Diagnostic Value of Indirect Hemagglutination in the Seroepidemiology of *Shigella* Infections

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To evaluate the usefulness of the indirect hemagglutination (IHA) test in the epidemiological investigation of shigellosis, single serum specimens were tested from 50 patients with *Shigella dysenteriae* 1 (Shiga bacillus) infections, 103 asymptomatic contacts of these cases, 267 adult and 100 student control, and serum specimens collected during two outbreaks caused by *S. sonnei* and one outbreak due to *S. flexneri* 6. In patients with *S. dysenteriae* 1, 74% demonstrated titers of $\geq 1:40$, with 50% showing titers of $\geq 1:160$, whereas in the controls 10.4% had titers of $\geq 1:40$ and only 0.3% had titers of $\geq 1:160$. IHA titers in serum specimens collected from patients with *S. sonnei* and *S. flexneri* 6 were too low to be considered diagnostic for individual patients, but were useful in analysis of group results. Groups of ill individuals yielded titers significantly higher than non-ill groups; however, titers from ill groups were usually less than 1:40. The IHA test for *S. dysenteriae* 1 antibodies serves as a valuable adjunct to the diagnosis of Shiga bacillus dysentery. In our laboratory, an IHA titer of 1:40 or 1:80 is a "borderline positive." Shiga bacillus dysentery is strongly indicated when IHA titers are $\geq 1:160$.

The indirect hemagglutination (IHA) test has been demonstrated by many investigators to be more sensitive in titrating antibody to *Shigella* than the bacterial agglutination test (7, 12, 19, 21, 23-25). Detection of *Shigella* antibody by IHA has proved to be valuable both for diagnostic confirmation of individual cases of shigellosis (12, 19, 22) and in epidemiological investigations (9, 14, 18). An adaptation of the microtiter technique has shown that the IHA test for *Shigella* can be performed without loss of sensitivity and have the additional advantages of speed and use of less materials (10, 13).

The IHA test using cell wall O polysaccharide from homologous *Shigella* serotypes was used in the investigation of individual cases of *Shigella dysenteriae* 1 (Shiga bacillus) that occurred mainly in the western United States and in three outbreaks of gastrointestinal illness caused by *S. sonnei* and *S. flexneri* 6. The methods of, serological response to, and diagnostic value of the IHA test in the epidemiology of *Shigella* infections are described in this report.

MATERIALS AND METHODS

Antigens. The polysaccharide extracts were prepared by the method of the Walter Reed Army Insti-

tute of Research (8) and Young et al. (25). Briefly, this consisted of heating smooth, agar-grown saline suspensions of the bacteria together with an equal quantity of 0.1 N NaOH at 100 C for 70 min. The material was then cooled, adjusted to pH 7.0, dialyzed, centrifuged, and supernatant was filtered through an asbestos filter (Seitz). Each lot of antigen was standardized with hyperimmune rabbit antisera to determine the optimal antigen dilution giving a high stable titer. Antigens were prepared from *S. dysenteriae* 1, *S. sonnei*, and *S. flexneri* 6. The *S. sonnei* and *S. flexneri* 6 isolates for antigen preparation were obtained from patients involved in the outbreaks.

Sera. Serum for *S. dysenteriae* 1 IHA testing was obtained from cases and their household contacts. A case of *S. dysenteriae* 1 was defined as a patient with a positive culture for the Shiga bacillus or a household contact of a culture-proved case who had bloody or mucous diarrhea. Sera from 50 cases and 103 household contacts were studied. Of the 50 cases, 35 were in Mexican-Americans residing in California or Texas, most of whom had recently traveled to or were close contacts of recent travelers to Mexico. The other 15 cases were in residents from various areas of the United States. Eleven had a history of recent travel to Mexico, one to Guatemala, and one to other areas outside the United States; one gave a history of no recent travel and a travel history was not available from another. Serum from two groups of controls were also tested in this study: (i) 100 specimens from students at Decatur High School,

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Decatur, Ga., who had no known exposure to *S. dysenteriae* 1, and (ii) 267 specimens randomly selected from sera submitted to the Texas State Department of Health for syphilis testing that were not from persons with known cases of dysentery.

