Persistence of Antibody to Hepatitis B Surface Antigen

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Sera from military personnel found to have antibodies to hepatitis B surface antigen (anti-HB_s) in an epidemiological study of a hepatitis B outbreak were tested for persistence of that antibody 1 year later. Initially, 64% of the anti-HB_s-positive sera reacted in passive hemagglutination tests with erythrocytes coated with hepatitis B surface antigen of both ayw and adw subtypes; the remaining sera reacted only with adw-coated erythrocytes (19%) or ayw-coated erythrocytes (17%). After 1 year, anti-HB_s was detectable by passive hemagglutination tests in 87% of individuals with initial antibody to both subtypes but in only 41% and 16% ($P < 0.001$) of those initially reacting only to adw- or ayw-coated erythrocytes, respectively. Seropositivity for anti-HB_s correlated best with history of contact with jaundiced people (20.3%) and duty in Asia.

Two major antibody systems comprise the spectrum of antibodies to hepatitis B antigen (HB_sAg). The group of antibodies to hepatitis B surface antigen (anti-HB_s) includes the common determinant a and the mutually exclusive determinants y or d (8) and w or r (3); antibody response to the core antigen of the Dane particle (anti-HB_c) has been recently described (2, 6).

Prevalence studies of anti-HB_s in different populations have shown that anti-HB_s is more common in lower socioeconomic groups (4), institutionalized populations (13), health personnel, and populations of underdeveloped countries (10, 11, 12). Anti-HB_s appears to correlate with immunity to hepatitis B. Persistence of anti-HB_s after hepatitis B virus (HBV) infection has not been well studied and has important implications in the evaluation of the immunogenicity of natural HBV strains and of candidate hepatitis B vaccines.

In this study, we followed a group of military personnel over a 1-year period to determine the persistence of anti-HB_s with specificities for a, y, and d antigens of HB_sAg. The military personnel had been shown to have anti-HB_s during an investigation of a hepatitis B outbreak (1).

MATERIALS AND METHODS

Collection of sera. Initial sera were obtained from a cohort of Army personnel and civilian employees at Fort Hood, Tex. during a study of the prevalence of HB_sAg and anti-HB_s at the time of a hepatitis B outbreak in early 1973 (1). None of these

persons was ill with hepatitis. Ninety-five percent of clinical hepatitis B cases at Fort Hood were of subtype ayw (1); however, only eight of the 30 soldiers with HB_sAg detected in prevalence surveys had HB_sAg/ayw. Five-hundred twenty-three individuals of 3,335 tested during the prevalence surveys were found to have anti-HB_s by the passive hemagglutination (PHA) test. After 1 year, a second sample of serum was obtained from anti-HB_s-positive individuals still remaining at Fort Hood who agreed to be rebled. Paired sera were tested together for anti-HB_s as described below.

Anti-HB_s test. The PHA test was performed according to the method of Vyas and Shulman (14). Sera were tested with erythrocytes coated with HB_sAg of subtype ayw and with subtype adw (obtained from Electronucleonics Laboratory Inc., Bethesda, Md.).

Four patterns of anti-HB_s were observed using PHA: (i) sera reactive to both HB_sAg/adw and ayw subtypes at a titer $\geq 1:32$; (ii) sera reactive to both subtypes but at a titer of 1:8 or 1:16; (iii) sera reactive only with HB_sAg/adw subtype (anti-d); (iv) sera reactive only with HB_sAg/ayw (anti-y). All sera found to be anti-HB_s positive (titer $\geq 1:8$) initially were retested at least once before being considered positive. To confirm PHA test results, a second equally sensitive test, radioimmune assay (RIA) (Abbott's AUSAB), was used to test the follow-up sera.

