal communication, 1976) and detected the two main specificities (e_1 and e_2)[16] and probably another unidentified one (G. Le Bouvier: personal communication, 1976). Third, we used a pattern in Ouchterlony plates that increases the sensitivity of the technique.[15] Moreover, e antigen was also tested in each serum by CIEP; 7 of the 23 (30.4%) e-positive subjects were identified by this technique only, which demonstrates the increase in sensitivity over the Ouchterlony technique, as reported recently.[8]

In eight subjects who were found to be e-positive, but in whom e antigen was not detected in all their serum samples, no consistent pattern of distribution of e-positive and negative sera was found according to the time of collection of the sample: it appears that there are fluctuations in the quantities of both antigen and antibody in the blood, and that these are at times below detectable levels with the techniques we have used. We identified two subjects with coexisting e and anti-e: this is similar to our previous findings[17] with HB_sAg and anti-HB_s in some carriers and is a frequent phenomenon in cases of persistent infection.[18] This might in fact be a limiting factor for the detection of e antigen and antibody in some subjects. One might hypothesize that all HB_sAg carriers are positive in the e system, but that some might go undetected because of the present technical limitations.

Table II—Distribution of 368 HB_sAg carriers by ethnic group, e positivity and transaminase values

Ethnic group	No. of subjects	No. of subjects positive for: e	No. of subjects positive for: e with high transaminase values
French	330	14	9
English	9	2	2
Italian	8	3	1
Asiatic*	10	4	0
Others†	11	0	—

*2 born in China and 8 in Vietnam.
†2 born in Belgium, 2 in Tunisia and 1 each in Haiti, Greece, Russia, Lebanon, Morocco, Algeria and Egypt.

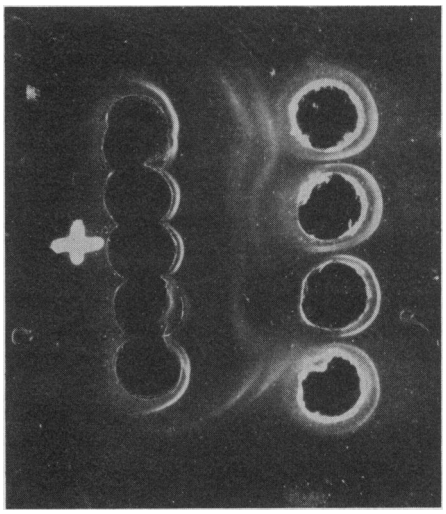

FIG. 1—Testing for e and anti-e system. Anodic trough is filled with standard anti-e. Cathodic side wells 1, 2 and 4 are filled with standard e and well 3 is filled with the serum of one e-positive subject. Standard e shows two clearly separated precipitin bands, with possibly a redoubling of the most anodic of the two. Serum of e-positive subject shows line of identity with the most anodic line.

High transaminase values were found more frequently in the e-positive subjects. This is in keeping with some previous reports of association of e antigen with high transaminase values[11] or more direct evidence of liver disease.[4,7,8] We have reported previously[19] the results of biopsies done in 31 carriers among our population and in only 3 of them was the liver found to be normal by the criteria used. Among these 31 carriers in whom histologic features of the liver were known, 3 were found to be e-positive: 1 of the 2 with chronic aggressive hepatitis and 2 of the 15 with the most minimal lesions of chronic persistent hepatitis. There is thus no clear association with the most severe involvement of the liver and e-positivity in our population. On the other hand, 4 of the 10 Asiatics in our series were e-positive and none of them ever had high transaminase values. The group is admittedly small but has to be interpreted with data existing in the literature. Indeed, e antigen was reported with abnormally high frequency in asymptomatic HB_sAg carriers among the Eskimos (34%)[11] and Japanese (43%).[12] The latter study, on pregnant women, showed that e antigen in the mother was associated with HBV infection in the children, while the reverse was seen when anti-e was present. It is well established that proportions of HB_sAg carriers vary widely among different populations.[20] Within our population, we have shown previously[21] that the principal mode of transmission appears to be horizontal, since about half of the HB_sAg carriers detected among blood donors were orphans who had lived in institutions as newborns or babies. In other populations like the Japanese, with very high proportions of HB_sAg carriers who are totally asymptomatic,[12] the infection might be mainly transmitted vertically from the mother to her child with a very high degree of immunologic tolerance and no sign of liver disease, as opposed to mainly horizontal transmission in populations in which the immunologic tolerance might be only partial, with signs of more or less severe liver involvement. It is possible that this apparent prejudice for the individual might be counterbalanced on epidemiologic grounds by the fact that a more perfect degree of tolerance appears to increase e-positivity and infectivity.

