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Familial Clustering of Hepatitis B Surface Antigen among Panamanian Indians

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Several authors have suggested that the host genome determines the occurrence of chronic HB_s Ag (hepatitis B surface antigen). In attempts to evaluate this possibility, total infection rate and the combined frequencies of HB_s Ag and antibody to HB_s Ag in the population have not been analyzed. Using counterelectrophoresis to assay HB_s Ag and radioimmunoprecipitation to measure antibody to HB_s Ag, we tested sera from 255 Panamanian Guaymi Indians. They represented 48 families and 32 living units. Clusters of chronically antigenemic individuals were found in families. Clusters of infection were not found in families or living units. Differences in family composition (age and sex) did not explain the increased occurrence of HB_s Ag. These findings support the hypothesis that some humans have an inherited susceptibility to chronic infection with hepatitis B virus after exposure.

Hepatitis B surface antigen (HB_s Ag) was initially regarded as a polymorphic serum antigen. Before suggesting that this antigen was related to hepatitis, Blumberg et al. [1] demonstrated an increased frequency of antigenemia in certain families. Their later studies [2] showed familial clustering of HB_s Ag in a pattern consistent with monofactorial autosomal recessive inheritance. Subsequently, Carbonara et al. [3] published similar findings from Sardinia.

At the time of these genetic studies, no sensitive tests existed for the measurement of antibodies to HB_s Ag (anti-HB_s). Although HB_s Ag clustered in families, it was not determined whether host genome or increased exposure within index families was the cause. In a recent study by Szmuness et al. [4] among family contacts of chronically antigenemic blood donors in New York, it was suggested that both environmental and genetic factors contributed to hepatitis B infections. Similar studies have not been reported from primitive populations. In this paper we report on studies on the frequencies of HB_s Ag and anti-HB_s among families of Panamanian Guaymi Indians.

Materials and Methods

Population groups. In 1970 and 1971 we conducted a survey to study HB_s Ag and anti-HB_s in sera of members of the three Indian tribes in Panama [5]. The highest frequency of HB_s Ag was among the Guaymi Indians, who live in the Pacific coastal mountains. During the survey we obtained village cooperation by establishing medical clinics to administer measles vaccine and to provide medical care. Blood was drawn from those attending the clinic. We recorded each subject's name, age, sex, and dwelling site as well as

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the names, ages, sexes, and residences of siblings, spouses, children, and parents. To obtain blood from all available family members, we visited individual homes and took samples from people who had not attended the clinic. The data for families and residences were verified and amplified during a four-month period by visiting each village again and questioning family members.

Serologic testing. All sera were assayed for HB_s Ag by counterelectrophoresis as previously described [5]. Tests for anti-HB_s were performed at the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, with use of radioimmunoprecipitation [6, 7].

Statistical analysis. Two hundred fifty-five people, comprising 48 families and 32 living units, made up the study population. All families analyzed contained at least three blood relatives, including one parent and one child. All available members of the immediate family (parents, offspring, siblings) were included. Two hundred four of the population were included in families. Similarly, a living unit was defined as containing at least three persons, not necessarily blood relatives. Two hundred eight people were included in such living units.

Since there was no reason to expect fewer carriers in the families of index cases, we used a one-sided significance test [8] to analyze HB_s Ag clustering. The proportion of HB_s Ag in index families (at least one antigenemic member) was compared with its proportion in the entire population. We used a similar method to test whether there were clusters of infection or of persons with either HB_s Ag or anti-HB_s within families or living units.

To calculate the proportion of HB_s Ag in the population, we used the total number of HB_s Ag-positive people as the numerator and the number of only those with known past infections (all those positive for HB_s Ag or antibody to HB_s Ag) as the denominator. In calculation of the frequency of antigenemia in index families, the numerator equaled the number of HB_s Ag-positive people minus the number of index families. The denominator was the number of past infections in index families minus the number of index families. (We subtracted the index families to eliminate ascertainment bias [9].)

Results

Only 87 of 255 persons tested for both HB_s Ag and anti-HB_s showed evidence of infection, and 76 of these people were in analyzed families. Fifteen (proportion, 0.17) were antigenemic. These antigenemic people belonged to eight index families that contained 26 currently or previously infected individuals. Thus the adjusted proportion of HB_s Ag in the index families was 0.39 [(15–8)/(26–8)]. This finding supports the hypothesis that HB_s Ag clustered in families ($P < 0.05$). The composition (age and sex) and the distribution of HB_s Ag and anti-HB_s in the families studied are summarized in table 1.

It was thought that antigenemia might cluster in families with a high rate of infection. We therefore compared the infection rate of the total population ($87/255 = 0.34$) with the adjusted proportion of infection in index families (0.23). The adjusted proportion [(76–38)/(204–38)] took into account 76 infected people in 38 families among whose members (total, 204) at least one had detectable HB_s Ag or anti-HB_s. This com-

Table 1. Prevalence of HB_s Ag (hepatitis B surface antigen) and antibody to HB_s Ag in Guaymi Indian families.

