Distribution of Antibody to Hepatitis A Antigen in a Population of Commerical Plasma Donors

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Plasma samples from 245 regular plasma donors for Plasma Alliance, Inc. (Knoxville, Tenn.) were tested for the presence of antibody to hepatitis A antigen. Antibody was detected at an immune adherence hemagglutination titer of 1:10 or greater in 37% (91 of 245) of the donors. A statistically significant difference in the frequency of anti-hepatitis A was found between the 18- to 29-year-old group (30%) and the 30- to 49-year-old group (57%). No significant differences between whites and blacks or males and females were observed. Eighty percent of the donors were in the age range of 18 to 29 years, whereas 48% of the higher-titered plasmas (1:1,000 or greater) were in the 30 to 49 age group. By preselection of donors, it would be possible to produce an immune serum globulin with a specific anti-hepatitis A titer.

The use of commercial human serum immunoglobulin has been commonly accepted as prophylaxis for hepatitis A (HA) virus infection without the availability of an adequate means to measure anti-HA activity before actual inoculation. Recently, Miller et al. (12), using an immune adherence hemagglutination assay (IAHA), tested several lots of commercial immunoglobulin and found that all of them had detectable levels of antibody to HA antigen (HA Ag). Although the average titer was quite high, there was some variation between lots, and Miller subsequently found additional lots with a very low titer or no detectable anti-HA titer (personal communication). Data from several sources suggest that about 47% of the adult population of the United States have anti-HA (11, 15, 16; W. T. Hall, D. L. Madden, F. K. Mundon, D. E. L. Brandt, and N. A. Clarke, *Am. J. Epidemiol.*, in press). In this report we examine the frequency of anti-HA in a commercial plasma donor population and the titer of circulating antibody. Finally, we suggest that by preselection of donors, it would be possible to produce immune serum globulin that has a known specific potency against HA virus, which could possibly result in the reduction of dose volumes required for treatment of patients exposed to HA.

MATERIALS AND METHODS

Donors. Plasma samples from 245 plasma donors at Plasma Alliance, Inc. (Knoxville, Tenn.) were tested for anti-HA and antibody to hepatitis B sur-

face antigen (anti-HB_s). The plasma donor population was composed of 207 whites and 38 blacks, 150 males and 95 females, ranging in age from 18 to 56. Most of these donors were students at the University of Tennessee. All had been previously tested and found negative for hepatitis B surface antigen. All donors had undergone plasmapheresis previously and form part of the nucleus of donors currently being used.

HA tests. The IAHA, as described by Miller et al. (12), was used for the detection of anti-HA. The HA Ag for the IAHA test was obtained from a stool specimen of a patient with subclinical viral hepatitis type A, which had been found to be positive for HA Ag by radioimmunoassay and immune electron microscopy (6a). The purification of this antigen for IAHA and its specificity for antibody to HA Ag have been described elsewhere (Hall et al., *Am. J. Epidemiol.*, in press). Each plasma was heated to 56°C for 30 min to inactivate complement and was tested for anti-HA at dilutions of 1:10, 1:100, and 1:1,000 against 4 IAHA units/0.25 ml of HA Ag. Specimens positive at 1:1,000 were titrated to the end point. Controls for anti-complementary activity were run on all samples. Only those samples that caused a 4+ agglutination at a given dilution were considered positive.

Hepatitis B tests. The commercial passive hemagglutination test sold by Electro-Nucleonics Laboratories, Inc. for the detection of anti-HB_s was used according to the instructions supplied by the manufacturer. End point titers were determined for all anti-HB_s-positive plasmas.

RESULTS

Anti-HA. Antibody to HA Ag at a titer of 1:10 or greater was detected in 91 of the 245 (37%)

regular plasma donors tested. Ninety of these positive samples were also reactive at a dilution of 1:100. Table 1 contains a summary of the frequency of anti-HA in this population according to age, sex, and race. There is a statistically significant ($P < 0.01$) increase in the frequency of anti-HA between the 18- to 29-year-old group (30%) and the 30- to 49-year-old group (57%). There was no significant difference between the frequency of anti-HA in whites and blacks or in the frequency of anti-HA between the white sexes. There was not a sufficient number of black females for statistical comparison.

