Antibodies to Hepatitis A Virus: Patterns by Two Procedures

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Antibody to hepatitis A virus demonstrable by immune electron microscopy appeared early but remained at low levels for several weeks. Antibody detectable by immune adherence hemagglutination was delayed.

Deinhardt et al. (1) reported propagation of hepatitis A virus (HAV) in marmosets in 1967, but confirmation was delayed until 1973 (7). Miller and co-workers (5) then developed an immune adherence hemagglutination (IAHA) procedure for antibody (anti-HA) using livers of infected marmosets as a source of antigen. This approach to serological diagnosis, however, became unusable because of an embargo on these primates.

Fortunately, in 1973, Feinstone and co-workers (3) found that immune electron microscopy (IEM) could identify virus-like particles in stools for humans with type A hepatitis. By IEM, they could similarly detect anti-HA. The National Institutes of Health group developed an IAHA test using HAV from stools of patients with type A hepatitis (6). They demonstrated a close correlation between quantitative scores (0 to 4+) for anti-HA on IEM and IAHA titer (2). They found, however, that IEM detected anti-HA somewhat earlier than did IAHA (2, 6).

An epidemic of type A hepatitis among multiply handicapped children in Los Angeles permitted us to compare the anti-HA response by both IEM and IAHA. The children were bled on 10 to 12 occasions over a 4-month period at

intervals determined by their status and that of the epidemic. The course of their hepatitis was followed by measuring serum alanine aminotransferase by a kinetic method; results were expressed as international units per liter at 37°C.

The IEM procedure was that described by Feinstone et al. (3). A 2% partially purified stool suspension was derived from a child in the epidemic. The average number of particles per square was 155. Specimens were scored for anti-HA (0 to 4+) under code after examination of five squares. They were also tested by IAHA, using an adaptation of the technique of M-yumi et al. (4). The HAV was from the same stool, purified by isopycnic banding in cesium chloride. Our IAHA procedure gave a titer of 1:512 with the National Institute of Allergy and Infectious Diseases standard (V-811-501-573); Hilleman and co-workers obtained a value of 1:1,280 with their IAHA system.

The antibody patterns exhibited by the patients we observed (Table 1) emphasize two points. First, in agreement with the reported studies of two experimentally infected chimpanzees (2, 6), we observed very early appearance of anti-HA by IEM. We did not observe,

TABLE 1. Antibody to HAV in three children: patterns demonstrable by IEM and IAHA

Days elapsed ^a	Patient A			Patient B			Patient D		
	ALT	IEM	IAHA ^b	ALT	IEM	IAHA ^b	ALT	IEM	IAHA ^b
-4	20 ^c	0	<10	42 ^c	0	<10	38 ^c	0	<10
0	56 ^c	0	<10	60 ^c	0	<10	89 ^c	0	<10
4	199	0-1+	<10	92 ^c	1-2+	<10	382	0-1+	<10
11	1,615	1-2+	<10	1,555	1-2+	<10	2,140	1-2+	<10
18	226	1-2+	<10	2,190	1-2+	<10	844	2-3+	<10
25	77	1-2+	<10	394	1-2+	<10	143	2-3+	<10
39	40	2+	<10	69	2+	<10	30	3+	100
54	20	2+	6,400	59	2+	800	50	2-3+	800
80	6	3+	12,800	65	4+	6,400	14	2-3+	3,200
109	12	3+	12,800	84	4+	12,800	17	3-4+	3,200

^a From first observed abnormality of alanine aminotransferase (ALT) (upper limit = 45 IU/liter).

^b Reciprocal of last dilution giving 3+ reaction.

^c HAV demonstrated in stool.

however, the rapid increase in IEM scores to 2 to 3+ by 7 to 10 days later, perhaps due to differences in response between humans and non-human primates. This point is important if IEM alone is used, because a low titer of anti-HA in the first specimen and again 2 to 4 weeks later could lead to the erroneous conclusion that the patient's illness was not type A hepatitis. Second, the interval between anti-HA positivity by IEM and by IAHA is several weeks. Low-level IEM positivity accompanied by IAHA negativity, therefore, may permit a provisional diagnosis of type A disease even if convalescent specimens cannot be obtained (e.g., in fatal fulminant hepatitis). In this situation, specificity of IEM must be confirmed by retesting with purified HAV demonstrably free of extraneous agents, especially enteroviruses.

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