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Hepatitis B Surface Antigen-Specific Cell-Mediated Immune Responses in Human Chronic Hepatitis B Surface Antigen Carriers

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The presence of hepatitis B surface antigen (HB_sAg) and antibody (anti-HB_s), hepatitis B e antigen (HB_eAg) and antibody (anti-HB_e), the nature of T-cell function, and specific cell-mediated immunity to HB_sAg were determined and evaluated serially in groups of subjects with chronic HB_sAg carrier states and in seronegative controls. The techniques of in vitro lymphocyte transformation, spontaneous rosette formation, radioimmunoassay, reverse passive hemagglutination, passive hemagglutination, rheophoresis, and liver function tests were employed for these studies. For the lymphocyte transformation assay, multiple concentrations of phytohemagglutinin and purified HB_sAg were used as stimulants. Cell-mediated immunity to HB_sAg was detectable in 50% of the chronic HB_sAg carriers (responders) at one or more concentrations of HB_sAg. The remaining carriers (nonresponders) and controls failed to manifest HB_sAg-specific lymphocyte transformation activity. The profile of the responders was characterized by elevated serum glutamic pyruvic transaminase levels, the presence of anti-HB_e, high HB_sAg titers, and the conspicuous absence of HB_eAg in the serum. The nonresponders were characterized by normal serum glutamic pyruvic transaminase levels, the presence of HB_eAg and anti-HB_e, and lower HB_sAg titers. These observations demonstrate the presence of specific cell-mediated immunity to HB_sAg in chronic HB_sAg carriers who manifest biochemical evidence of liver disease.

Hepatitis B virus (HBV) infection results in a broad spectrum of clinical manifestations involving the liver and other organs (31, 32). One such clinical entity associated with HBV infection is the development of chronic hepatitis B surface antigen (HB_sAg) carrier state. Frequently, the chronic HB_sAg carriers are asymptomatic and demonstrate mild or no biochemical abnormalities. However, recent studies have demonstrated histological patterns compatible with chronic aggressive or chronic persistent hepatitis in some clinically asymptomatic carriers (1, 3, 32, 35).

The cell-mediated immune (CMI) response to HB_sAg in chronic HB_sAg carriers has been studied by a number of investigators (7, 8, 12, 17-21, 33). These studies have indicated that "healthy" chronic HB_sAg carriers free of histological and or biochemical evidence of disease do not mount a detectable CMI response to HB_sAg. Conflicting results have been presented about the presence or absence of a CMI response to HB_sAg in clinically symptomatic or asymptomatic chronic HB_sAg carriers with histological and/or biochemical evidence of liver damage (7, 8, 21, 33). It has not been established which chronic HB_sAg carriers mount a CMI response to HB_sAg.

The present studies were designed to evaluate the prevalence and temporal kinetics of HB_sAg-specific CMI responses in clinically asymptomatic chronic HB_sAg carriers by employing the in vitro lymphocyte transformation assay (LTF).

MATERIALS AND METHODS

Study population and specimen collection. The study population was drawn from the resident population of an institution for mentally handicapped children and adults. The institution has about 1,500 residents. The HBV serological status of these residents has been studied intensively as part of an ongoing epidemiological program over several cross-sectional and longitudinal surveys for the past 4 to 5 years. HBV infection is endemic in this population. Approximately 10% of the residents have been found to be positive for HB_sAg, and 35% have been found to be positive for HB_sAg antibody (anti-HB_s) in the serum.

For the evaluation of CMI to HB_sAg, 53 chronic HB_sAg carriers and 10 age- and sex-matched HB_sAg

and anti-HB$_s$ seronegative subjects were selected from the resident population. The chronic HB$_s$Ag carriers were all seropositive for HB$_s$Ag and seronegative for anti-HB$_s$. The seronegative controls demonstrated no serum HB$_s$Ag or anti-HB$_s$ activity. The HBV serological and clinical status of both groups were documented by repeated testing and observation over several years. None of the subjects had manifested any clinical illness suggestive of acute or chronic hepatitis.

A single sample of 10 to 15 ml of heparinized blood was collected from each of the 53 chronic HB$_s$Ag carriers and 10 seronegative controls. Subsequently, 17 of these chronic HB$_s$Ag carriers were chosen, on the basis of the results obtained from the initial survey, to be tested serially. For this purpose each of these 17 subjects was tested one to four additional times, 4 to 32 weeks after the initial testing.

