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Development of Immunity Against Hepatitis A Virus by Subclinical Infection (39511)[1]

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Inapparent infections are a major source of virus for maintenance and spread of the transmissible viral diseases of man. This is especially true for those diseases in which humans are the only reservoir of infection, such as for poliomyelitis, and probably for hepatitis as well. It has been known for many years that viral hepatitis may be subclinical (1, 2) but the lack of means for measuring either virus or antibody precluded any precise assay of the incidence of such infection.

The development in recent years of methods for detecting hepatitis B antigen and antibody provided reliable means for diagnosis of inapparent hepatitis B infections. Recently, serum neutralization, immune adherence (IA), and complement-fixation (CF) test procedures for detecting antibody against hepatitis A virus were described by our group (3-7) and these, together with the immune electron microscopy technique of Feinstone *et al.* (8), opened the way to studies of inapparent infection in hepatitis A as well. Heretofore, such infections could be surmised only from the presence of abnormalities of liver function that might be caused, in fact, by any of a number of different viruses.

This report presents the serological test results for hepatitis A and B in a number of patients who presented transient serum transaminase elevations and who were diagnosed as having subclinical hepatitis. The patients were identified in the course of longitudinal epidemiologic studies of hepatitis carried out in an endemic hepatitis area in Costa Rica (9).

Materials and methods. Twenty-five persons with subclinical hepatitis, from whom appropriate serum samples were available, were selected for study. The presumptive diagnosis of subclinical hepatitis was based on serum glutamic pyruvic transaminase (SGPT elevations found during weekly or biweekly monitoring of family contacts of clinically overt cases of viral hepatitis. In most individuals, the diagnosis was supported by measurements for serum bilirubin, for immunoglobulin M (IgM), and for hepatitis B (HB_sAg) antigenemia.

Tests for hepatitis A antibody were performed by the IA test on the first serum specimen that showed an elevated transaminase level (acute specimen) and on a serum sample taken from the same individual about 90 days later (convalescent specimen). In some cases, serum samples taken earlier than the acute specimen were available for study. Hepatitis A antigen was prepared from the livers of infected *Saguinus mystax* marmosets. The IA test was carried out as described earlier (6, 10). The IA tests were performed at West Point, Pennsylvania. The sera were submitted under code from Costa Rica and the code was not broken until the test results had been reported.

Assays for HB_sAg were carried out by radioimmunoassay (11), and antibody against hepatitis B antigen (anti-HB_s) was measured by the passive hemagglutination test (12). Persons with HB_sAg were examined for development of anti-HB_s at 6- and 12-month intervals after the first transaminase elevation.

The tests for SGPT were carried out by

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the method of Reitman and Frankel (13) and for IgM by the method of Mancini *et al.* (14). Values exceeding 75 U/ml and 200 mg/100 ml (200 international units), respectively, were considered to be abnormal and of diagnostic significance.

Results. The findings in the tests of the sera from the 25 cases of clinically inapparent hepatitis are shown in Table I. All persons except the four subjects who showed hepatitis B antigenemia had been diagnosed tentatively as hepatitis A cases. The IA tests showed that 18 of the cases developed hepatitis A antibody. In all but three of these cases (No. 11, 18, and 21), the first sample of serum tested was free of detectable hepatitis A antibody. In these three individuals with low initial IA titers, the acute serum specimens had been taken 2–3 weeks after onset of serum enzyme elevation. All 18 persons developed high titers of hepatitis A antibody during convalescence.

Four of the 25 cases were diagnosed as hepatitis B (No. 1, 2, 4, and 5) because HB_sAg was detected in their acute phase bloods; this was transient and disappeared within 3 weeks. All had resided in households where clinical hepatitis B had occurred. All had demonstrable hepatitis A antibody initially and there was no change in titer during convalescence.

Three of the 25 cases (No. 3, 6, and 24) had hepatitis B antibody at 1:8 to 1:32 titer in their acute phase sera, and there was no change in titer during convalescence. Two of these three persons (No. 6 and 24) had hepatitis A antibody in their acute sera and this did not increase in convalescence. Case 3 was without hepatitis A antibody initially and this person did not develop such antibody until 1 year later, at which time he was convalescent from a subsequent clinically apparent hepatitis A infection. The evidence indicates that these three persons had neither hepatitis A nor B and suggests that they may have suffered from hepatitis of different etiology (10, 15).

Serum samples were available over a fol-

TABLE I. DEVELOPMENT OF ANTIBODIES AGAINST HEPATITIS A VIRUS IN SUBCLINICAL CASES.