The frequency of anti-HA in this population according to IAHA titer is summarized in Table 2. Approximately 32% (29 of 91) of the positive donors, or 12% (29 of 245) of all donors, had an anti-HA IAHA titer of at least 1:1,000. Whereas the 30- to 49-year-old group contained only 21% (51 of 245) of the donor population tested, 48% (14 of 29) of the high-titered plasma donors were in this group.

Anti-hepatitis B. The frequency of anti-HB_s by age, race, and sex is tabulated in Table 3. The present study was performed on regular, paid plasma donors, and, therefore, none of the samples was positive for hepatitis B surface antigen, since such samples would not be admitted to the donor program. However, 8.6% (21 of 245) of the donors were positive for anti-HB_s. Of these 21, 33% (7 of 21) also had anti-HA. There was no significant distribution of anti-HB_s as related to age, sex, or race.

DISCUSSION

Commercially produced human serum immunoglobulin has long been used as an accepted method of prophylaxis for HA. The premise that this immunoglobulin was effective in conferring passive immunity against HA was based on several assumptions recently summarized by Grady (5): "(1) hepatitis A is a common infection of childhood, though often inapparent; (2) long lasting natural immunity is implied by the rarity of second attacks of

overt disease; (3) immunity would be mediated via circulating antibody which would still be present in serum of a significant proportion of persons old enough to donate blood from which immune plasma could be obtained."

Combined data from several reports (11, 15, 16; Hall et al., *Am. J. Epidemiol.*, in press) of the distribution of anti-HA in populations representing every American socioeconomic level indicate that approximately 47% of the adults tested (896 of 1,908) have antibody to HA Ag at concentrations detectable by IAHA. This incidence is age related and increases from 10 to 15% in children under 15 years of age to almost 75% by age 50. In contrast to the figures reported for the United States, Villarejos et al. (16) found 80% of Costa Ricans with circulating anti-HA by age 9. Published estimates of HA virus infections in the United States indicate that either most cases are subclinical or the disease is greatly misdiagnosed or under-reported (3, 10).

Long-lasting immunity is implied. Circulating anti-HA is known to persist for several years (4; Hall et al., *Am. J. Epidemiol.*, in press), and recent studies verify the enduring natural immunity provided by this circulating antibody (Hall et al., *Am. J. Epidemiol.*, in press). In a retrospective study of an epidemic of HA at the Lynchburg Training School and Hospital, Lynchburg, Va., 80% (180 of 225) of the patients without detectable anti-HA in the 10 wards most seriously affected developed hepatitis, whereas none of the 160 patients with pre-existing anti-HA contracted the disease. Epidemiological evidence indicated that many of these patients with anti-HA had developed their immunity 18 years before, possibly as a result of a previous epidemic (7).

Passive immunity also prevents hepatitis. Miller et al. (12) tested 24 lots of commercial immunoglobulin produced by Merck Sharpe and Dohme and found an average anti-HA IAHA titer of approximately 1:5,700. That such a titer is protective against infectivity by HA virus was suggested by the neutralization stud-

TABLE 1. Frequency of antibody to HA Ag among a group of regular plasma donors at Plasma Alliance, Inc., according to age, race, and sex

Age (yrs)	No. of donors positive for anti-HA/no. tested				
	White		Black		Total
	Male	Female	Male	Female	
18-29	27/89 (30) ^a	18/68 (26)	11/29 (38)	1/1 (100)	57/187 (30)
30-49	13/21 (62)	14/22 (64)	1/5 (20)	1/3 (33)	29/51 (57)
≥50	4/6 (67)	1/1 (100)	0/0 (0)	0/0 (0)	5/7 (71)
Total	44/116 (38)	33/91 (36)	12/34 (35)	2/4 (50)	91/245 (37)

^a Number in parentheses gives percentage of donors positive for anti-HA for respective group.