Detection and quantitation of HB$_s$Ag and anti-HB$_s$. Solid-phase radioimmunoassay was used for the detection of HB$_s$Ag. This test was performed by using commercially available kits (Austria-II, Abbott Laboratories, North Chicago, Ill.), as described by Ling and Overby (22). For the quantitation of serum HB$_s$Ag, a reverse passive hemagglutination test system (2) kindly donated by Wellcome Research Laboratories, Beckenham, England, was employed. To detect HB$_s$Ag during the purification procedure (described below) and to quantitate the purified HB$_s$Ag, counterelectrophoresis (CEP) was employed as described by Gocke and Howe (13). For the quantitation of purified HB$_s$Ag, serial twofold dilutions were tested by CEP. The highest dilution demonstrating a positive reaction was assigned the value of one CEP unit of HB$_s$Ag. The technique of passive hemagglutination (34) (Electronucleonics Laboratories, Inc., Bethesda, Md.) was employed for the detection of anti-HB$_s$.

Detection of HB$_e$Ag and anti-HB$_e$. For the detection of hepatitis B e antigen (HB$_e$Ag) and antibody (anti-HB$_e$), the serum samples were tested by rheophoresis, employing reference human sera with known HB$_e$Ag or anti-HB$_e$ activity (10, 24).

Purification of HB$_s$Ag. By employing the method of Mistretta et al. (25), the HB$_s$Ag was purified from HB$_s$Ag-positive human plasma, kindly provided by the Buffalo chapter of the American Red Cross.

To remove fibrinogen, calcium chloride was added to the plasma and incubated for 2 h at 37°C. The recalcified plasma was centrifuged. The supernatant was then decanted, pooled, and diluted 10-fold with 0.02% pepsin (Worthington Biochemicals Corp., Freehold, N.J.) in 0.02 N HCl. The pH of the mixture was adjusted to 2.3 with 1 N HCl and incubated at 37°C for 4 h. After incubation the pepsin digest was dialyzed at 4°C against 10 volumes of phosphate-buffered saline, pH 7.4, for 72 h with two changes of the buffer. The dialyzed material was then concentrated 50-fold by ultrafiltration with an Amicon model 202 ultrafiltration apparatus with an Amicon XM 100 membrane. After concentration the resulting preparation was layered onto three preformed linear cesium chloride gradients, 1.4 to 1.1 g/ml. The gradients were centrifuged in a Beckman model L-50 ultracentrifuge with an SW-25 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 47,000 × g for 21 h. Fractions (1 ml) were collected, and all fractions were tested by CEP for the presence of HB$_s$Ag. Fractions positive for HB$_s$Ag were pooled, dialyzed against three changes of phosphate-buffered saline at 4°C, and subjected to a second cesium chloride centrifugation as described above. The pooled, dialyzed HB$_s$Ag-positive fractions served as the purified HB$_s$Ag. On immunoelectrophoresis with rabbit anti-normal human sera (Behring Diagnostic), the purified HB$_s$Ag demonstrated faint nonspecific precipitation reactions. Immunoelectrophoresis of the purified HB$_s$Ag and the original serum samples developed with a horse anti-HB$_s$ revealed identical precipitin lines in the α_2-β globulin region. The purified HB$_s$Ag was pooled, filtered through a 0.22-μm filter (Millipore Corp., Bedford, Mass.), aliquoted, and stored at −20°C for further use.

LTF. The LTF employed in this study was a microwhole-blood culture system as described by Pauly and Han (29). For this test, heparinized venous blood (phenol-free sodium heparin, 50 U/ml) was collected. After the determination of a total leukocyte count and a differential count, the cells were suspended in serum-free RPMI-1640 medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with glutamine and containing penicillin and streptomycin at a final lymphocyte concentration of 1×10^5/ml. With a repeating micropipette dispenser (Clay Adams, Parsypany, N.J.), 0.2 ml of the cell suspension was added to a U-bottomed well of a sterile microtiter plate (no. 220-24A, Cooke Laboratory Products). Before the addition of cells, 0.02 ml of RPMI-1640 or an appropriate dilution of HB$_s$Ag or phytohemagglutinin (PHA) was added to triplicate sets of wells. After the addition of cells, the microtiter plates were sealed with a self-adhering polyester film (Cooke Laboratory Products), covered with a polystyrene lid (Cooke Laboratory Products), and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. On day 5 of culture, 0.2 μCi of [*methyl*-^{3}H]thymidine (specific activity, 1.9 μCi/mmol, Schwartz-Mann, Orangeburg, N.J.) in 0.02 ml of RPMI-1640 was added to each culture with a repeating microtiter syringe (Hamilton Co., Reno, Nev.).