Serum specimens from a variety of outbreaks of gastrointestinal illness were studied. (i) Forty-two paired sera were collected 9 days apart (17 and 26 days after the outbreak) from crewmen aboard a Norwegian supertanker that had experienced an outbreak of shigellosis due to *S. sonnei* in January and February 1970 (5). Sera from 21 crewmen from another Norwegian ship in the same port were collected as controls. (ii) Eighty-four single specimens were collected from passengers and boatmen who had taken Colorado River raft trips in the summer of 1972. Seventy-two specimens were from those who had traveled on trips on which acute gastroenteritis occurred, and 12 were from those who were on a trip from which no illness was reported. An epidemiological investigation of 13 raft trips was carried out to define the cause and means of transmission (16). Persons on 12 of the 13 trips had symptoms compatible with shigellosis, and *S. sonnei* was isolated from the stool of one person each on seven of the trips. Persons on a raft trip (trip A) that took place 3 weeks earlier experienced somewhat different clinical symptoms. The serum samples were obtained 7 to 12 weeks after the trips (serum from passengers on trip A was collected 3 weeks later than serum from other passengers). (iii) A total of 113 paired sera were collected from crew aboard a cruise ship on which an outbreak of gastroenteritis occurred in June 1973 (17). Ninety percent of the 650 passengers and at least 35% of the 299 crew were ill; *S. flexneri* 6 was isolated from rectal swabs from 8 passengers and 33 crew members. The follow-up serum samples were collected approximately 3 weeks after the first serum samples. Control specimens consisted of 20 serum specimens from crew members on a ship docked next to the implicated ship. No reported illness had occurred on the control ship.

Monospecific serum used in the block or checkerboard titration for standardization of antigens was obtained from the Biological Products Division, Center for Disease Control. This serum was prepared by the method outlined by Edwards and Ewing (6).

IHA. The IHA test of Young et al. (25) performed by the microtechnique of Lee et al. (13) and modified by Cáceres and Mata (3) was used to detect antibodies. Fresh human type O Rh-negative cells preserved in Alsever solution (2) were used as the antigen carrier. Duplicate testing was performed on 529 serum specimens. More than one tube variation between duplicate measurements occurred in only 1.1% of the samples.

RESULTS

Serological response to *S. dysenteriae* 1 in cases, asymptomatic contacts, and controls. Antibody levels varied in patients that had *S. dysenteriae* 1 (cases) with titers ranging from negative (<1:10) to 1:1,280. In the group of 50

cases, 74% had titers of 1:40 or greater (Fig. 1). Twenty-four percent (12 cases) had titers in the 1:40 to 1:80 range. The specimens from cases were collected on various days after onset of symptoms, ranging from 6 to approximately 255 days, with 41 (82%) of 49 specimens collected between 10 and 60 days after onset (date of onset of symptoms was not available in one case). Mean titers were highest when serum was collected 31 to 50 days after onset of illness (Fig. 2). Individual titers are indicated in Table 1. In 9 of the 50 cases, *S. dysenteriae* 1 isolates were not obtained. Six of these 9 had titers of $\geq 1:40$.

Antibody titers of the 103 asymptomatic household contacts of patients ranged from negative (<1:10) to 1:640. Over 29% of the close contacts had titers of 1:40 or greater (Fig. 1). Most of these sera were collected at approximately the same time specimens were collected from cases, i.e., between 10 and 60 days after onset of symptoms in the case.

An average of 10.4% of the specimens from the 367 controls showed titers of 1:40 or greater. The adult controls from Texas had a slightly higher reaction rate at $\geq 1:40$ than the students from Georgia, 11.9% compared with 9.0% (Fig. 1). Only 1 of the 367 controls had a titer as high as 1:160.