Conclusion

We conclude that markers for the e system are always associated with markers of past or present infection with HBV. Among HB_sAg carriers, there is a wide variation of distribution of e antigen among ethnic groups and this might be related to the mode of transmission of HBV infection. It might be hypothesized that, with increasing sensitivity of the techniques, all HB_sAg carriers could be classified as having e or anti-e, and this might be of considerable epidemiologic importance.[22]

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References

1. MAGNIUS LO, ESPMARK A: A new antigen complex co-occurring with Australia antigen. *Acta Pathol Microbiol Scand* [B] 80: 335, 1972
2. Idem: New specificities in Australia antigen positive sera distinct from the Le Bouvier determinants. *J Immunol* 109: 1017, 1972
3. NIELSEN JO, DIETRICHSON O, JUHL E: Incidence and meaning of the "e" determinant among hepatitis-B-antigen positive patients with acute and chronic liver diseases. *Lancet* 2: 913, 1974
4. MAGNIUS LO, LINDHOLM A, LUDIN P, et al: A new antigen–antibody system. Clinical significance in long-term carriers of hepatitis B surface antigen. *JAMA* 231: 356, 1975
5. EL SHEIKH N, WOOLF IL, GALBRAITH RM, et al: e antigen–antibody system as indicator of liver damage in patients with hepatitis-B antigen. *Br Med J* 4: 252, 1975
6. ELEFTHERIOU N, THOMAS HC, HEATHCOTE J, et al: Incidence and clinical significance of e antigen and antibody in acute and chronic liver disease. *Lancet* 2: 1171, 1975
7. FEINMAN SV, BERRIS B, SINCLAIR JC, et al: e antigen and anti-e in HBsAg carriers. Ibid, p 1173
8. TREPO C, MAGNIUS L, SCHAEFER RA, et al: Detection and clinical significance of e antigen and anti-e antibody in hepatitis B surface antigen carriers. *Gastroenterology* 69: 872, 1975
9. SKINHØJ P, COHN J, BRADBURNE AF: Transmission of hepatitis type B from healthy HBsAg-positive mothers. *Br Med J* 1: 10, 1976
10. SCHWEITZER IL, EDWARDS VM, BREZINA M: e antigen in HB_sAg-carrier mothers (C). *N Engl J Med* 293: 940, 1975
11. MAYNARD JE, BARRETT DH, MURPHY BL, et al: Relation of e antigen to hepatitis B virus infection in an area of hyperendemicity. *J Infect Dis* 133: 339, 1976
12. OKADA K, KAMIYAMA I, INOMATA M, et al: e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. *N Engl J Med* 294: 746, 1976
13. MOORE BPL, MEADE D: Counter-immunoelectrophoresis for detection of hepatitis B antigen and antibody: a technique for large-scale use. *Can J Public Health* 63: 453, 1972
14. VYAS GN, SHULMAN NR: Hemagglutination assay for antigen and antibody associated with viral hepatitis. *Science* 170: 332, 1970
15. CROWLE AJ: *Immunodiffusion*, New York and London, Acad Pr, 1973, p 410
16. WILLIAMS A, LE BOUVIER G: Heterogeneity and thermolability of 'e'. *Bibl Haematol* 42: 71, 1976
17. RICHER G, HOULE G, VIALLET A, et al: Antibodies against coat HBAg. *Lancet* 1: 356, 1974
18. LEHMANN-GRUBE F: Persistent infection of the mouse with the virus of lymphocytic choriomeningitis. *J Clin Pathol* 25 (suppl 6): 8, 1972
19. VILLENEUVE JP, RICHER G, CÔTÉ J, et al: Chronic carriers of hepatitis B antigen (HBsAg): histological, biochemical, and immunological findings in 31 voluntary blood donors. *Am J Dig Dis* 21: 18, 1976
20. BLUMBERG BS, SUTNICK AI, LONDON WI, et al: Australia antigen and hepatitis: a comprehensive review. *CRC Crit Rev Clin Lab Sci* 2: 473, 1971
21. RICHER G, DESROCHERS M, GUÉVIN R, et al: Hepatitis B antigen in Montréal blood donors: childhood institutionalization as an epidemiologic factor. *Can Med Assoc J* 112: 49, 1975
22. e and anti-e (E). *Lancet* 2: 1191, 1975