RIA inhibition of HB_sAg subtypes. To confirm the specificity of sera reacting only with cells coated with HB_sAg of one of the two subtypes, ayw or adw, sera in each of the four anti-HB_s categories were tested by a radioimmune assay inhibition (RIAI). Block titrations were performed using three different HB_sAg subtypes, i.e., ayw, adw, and adr, against human sera as well as specific rabbit anti-HB_s. A standard dilution of each HB_sAg subtype was made in saline (1:100 dilution of adw and ayw and 1:1,000

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of adr). Antibody was diluted serially from 1:8 to 4,096. One-tenth milliliter each of antigen and antibody was mixed in a 75-mm glass tube and allowed to incubate for 2 h. After incubation, 0.1 ml of the mixture was transferred to an Ausria I (Abbott Laboratory) tube and tested for the presence of HB_sAg (7, 9). Controls containing antigen or antibody alone and diluent were included in each test. In addition, a serum without anti-HB_s was diluted 1:2 in saline and tested in 10 Ausria tubes in replicate. A 25% or more reduction in counts per minute of the test specimen as compared with the mean count of these 10 control replicates was considered to be significant inhibition.

RESULTS

In the initial 1973 prevalence study at Fort Hood, 523 of the 3,335 personnel tested (15.6%) had anti-HB_s. After 1 year serum was obtained on 217 (41.5%) of the 523 soldiers and civilians originally positive for anti-HB_s. There was no difference in the distribution of anti-HB_s specificity of original serum between those personnel bled 1 year later and the total group originally determined to have anti-HB_s (Table 1).

The persistence of anti-HB_s, as detected by PHA, over 1 year for the four serological categories described is shown in Table 2. Of persons with sera reactive to both antigen subtypes at titer $\geq 1:32$, 92% had anti-HB_s persisting at 1 year; a lower proportion (74%) of those with PHA titers of 1:8 or 1:16 to both subtypes had persistent anti-HB_s. Seventy-nine soldiers had anti-HB_s reactive with only one subtype initially; 41 reactive with erythrocytes coated with HB_sAg/adw, and 38 reactive with erythrocytes coated with HB_sAg/ayw. Of those with adw reactivity only, 59% had persistent anti-HB_s; a much smaller proportion of personnel with ayw reactivity (16%) had anti-HB_s 1 year later. Human sera of only anti-d or only anti-y activity in original sera maintained this specificity over a 1-year period; none became reactive to the heterologous subtype. Results determined by PHA on the 1-year follow-up sera were similar to those obtained by RIA (AUSAB).

TABLE 1. Distribution of anti HB_s specificity in Fort Hood prevalence survey, February and March 1973

Anti-HB _s status	No. (%) of personnel with anti-HB _s	No. (%) of personnel with serum obtained 1 year later
Anti-HB _s to both adw- and ayw-coated erythrocytes	346 (66)	138 (64)
Anti-HB _s to adw-coated erythrocytes only	89 (17)	41 (19)
Anti-HB _s to ayw-coated erythrocytes only	89 (17)	38 (17)
Total anti-HB _s positive	524	217

TABLE 2. Persistence of anti-HB_s after 1 year according to specificity of initial sera for adw- or ayw-HB_sAg-coated erythrocytes^a

Anti-HB _s status of initial serum	No. with anti-HB _s initially	No. (%) with anti-HB _s persisting at 1 year
$\geq 1:32$ titers to both adw- and ayw-coated erythrocytes	99	91 (92)
Titers 1:8-1:16 to both adw- and ayw-coated erythrocytes	39	29 (74)
Titer $\geq 1:8$ to adw-coated erythrocytes only	41	24 (59)
Titer $\geq 1:8$ to ayw-coated erythrocytes only	38	6 (16)
Total	217	150 (69)

^a Sera initially positive to one or both types of cells, if positive 1 year later, reacted with the same specificity.

Of 147 sera available for testing by RIA, 110 were positive as compared with 113 by PHA.

To test the specificity of sera with monotypic PHA reactivity, RIAI against HB_sAg of subtypes ayw, adw, and adr were undertaken with representative human sera and with hyperimmune rabbit antisera raised against purified antigens of each of these subtypes. Hyperimmune sera produced precipitating antibody to all three antigens of the immunizing subtype. Comparable specificity for the anti-d and anti-y as determined by PHA was evident by the RIAI technique in that reactions for subtype antibody were only to homologous antigens by this second test (Table 3). Table 3 indicates the inhibiting titers of one sera from each group, but three sera from each category were tested and gave comparable results.