After an additional 18 h of incubation, the cells were harvested with a Multiple Automated Sample Harvester (MASH, model II, Microbiological Associates, Bethesda, Md.). The cells were washed with approximately 4 ml of 3% acetic acid and deposited onto a glass-fiber strip (Reeves Angle, Clifton, N.J.). Small circular disks containing the cells of individual cultures were removed with forceps and transferred to mini-plastic liquid scintillation counting vials (Fisher Scientific, Fairlawn, N.J.). Two drops of 30% hydrogen peroxide were then applied to each disk and allowed to evaporate at room temperature for 2 h. The samples were then processed for liquid scintillation counting. First, 0.3 ml of the tissue-solubilizer Protozol (New England Nuclear Corp., Boston, Mass.) was added to each vial. The vials were placed in a drying oven at 60°C for 20 min. After cooling, 3.5 ml of diluted toluene-based Liquaflour (New England Nuclear) was added. Before counting in a Nuclear Chicago Mark I liquid scintillation counter, the vials were allowed to cool and dark adapt for 24 to 48 h at 4°C. The results of the LTF assay were expressed as stimulation indexes (SI), which were calculated as follows: SI = mean counts per minute of stimulated cultures/mean

counts per minute of unstimulated cultures.

For the LTF assay the mitogen, PHA, and purified HB_sAg were employed as stimulants. A purified form of PHA (Burroughs Wellcome Co., Tuckahoe, N.J.) was diluted in RPMI-1640, aliquoted, and stored at −20°C. For lymphocyte stimulation, PHA was added to microcultures to obtain final concentrations of 8.0, 2.0, 1.0, 0.25, 0.125, and 0.0625 μg/ml. Purified HB_sAg was obtained as described above, diluted in RPMI-1640, and employed at 1.0, 0.5, 0.25, 0.125, and 0.0625 CEP units/ml.

E-rosetting. Spontaneous rosette formation (E-rosette) with sheep erythrocytes was employed to enumerate the percentage and absolute number of T-cells in the peripheral blood, employing the technique described by Hepburn and Ritts (15). The percentage of E-rosette-forming lymphocytes was then determined with a Leitz Ortholux microscope equipped with phase-contrast optics. Only mononuclear cells having three or more sheep erythrocytes closely associated with their surface were considered rosettes. The absolute number of E-rosette-forming lymphocytes was calculated, based on the percentage of E-rosette-forming lymphocytes and the total lymphocyte count of the original sample of blood.

SGPT levels. The serum glutamic pyruvic transaminase (SGPT) levels were determined by a kinetic assay with commercially available kits (stazyme GPT, Worthington Biochemicals Corp.). The values were expressed in international units per liter at 30°C. The upper limit of normal in this laboratory is 25 IU.

RESULTS

Based on the initial LTF testing of the study population, two groups of chronic HB_sAg carriers were recognized. The first group included 27 (51%) of the 53 chronic HB_sAg carriers who manifested significant in vitro lymphoproliferative activity to HB_sAg (responders). Significant lymphoproliferative activity was defined as LTF activity resulting in a SI of greater than or equal to 3, at one or more concentrations of HB_sAg. The second group, 26 (49%) of the 53 chronic HB_sAg carriers, did not manifest any significant CMI activity when tested initially and were termed nonresponders. No lymphoproliferative activity in response to HB_sAg was observed in lymphocyte cultures obtained from the 10 seronegative controls.

For the sake of clarity in the ensuing presentation of Results and Discussion, the term responder refers to a chronic HB_sAg carrier who on initial testing demonstrated in vitro lymphoproliferative activity to HB_sAg, and nonresponder refers to a chronic HB_sAg carrier who did not manifest such a response at the time of initial testing.

The SIs observed at the initial testing of nonresponder chronic HB_sAg carriers and seronegative controls are shown in Fig. 1. The individual and mean SI observed in both groups were similar, regardless of the concentration of the antigen used for stimulation. The SIs of the responders at initial testing are presented in Fig. 2. Significant lymphoproliferative activity, with at least one concentration of HB_sAg, was observed in all responders. However, the individual responses manifested variability relative to the concentration of HB_sAg used for in vitro stimulation.