ies of Krugman (8). MS-1 serum, known experimentally to be highly infectious, was completely neutralized by *in vitro* incubation with immunoglobulin having an IAHA titer of 1:3,200. What effect such an immunoglobulin would have *in vivo* after injection is not known, but several studies have shown the successful use of immunoglobulin derived from outdated whole blood from volunteers (1, 2) and from paid plasma donors (9). Our recently completed retrospective serological analysis of a prospective trial of immunoglobulin (IAHA titer, 1:10,000) during an epidemic of HA indicates that 0.02 ml/454 g of body weight was effective in preventing disease. Although the sample size was small, the results were statistically significant ($P < 0.005$). Twelve of 19 (63%) patients without detectable IAHA anti-HA, and who did not receive immunoglobulin, developed hepatitis, whereas only 4 of 25 who received immunoglobulin developed disease. Bilirubin and serum glutamic pyruvic transaminase levels indicated that these four patients had probably been infected with the virus before receiving immunoglobulin (Hall et al., *Am. J. Epidemiol.*, in press).

Long-lasting immunity implies that antibody would be present in a significant proportion of the population old enough to give plasma. Actual evidence indicates that almost one of every two or three persons theoretically available to

contribute plasma would have anti-HA. However, since the frequency of antibody is age dependent, the older the donor, the more likely anti-HA is to be found. Thus, assuming a 25-fold concentration of commercial immunoglobulin over circulating immunoglobulin (6), a plasma pool from 12% of the Plasma Alliance, Inc. donors with an anti-HA $\geq 1:1,000$ would require only a 2.5-fold concentration to attain a titer comparable to that found most often by Miller et al. (12) in commercial immunoglobulin and superior to that successfully used by Krugman (8).

Ringertz (14) has reviewed a number of published investigations that used immune serum globulin for the prevention of HA. Doses of immunoglobulin from 0.01 to 0.15 ml/454 g of body weight have been effectively used without apparent correlation between dose level and protection provided. The variation and inconsistency of the noted dose-response levels become much more intelligible in light of the now-recognized, lot-to-lot differences in antibody titer. Mosley et al. (13) previously pointed out that such differences in protective effect might exist between different lots of immunoglobulin but lacked a reliable method for measuring the amount of specific anti-HA antibody present.

The frequency of anti-HB_s could not be correlated with the frequency of anti-HA. In this study, the frequency of anti-HA was essentially the same for the population with anti-HB_s (33%) as for that without it (37%). The chi-square analyses of these data were not significant at the $P < 0.1$ level. This is in contrast to the report of Szmunn et al. (15), who found that in a study of 947 New York City residents from a variety of socioeconomic classes those positive for anti-HB_s were 1.5 times as likely to have anti-HA as people negative for anti-HB_s.

The recent development of serological assays for detecting anti-HA has made it possible to test the assumptions on which past immunoglobulin prophylaxis of HA have been based.

TABLE 2. Frequency of antibody to HA Ag according to age and titer among a group of plasma donors at Plasma Alliance, Inc.

Age (yrs)	Reciprocal of IAHA titer					
	10	100	1,000	2,000	3,000	6,000
18-29	1 ^a	41	7	1	5	2
30-49		15	8	2	3	1
≥50		5				

^a Number of samples positive for anti-HA at the indicated dilution but negative at the next higher dilution.

TABLE 3. Frequency of antibody to hepatitis B surface antigen among a group of regular plasma donors at Plasma Alliance, Inc.

Age (yrs)	No. of donors positive for anti-HB _s /no. tested					% Positive
	White		Black		Total	
	Male	Female	Male	Female		
18-29	8/89 (2) ^a	6/68 (0)	1/29 (0)	0/1 (0)	15/187 (2)	8
30-49	1/21 (1)	4/22 (3)	1/5 (1)	0/3 (0)	6/51 (5)	12
≥50	0/6 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/7 (0)	0
Total	9/116 (3)	10/91 (3)	2/34 (1)	0/4 (0)	21/245 (7)	8.6

^a Number in parentheses in number of donors possessing antibody to both HA and hepatitis B antigens in respective age group.

Initial results of these tests have confirmed the soundness of the assumptions and presage the future prescription of immunoglobulin by taking into account anti-HA titer and not merely volume alone.

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