Table 4 compares the proportion of soldiers in each of the four anti-HB_s classifications who had previous overseas tours, a past history of hepatitis, recent contact with a jaundiced person, or a history of previous blood transfusion. The mean age of soldiers in each antibody group is also shown. Soldiers with monotypic reactivity to adw or ayw erythrocytes were less likely to have an overseas tour of duty ($P < 0.001$) than soldiers with cross-reactivity (anti-a). There was no significant difference however in recent contact with jaundiced persons among the different serological groups ($P = 0.08$, not significant) by chi-square test. The mean age of soldiers with only y reactivity (23.7 years) was significantly lower than that of the other three groups taken together ($P \leq 0.01$) (t test).

DISCUSSION

Using the PHA test, sera found to be positive for anti-HB_s (titer $\geq 1:8$) were arbitrarily divided into four groups based on observed differ-

TABLE 3. *Inhibition titers of control hepatitis B antigen subtypes by hyperimmune subtype-specific antisera and human sera positive for anti-HB_s by PHA*

Types of PHA reaction	Serum no.	RIA titer		
		HB _s Ag/ayw	HB _s Ag/adw	HB _s Ag/adw
Anti-HB _s titer ≥32 to both adw- and ayw-coated erythrocytes	M 938	1:8	1:8	1:8
Anti-HB _s titer ≥8-16 adw-coated erythrocytes only	E 1205	<1:8	1:8	1:8
Anti-HB _s titer ≥8 to ayw-coated erythrocytes only	M 307	1:8	<1:8	<1:8
Rabbit hyperimmune anti-HB _s /ayw	R 228	1:1,024	1:128	1:64
Rabbit hyperimmune anti-HB _s /adw ^a	R 181	1:128	1:2,048	1:2,048
Rabbit hyperimmune anti-HB _s /adr	R 560	1:64	1:512	1:512

^a Rabbit hyperimmune sera contained precipitating antibody to a determinant as well as homologous subtype determinants.

TABLE 4. *Comparison of epidemiological variable in individuals with anti-HB_s and serological category*

Anti-HB _s status	Overseas tour				Clinical hepatitis history			
	No.	Asia ^a	Europe	None ^b	History of hepatitis	Recent contact with jaundiced person	History of blood transfusion	Mean age
Anti-HB _s to both adw- and ayw-coated erythrocytes, titer ≥32	99	54 (54.6%)	14 (14.1%)	31 (31.3%) ^c	2 (2.0%)	18 (18.2%)	4 (4.0%)	34
Anti-HB _s to both adw- and ayw-coated erythrocytes, titer 1:8 or 1:16	39	27 (69.2%)	0	12 (30.8%)	3 (7.7%)	5 (12.8%)	1 (2.6%)	32
Anti-HB _s to adw-coated erythrocytes only. Titer 1:8 or greater	41	13 (31.7%)	3 (7.3%)	25 (61%)	2 (4.9%)	13 (31.7%)	3 (7.3%)	36
Anti-HB _s to ayw-coated erythrocytes only. Titer 1:8 or greater	38	12 (31.6%)	2 (5.3%)	24 (63.1%)	0	8 (21%)	1 (2.6%)	23.7 ^d
Total	217	106 (48.8%)	19 (8.8%)	92 (42.4%)	7 (3.2%)	44 (20.3%)	9 (4.1%)	

^a Vietnam primarily.

^b Mostly new recruits—United States only.

^c $P < 0.001$ overseas tour versus none.

^d $P < 0.01$.

ences in the specificity of the PHA reaction. The first two groups represented anti-HB_s that reacted with erythrocytes coated with both HB_sAg subtypes adw and ayw with a titer of ≥1:32, 1:8, or 1:16, respectively. The remaining two groups of serological reactions included sera that agglutinated either adw- or ayw-HB_s-Ag-coated erythrocytes. Our interpretation of these serological reactions is that (i) sera reacting to both types of coated erythrocytes contain anti-HB_s to the a antigenic determinant of HB_sAg (and perhaps to the w determinant) in equal or higher titers than to d or y; and (ii) those sera reacting to only adw- or ayw-coated erythrocytes have subtype-specific anti-HB_s to d or y antigenic determinants with undetectable antibody to a or w antigens.