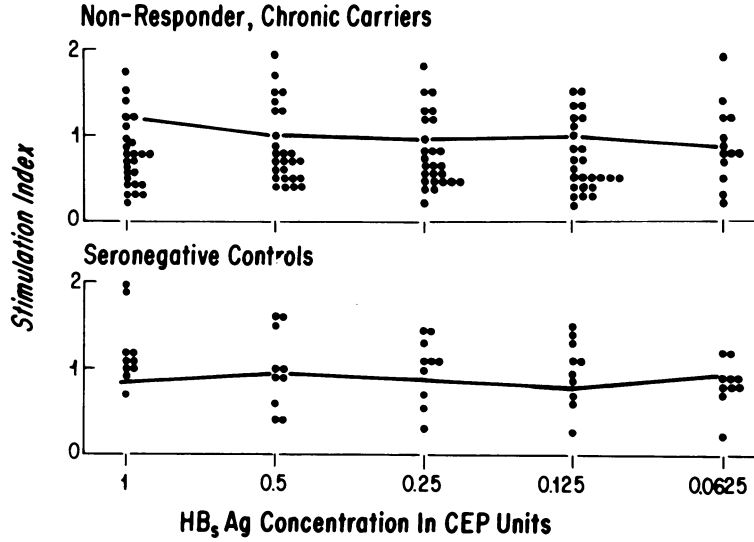

FIG. 1. *Individual SIs of lymphocyte cultures from nonresponder chronic HB_sAg carriers and seronegative controls to various concentrations of HB_sAg. Each point represents an individual response at the indicated concentrations. The solid lines represent the mean SIs.*

Analysis of the frequency and distribution of the lymphoproliferative response to various concentrations of HB$_s$Ag (Table 1) revealed that 56% of the responders demonstrated significant activity at only one concentration of the antigen and 44% responded to multiple HB$_s$Ag concentrations. It is also important to note that only four responses were observed at the highest antigen concentration and only one response was observed at the lowest. The majority (89%) of the lymphoproliferative responses were observed at the three intermediate antigen concentrations.

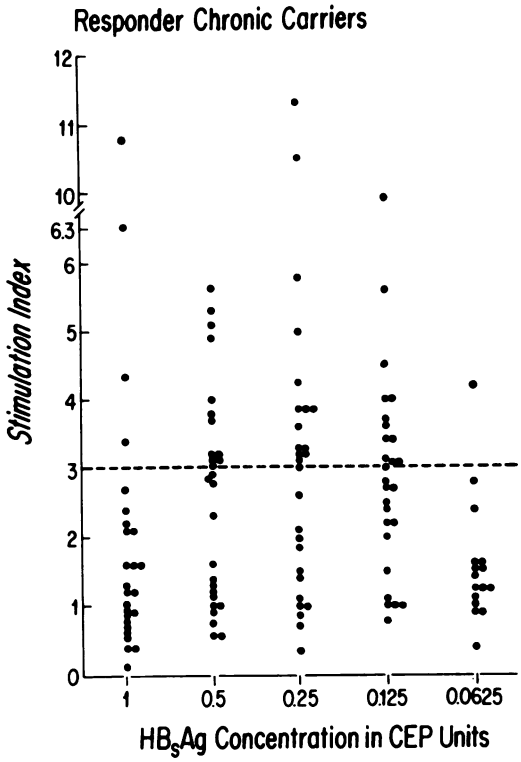

FIG. 2. *SIs of lymphocyte cultures from responder chronic HB$_s$Ag carriers at different concentrations of HB$_s$Ag. Each point represents the response of a single individual at the given concentrations.*

Seventeen randomly selected chronic HB$_s$Ag carriers (nine responders and eight nonresponders) were tested subsequently one to four additional times, 4 to 32 weeks after the initial testing. As shown in Table 2, 33% of the initial responders manifested a response to HB$_s$Ag at every subsequent testing (persistent responders). However, 66% of the initial responders failed to respond subsequently, and 25% of the initial nonresponders subsequently manifested a response at least once during the serial evaluation (intermittent responders). The majority (75%) of the nonresponders consistently failed to manifest lymphoproliferative activity to HB$_s$Ag (persistent nonresponders). SIs of serially tested persistent responders and representative intermittent responders are presented in Table 3. Responses to PHA at each testing were similar for a given individual. Subjects responding on more than one occasion demonstrated lymphoproliferative activity at the same HB$_s$Ag concentration or within one serial twofold dilution at each testing. These observations demonstrate the reproducibility of the LTF assay.