Family group*	No. tested	No. with antigen	No. with antibody	Total no. infected
Antigen-positive (8)	36	15	11	26
Parent/spouse				
Father	5	2	2	4
Mother	7	2	4	6
Offspring				
<15 years old	14	5	3	8
≥15 years old	10	6	2	8
Infected but antigen-negative (30)	137	0	50	50
Parent/spouse				
Father	21	0	15	15
Mother	19	0	9	9
Offspring				
<15 years old	67	0	12	12
≥15 years old	30	0	14	14
Uninfected (10)	31	0	0	0
Parent/spouse				
Father	0	0	0	0
Mother	8	0	0	0
Offspring				
<15 years old	18	0	0	0
≥15 years old	5	0	0	0

* Numbers in parentheses indicate number of families.

parison did not support the hypothesis that infection clustered in families ($P > 0.5$). However, the infection rate in antigenemic index families was 0.64 [(26–8)/(36–8)] in contrast to 0.19 in infected families without antigen carriers [(51–30)/(137–30)] ($P < 0.001$). This result indicates that families with carriers suffered significantly more hepatitis B infection than families without carriers.

Since infection could also cluster by residence, we compared infection in the total population (0.34) to the adjusted proportion of infection in index living units (0.25). In this calculation we used 81 infected persons in 32 living units containing a total of 228 members, with at least one member of each unit infected. Again the data did not support the clustering hypothesis ($P > 0.5$).

Two or more families comprised 16 of the 32 living units. Statistically significant differences in overall rates of infection could not be demonstrated between families in the same living unit. Similarly, there was no spatial clustering of infected living units within villages.

Discussion

Studies of HB_s Ag in free-living population groups have revealed significantly different prevalences in different geographic regions and races [10, 11]. Since anti-HB_s was not measured concurrently, we cannot know whether the different frequencies of antigenemia reflected diverse host or viral genomes, variable routes of infection, differences in age at exposure, or discrepant magnitudes of exposure. We have previously shown [5][1] that three genetically distinct Panamanian Indian tribes, the Guaymi, the Chocó, and the Cuna, responded differently upon exposure to HB_s Ag. Although the Chocó and the Cuna were more frequently exposed in all age groups, they had a significantly lower frequency of chronic antigenemia than the Guaymi. All carriers from these populations had the **adw** subtype, so no evidence exists that the strain of virus involved determined chronic antigenemia. Similarly, we did not encounter obvious differences in route or dose of inoculation. Age of exposure was also similar in all groups of Indians studied.

This study strengthens the argument that host genome may partly determine the chronic HB_s Ag carrier state. Examining a single cultural unit in a small geographic area, we clearly established that HB_s Ag clustered in families, and that 11 of 15 chronically infected Indians were siblings (69%). However, environmental factors may also be important, as is indicated by the overall higher rate of infection in families with chronic carriers of HB_s Ag than in antibody-positive families. Szmuness et al. [4] presented similar findings in their study of hepatitis B infection among family contacts of volunteer blood donors in New York. We sampled eight of the antigenemic Guaymi again after a one-year interval, and all eight remained antigenemic with the same titer. This finding indicates that HB_s Ag-positive Guaymi Indians probably represented true chronic infections. However, we do not know the duration of the chronic carrier state. Several studies showed a decline in prevalence of HB_s Ag with age and a concomitant increase in frequency of anti-HB_s [5, 12–14], a finding indicating that the chronic carrier state may be self-limited.

We assumed that HB_s Ag and anti-HB_s rates reflected rates of infection with hepatitis B virus. However, little is known about the persistence of anti-HB_s after infection. Not all patients who become HB_s Ag-negative produce detectable anti-HB_s (R. H. Purcell, unpublished observations). We sampled 29 antibody-positive Guaymi Indians a second time after 14 months and found that one had reverted to a negative response. Similarly, in their institutional study, Szmuness et al. [12] found that four of 79 patients positive for anti-HB_s had become negative after one to two years.

Using segregation analysis Blumberg et al. [1, 2] and Carbonara et al. [3] found the chronic carrier state to be compatible with a Mendelian recessive trait. They assumed that the entire population was exposed to HB_s Ag. This assumption would not be well founded among the Guaymi, other Panamanian Indians [5],[2] certain

[1] W. C. Reeves, C. J. Peters, and R. H. Purcell, "The Epidemiology of Hepatitis B Antigen among Panamanian Cuna Indians," manuscript in preparation.

[2] See footnote 1.

African groups [13], or institutionalized populations [12]. We did not apply the statistical methods of segregation analysis to our data because such tests require large samples, because binomial tests of segregation have a low ability to discriminate between genetic and nongenetic hypotheses, and because the tests assume complete testing of family members (especially all parents). Secondly, simple Mendelian recessive genetics seldom completely describes mechanisms of human disease; for example, Carbonara et al. [3] had to invoke incomplete penetrance to explain their data.

The observed familial clustering of HB_sAg might represent relatively recent (five to 10 years previous) infection within the family. However, the high prevalence of anti-HB_s may reflect the long-term exposure of the population to HB_sAg. Under hyperendemic conditions repeated exposures to HB_sAg would stimulate new antibody synthesis. Differentiation of this possibility from a genetic hypothesis will require long-term observation of the Guaymi and other primitive peoples.

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