Using a second serological test (RIAI), which involved inhibiting standard dilutions of HB_sAg of known subtypes adr, adw, and ayw, confirmation of this distinction was obtained (Table 3). Whether the anti-HB_s that reacted

with both adw- and ayw-HB_sAg-coated erythrocytes also contained y or d subtype-specific anti-HB_s was not examined because of the limited volume of sera available and because of the number of homologous and heterologous absorption steps required to examine this less significant point.

In three of the four categories of anti-HB_s response, the prevalence of antibody decreased significantly over 1 year. Only in the soldiers with relatively high titers to both subtypes of HB_sAg did prevalence of antibody remain relatively unchanged. In the other three groups, anti-HB_s was lost by 84% of those with anti-y, 41% of those with anti-d, and 26% of those with low-titered anti-a specificity. The decrease in antibody prevalence in these three groups did not appear to be related to PHA titer since titers of monotypic antibody were usually 1:8 or 1:16.

The more rapid loss of anti-y (84%) as compared with loss of anti-d (41%) may possibly

reflect the decreased immunogenicity of the y determinant in comparison with the d determinant. In guinea pigs immunized with HB_sAg ayw or adw subtypes, antibody to the y determinant is more difficult to produce than antibody to the d antigen. Persistence of specific antibody was not determined in these immunized animals (personal observation). A more likely possibility is that individuals with specific anti-y had more recent infections than individuals with anti-d. Consistent with this hypothesis is the almost exclusive detection of HB_sAg/ayw among hepatitis cases at Fort Hood during the epidemic and the younger median age of individuals with anti-HB_s of y reactivity (since hepatitis was limited almost exclusively to young enlisted personnel [1]). Although the time of acquisition of anti-HB_s is unknown for specific individuals, it is likely that older soldiers with anti-d were exposed to HBV at some more remote time than those with anti-y. Thus the more rapid decay in anti-HB_s of y rather than d specificity may be a reflection of more recent infections in the former and more distant and possibly repeated infections in the latter.

Regardless of the subspecificity of antibody involved, a large proportion of soldiers (31%) lost anti-HB_s over a 1-year period. In young soldiers with presumably recent infection with HB_sAg/ayw, 84% of those who developed only antibody to the y antigen no longer had detectable anti-HB_s 1 year later by PHA. Thus, a significant decay in anti-HB_s occurs in HBV infection or disease. The fall of anti-HB_s titers to undetectable levels in a significant proportion of persons with HBV infection must raise concern about the persistence of anti-HB_s in humans immunized with inactivated HB_sAg vaccines.

The above findings are not without precedent. In a survey of HBV infection in residents of Bangkok, Thailand, Grossman et al. (5) noted that 13% of the urban population studied acquired evidence of HBV infection over a 9-month period. Over two-thirds of these infections occurred in persons with preexisting anti-HB_s and were therefore secondary HBV infections. During the same time, 7% of individuals lost anti-HB. In persons under 14 years of age, in whom HBV infection was likely primary, 10% lost anti-HB_s. This decrease in anti-HB_s prevalence in the Thai urban population was less than that seen in soldiers at Fort Hood; but differences in the route, dose, and intensity of transmission between the two populations may account for the different rates of decay of antibody.

This difference in rates of loss of anti-HB_s may be a reflection of the Fort Hood epidemic being limited in time, as opposed to continual transmission and/or reinfection in Thai or institutionalized populations, so that a larger proportion of military cases were primary subclinical infections rather than secondary or tertiary infections. Clearly, our findings suggest that a prospective study of the decay of anti-HB_s after clinical and subclinical infection is needed. The significance of this decline in anti-HB_s response after subclinical HBV infection is unclear, since it remains to be determined whether loss of detectable anti-HB_s is equivalent to loss of immunity to HBV.

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