The in vitro response of peripheral blood lymphocytes from 27 responders, 26 nonresponder chronic HB$_s$Ag carriers, and the 10 seronegative controls to the mitogen PHA are shown in Fig.

TABLE 2. *Status of cellular immunity to HB$_s$Ag in subsequent testing relative to the status of immunity on the initial testing*

Status based on initial LTF test	No. of subjects	Status based on subsequent testing[a]		
		Persistent responders	Intermittent responders	Persistent nonresponders
Responders	9	3 (33)	6 (66)	0
Nonresponders	8	0	2 (25)	6 (75)
Total	17	3 (18)	8 (47)	6 (35)

[a] Number (%) of subjects.

TABLE 1. *Frequency of significant in vitro lymphoproliferative activity manifested by individual subjects to one or more concentrations of HB$_s$Ag and their distribution relative to the concentrations of HB$_s$Ag employed*

No. of HB$_s$Ag concns stimulating LTF response[a]	Subjects		No. of responses at HB$_s$Ag concn:[b]				
	No.	%	1.0	0.5	0.25	0.125	0.0625
1	15	56	2	3	4	6	0
2	7	26	0	4	6	4	0
3	4	15	1	4	4	2	1
4	1	4	1	1	1	1	0
5	0	0	0	0	0	0	0

[a] SI ≥ 3.
[b] Concentration in CEP units.

TABLE 3. *Representative lymphoproliferative responses of persistent responders and intermittent responders to HB$_s$Ag and PHA*

Group	Subject no.	Test wk	SI					
			PHA (1 μg/ml)	HB$_s$Ag concn[a]				
				1	0.5	0.25	0.125	0.0625
Persistent responders	37	0	172	1.6	**3.1**	**11.3**	**3.0**	1.0
		4	149	0.4	**3.7**	**3.6**	**4.0**	1.4
	492	0	247	0.4	**5.6**	**5.0**	2.9	1.6
		12	283	2.0	**3.5**	1.7	0.9	ND[b]
	565	0	828	2.9	2.7	2.9	**3.2**	**3.4**
		20	778	0.9	1.6	2.8	**3.9**	1.2
		24	707	0.7	0.7	**3.0**	0.8	ND
Intermittent responders	224	0	175	0.3	0.5	0.5	0.6	0.7
		16	150	1.0	**3.2**	1.9	1.1	0.9
		20	156	0.6	1.8	0.7	0.8	ND
		28	188	**3.4**	**4.0**	**3.0**	2.5	ND
	1,380	0	249	ND	1.0	2.7	**4.2**	1.5
		6	228	0.5	0.4	0.8	0.4	ND
		14	238	0.2	0.8	0.7	0.3	ND
	26	0	273	1.4	**3.9**	**8.3**	1.5	ND
		16	250	1.3	**5.3**	**5.8**	2.7	1.6
		18	169	1.4	0.4	1.5	1.4	ND
	229	0	162	2.4	**5.1**	**6.7**	1.7	1.2
		20	170	2.0	1.6	0.8	2.0	1.9
		32	107	1.5	1.1	0.9	1.0	ND

[a] HB$_s$Ag concentrations are in CEP units. Positive responses are in bold-face type.
[b] ND, Not done.

3. The mean SIs of the seronegative controls and the nonresponders were similar at all PHA concentrations. However, the LTF activity of responders was found to be somewhat elevated. The elevated PHA reactivity of responder chronic HB$_s$Ag carriers was statistically significant at 2, 1, and 0.25 μg of PHA per ml (Student's t test, $P < 0.01$). No differences were observed in either the percentage or absolute number of E-rosette-forming peripheral blood lymphocytes in responders (58.5% ± 9.9, 1,092 ± 440/mm^3), nonresponders (60.5% ± 9.0, 1,039 ± 446/mm^3), and seronegative controls (61.3% ± 11.8, 1,333 ± 765/mm^3).

Although clinical hepatitis was not observed in any subject, 17 of 21 responder HB$_s$Ag carriers demonstrated elevated SGPT (>25 IU) levels. Only 3 of 17 nonresponder antigen carriers manifested slightly elevated SGPT levels. The mean SGPT levels in responder antigen carriers (41 IU) were significantly greater ($P < 0.001$) than the mean SGPT levels observed in nonresponder (17.1 IU) antigen carriers (Fig. 4).