Case number	Sex	Age	SGPT (highest value)	IgM (mg/100 ml) acute	Immune adherence antibody titer			Hepatitis B antigen
					Pre onset[a]	Acute[b]	Convalescent[c]	
8	M	1	1660	>400	ND[d]	<5	≥6400	Negative
12	F	2	1260	>400	ND	<5	≥6400	Negative
9	M	2	920	>400	ND	<5	≥6400	Negative
13	F	1	830	210	ND	<5	≥10,240	Negative
23	M	2	830	290	ND	<5	≥6400	Negative
10	F	2	750	>400	ND	<5	≥10,240	Negative
15	M	6	680	>400	ND	<5	≥6400	Negative
7	F	2	415	ND	ND	<5	≥6400	Negative
16	F	2	415	180	<5	<5	≥6400	Negative
19	F	2	375	340	<5	<5	3200	Negative
14	F	5	345	>400	<5	<5	5120	Negative
17	M	2	310	>400	<5	<5	≥6400	Negative
22	F	7	230	150	ND	<5	3200	Negative
20	M	6	126	>400	<5	<5	3200	Negative
25	F	1	114	ND	ND	<5	≥6400	Negative
21	F	3	280	>400	<5	200	≥6400	Negative
11	F	6	310	ND	ND	800	≥6400	Negative
18	F	3	1030	>400	50	800	≥6400	Negative
2	F	6	100	110	ND	100	100	Positive
4	M	39	95	165	ND	400	400	Positive
1	M	7	120	ND	ND	≥6400	≥6400	Positive
5	M	7	1260	210	ND	≥6400	≥6400	Positive
3	M	2	134	>400	ND	<5	<5	Anti-HB_sAg positive
24	M	54	230	70	ND	100	100	Anti-HB_sAg positive
6	F	5	345	95	ND	≥10,240	≥10,240	Anti-HB_sAg positive

[a] Average: 14 days before onset.
[b] At time of first detection of SGPT elevation.
[c] Average: 90 days after onset.
[d] ND = not done.

low-up period of 2–7 years from the persons in Table I who had developed hepatitis A antibody. In all cases, the antibody titers remained undiminished from the levels as given in Table I.

It was of interest that the IgM values in hepatitis A cases were most often 200 or greater (13/15 cases) and the values in hepatitis B were less than 200 in two of three cases. This differentiating characteristic for IgM levels in hepatitis A and B cases has also been noted by Giles and Krugman (16).

Discussion. The sensitivity and specificity of the IA test for hepatitis A antibody have been demonstrated by our group (4, 6, 7, 10, 15) and have been confirmed in tests by Krugman *et al.* (17). In earlier studies (4, 6, 7) we had seen evidence, in a limited number of cases, for antibody development in subclinical hepatitis A infection. Also, Dienstag *et al.* (18) have shown antibody development, by immune electron microscopy, in similar subclinical hepatitis A cases characterized by serum enzyme elevations. The present findings confirm the reliability of the IA test for serologic diagnosis of hepatitis A, even in subclinical disease. Importantly, persons with subclinical hepatitis A, like those with clinically apparent illness, develop a high level of antibody against hepatitis A during convalescence from the disease and this antibody persists for at least 7 years. Such antibody likely equates with immunity to reinfection in hepatitis A, as Krugman *et al.* (19) have shown that resistance to reinfection follows both clinical and subclinical disease.

The lack of hepatitis A antibody response in the four cases of hepatitis B was in accord with expectation and served to emphasize the specificity of the test for hepatitis A antibody. The three cases of inapparent hepatitis for which no serologic diagnosis was made were probably neither hepatitis A nor B and may, indeed, have been cases of the still hypothetical "hepatitis C" (10, 15).

The newly developed IA test for hepatitis A, used in conjunction with the available tests for hepatitis B antigen and antibody, has opened the door to agent-specific serodiagnostic and epidemiologic investigations of these two important diseases of man. The ability to detect both hepatitis A and B now presents the opportunity for a more rational approach to studies aimed at propagating these agents in the laboratory.

Summary. A newly developed immune adherence (IA) test for hepatitis A antibody was applied to etiologic investigations of subclinical hepatitis cases diagnosed by serum enzyme elevations. Among 25 cases of subclinical infection occurring in Costa Rica, 18 seroconverted and were diagnosed as hepatitis A, four had HB_sAg and were diagnosed as hepatitis B, and three were neither hepatitis A nor B. The development of hepatitis A antibody in such cases equates with protection against the disease and confirms that immunity may result from clinically inapparent infection. Hepatitis A antibody in these subclinical cases was shown to persist for at least 7 years, the longest time period investigated. The antibody response in subclinical hepatitis A was comparable to that previously noted in clinical cases of the disease.

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