An evaluation of SGPT levels relative to the status of cellular immunity on serial testing demonstrated three patterns of SGPT levels: consistently normal SGPT levels (<25 IU), consistently elevated levels (>25 IU), or variable levels with elevated or normal SGPT values observed on one or more occasions. Persistent and intermittent responser antigen carriers demonstrated predominantly a variable pattern of SGPT levels. Persistent nonresponders consistently manifested normal SGPT levels (Table 4).

By employing the reverse passive hemagglutination assay, serum HB$_s$Ag was quantitated. The geometric mean serum HB$_s$Ag titers at the time of initial testing in responders (7,045) was sixfold greater than that observed in nonresponders (1,168), as shown in Fig. 5.

The occurrence of HB$_e$Ag and anti-HB$_e$ was determined in responder and nonresponder HB$_s$Ag carriers and in seronegative controls (Table 5). The incidence of detectable anti-HB$_e$ was approximately 50% in both responders and nonresponders. HB$_e$Ag was detected in 22% of nonresponders and was notably absent in responders. Neither HB$_e$Ag nor anti-HB$_e$ was detected

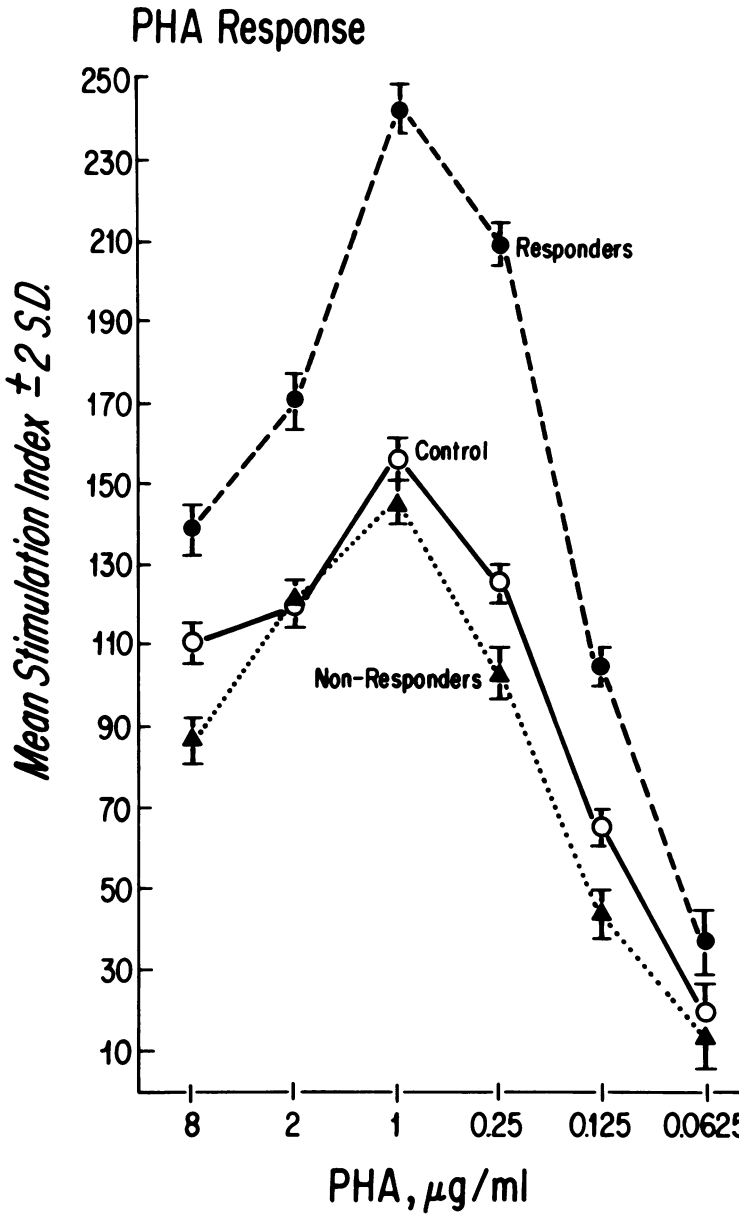

FIG. 3. *Geometric mean dose-response curves of PHA-stimulated lymphocyte cultures from 27 responder chronic HB$_s$Ag carriers* (- - -), *26 nonresponder chronic HB$_s$Ag carriers* (· · ·), *and 10 seronegative controls* (——).

in the control group. However, HB$_e$Ag and anti-HB$_e$ were undetectable in 47% of responders and 34% of nonresponders.

DISCUSSION

The LTF assay is generally accepted as an in vitro correlate of the CMI response. Although the induction of lymphoproliferative activity by mitogens has been shown to involve both T- and B-cells (4), the initial triggering of the proliferative responses to bacterial (purified protein derivative) and viral (mumps and rubella) antigens has been demonstrated to be primarily a T-cell response (5, 26).

The results of the initial testing for LTF activity in response to HB$_s$Ag indicated that approximately 50% of chronic HB$_s$Ag carriers manifested a HB$_s$Ag-specific CMI reponse. Although similar responses in chronic HB$_s$Ag carriers have been reported by a number of investigators, the

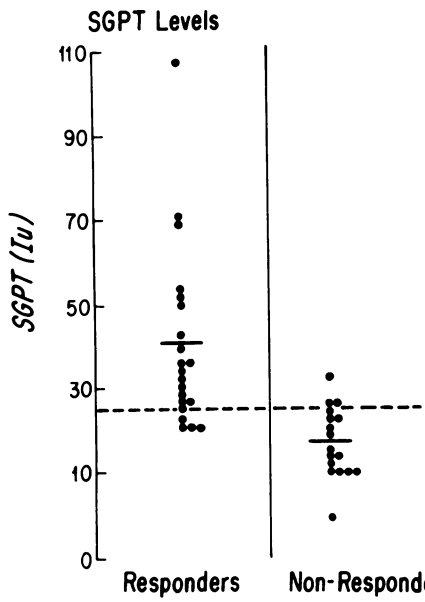

FIG. 4. *Levels of SGPT, expressed in international units, in responder and nonresponder chronic HB_sAg carriers. The upper limit of normal (25 IU) is indicated by the dotted line, and the solid bars indicate the mean SGPT levels for each group.*

TABLE 4. *Patterns of SGPT levels relative to the in vitro lymphoproliferative responses of serially evaluated chronic HB_sAg carriers*

Group	No. of subjects	No. of subjects with SGPT patterns:		
		Consistently normal[a]	Consistently elevated[b]	Variable[c]
Persistent responders	3	0	1	2
Intermittent responders	8	1	1	6
Persistent nonresponders	6	6	0	0

[a] Consistently normal, SGPT < 25 IU at each testing.
[b] Consistently elevated, SGPT > 25 IU at each testing.
[c] Variable, SGPT elevated (>25 IU) one or more times and normal (<25 IU) at other testings.

prevalence reported here is much higher than previously reported (7, 8, 18, 21, 33). The major difference between this study and the previous reports (7–9, 18, 21, 33) is one of methodology, specifically the use of multiple antigen concentrations to increase the sensitivity of the detection of CMI. If only one concentration of HB_sAg had been employed for in vitro stimulation, a prevalence of CMI to HB_sAg as low as 2% or as high as 28% would have been observed. These data suggest that the in vitro detection of CMI response to HB_sAg can be maximized by the use of multiple antigen concentrations.

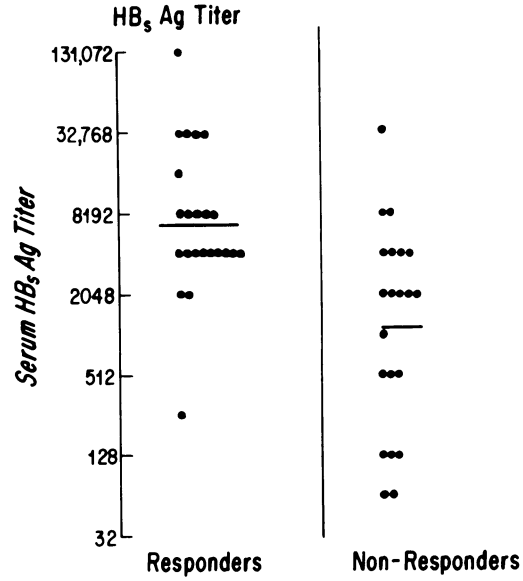

FIG. 5. *Serum HB_sAg titers as determined by reverse passive hemagglutination of responder and nonresponder chronic HB_sAg carriers. Each point represents an individual determination, and the horizontal bars indicate the geometric mean HB_sAg titer of each group.*

There are conflicting reports about the correlation of CMI response to HB_sAg in subjects with biochemical or histological evidence of liver damage and/or disease associated with chronic antigenemia (6–8, 10, 12, 18, 19, 21, 33). In the present study, elevated SGPT levels were observed in subjects who manifested a CMI response to HB_sAg. Because elevated SGPT levels are an indicator of liver injury, the present study demonstrates an association between the existence of HB_sAg-specific CMI responses and concurrent liver damage. This is supported by the observation that subjects who repeatedly failed to manifest CMI to HB_sAg (persistent nonresponders) also manifested consistently normal SGPT levels.

The present study also indicates that the CMI response observed in the chronic HB_sAg carriers is intermittent. This may be an indication of a qualitative defect in the CMI response to HB_sAg in at least some chronic HB_sAg carriers. This is the first report to recognize the intermittent nature of the CMI response in chronic HB_sAg carriers, suggesting a dynamic rather than a static relationship between HBV infection and the host immune response.

The mean serum HB_sAg titers were fourfold greater in responder chronic HB_sAg carriers than in nonresponder chronic HB_sAg carriers. This lends support to the concept that signifi-

TABLE 5. *Distribution of HB$_e$Ag and anti-HB$_e$ in the sera of responder and nonresponder chronic HB$_s$Ag carriers and seronegative controls*

Group	No. tested	HB$_s$Ag present		Anti-HB$_e$ present		HB$_e$Ag and anti-HB$_e$ absent	
		No.	%	No.	%	No.	%
Responders	15	0	0	8	53	7	47
Nonresponders	18	4	22	8	44	6	34
Seronegative controls	6	0	0	0	0	6	100

cant hepatocyte damage occurs in responder chronic HB$_s$Ag carriers. HB$_s$Ag has been detected both in the cytoplasm and on the surface (11, 14) of hepatocytes. Thus, an infected hepatocyte destroyed by immunological mechanisms should release HB$_s$Ag. Kohler et al. (20) evaluated the effect of immunotherapy on the chronic HB$_s$Ag carrier state by using lymphocytes and transfer factor in one subject and transfer factor alone in another. They observed that soon after infusion of lymphocytes or injection of transfer factor both SGPT levels and serum HB$_s$Ag titers increased.

The elevated reactivity of PHA observed in responder chronic HB$_s$Ag carriers occurred only in the range of optimal PHA concentration and did not involve a shift in the PHA dose-response curve. Clinically significant differences in PHA dose-response curves occur at suboptimal rather than optimal PHA concentration (16, 28). The observed difference in PHA response between the responders and nonresponders may represent a subtle stimulatory difference. The simultaneous occurrence of CMI to HB$_s$Ag and chronic antigenemia in responders may result in in vitro antigenic stimulation of lymphocytes and the subsequent amplification of in vitro lymphocyte reactivity. No significant differences were noted in the percentage or absolute number of peripheral blood T-cells among responder, nonresponder chronic HB$_s$Ag carriers, and the seronegative controls. This is consistent with the findings in regard to PHA activity, indicating no T-cell defect in chronic HB$_s$Ag carriers.

HB$_e$Ag was observed in subjects with normal SGPT levels (nonresponders) and was absent in subjects having elevated SGPT levels (responders). Anti-HB$_e$ was observed in approximately 50% of both groups. These observations would indicate that HB$_e$Ag is present in subjects free of liver damage but absent in subjects demonstrating liver abnormalities. Complicating this interpretation is the observation that approximately 40% of the chronic HB$_s$Ag carriers in the present study were seronegative for both HB$_e$Ag and anti-HB$_e$. This may result from technical difficulties in detecting HB$_e$Ag and anti-HB$_e$, namely the relatively low sensitivity of the method employed for detection and the possible existence of HB$_e$Ag/anti-HB$_e$ immune complexes in these subjects. This interpretation of the e system is contrary to previous reports which have associated the presence of HB$_e$Ag with liver damage and anti-HB$_e$ with a disease-free status (9, 10, 23, 27). However, these workers have also experienced difficulty in uniformly detecting HB$_e$Ag or anti-HB$_e$ in the majority of subjects. Thus, the interpretation of the e system may change when more sensitive methods for detection become available.

Based on the data presented, it is suggested that the pathogenesis of hepatocyte injury in asymptomatic chronic HB$_s$Ag carriers may be, to a large extent, mediated by cellular immune mechanisms directed against HB$_s$Ag.

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