Serological response to *S. sonnei* and *S. flexneri* 6 in gastrointestinal epidemics. Serological evaluation of serum from the 42 crew aboard the Norwegian supertanker and the 21 controls from another Norwegian ship revealed that 15 of 28 ill seamen had measurable titers to *S. sonnei*. A measurable titer was also demonstrated in an asymptomatic excretor of *S. sonnei*. Titers ranged from 1:4 to 1:64 with the second serum of the pair showing a drop in titer in most instances. No detectable antibody was demonstrated in the remaining well crew members or the controls.

Serological results to *S. sonnei* in passengers and boatmen on Colorado River raft trips (Fig. 3) showed that sick passengers on five trips on which illness occurred had a geometric mean titer (± 2 standard error) of $1:36 \pm 10$, compared with $1:15 \pm 5$ for well passengers on these trips; $1:10 \pm 7$ for ill passengers on trip A, and $1:10 \pm 4$ for passengers on a trip on which no illness was reported (16).

Serological testing for *S. flexneri* 6 antibodies in crew aboard the cruise ship showed a geometric mean titer in the ill group of 1:6.8 in the first specimen and 1:12.5 in the convalescent one (Fig. 4). The non-ill group had a geometric mean titer of 1:4.4 in the first serum and 1:5.4 in the second. The mean change from first serum to convalescent serum was significantly differ-

FIG. 1. Percentage of serum specimens with IHA antibody titers to *S. dysenteriae* type 1.

ent between the two groups at the 5% level (*t* test). The geometric mean titer for the control group (single specimen only) was 1:3.8. This was not significantly different from the first serum specimens of the ill and non-ill groups.

DISCUSSION

This study shows that the IHA test may serve as a valuable adjunct in the investigation of individual clinical cases of *S. dysenteriae* 1 and in epidemiological analysis of outbreaks of shigellosis caused by *S. sonnei* and *S. flexneri* 6. This serological test is useful primarily when there is supporting clinical and laboratory data.

The presence of hemagglutinating antibodies to the Shiga bacillus at levels of 1:40 or greater in human serum have been considered an indication of current or recent infection (10, 22). In

a nationwide serosurvey conducted in Guatemala in 1965, 3 years before a severe epidemic of *S. dysenteriae* 1, only 1.8% of 2,884 persons examined had antibodies to the organism at a titer of $\geq 1:40$. In 1954 in the United States, Neter and Gorzynski (20) noted that 18 of 60 randomly selected human serum specimens from blood donors, healthy pregnant women, and children suffering from various diseases other than intestinal infections showed *S. dysenteriae* 1 hemagglutinating titers of 1:5 to 1:80. Our investigation, compared with the Guatemala study, indicated that in both the southeastern and southwestern United States a greater percentage of the tested population (10.4 versus 1.8%) demonstrated titers of 1:40 or higher. The origin of these low-level antibodies remains undetermined. Cross-reacting antigens (heterologous) could account for the titers,

FIG. 2. Arithmetic mean titers of antibody to *Shiga bacillus*.

TABLE 1. Individual titers of antibody to *Shiga bacillus* in 49 cases

Reciprocal titer		Day of serum collection after onset				
		<10	10-30	31-50	51-70	>70
Negative	(<10)	1	1	1	1	1
	10	1	2	1		
	20	3		2		
	40	3	1			2
	80	1	4	1		
	160	3		1		2
	320	5	5	1	1	
	640	1				
	1,280	1	2	1		

^a Indicates number of antisera in each category.

as observed with other *Enterobacteriaceae* (6, 12, 19, 22, 26); however, specificity studies with monospecific rabbit antiserum and serum from shigellosis patients performed by Guatemalan investigators (19, 25) have indicated *S. dysenteriae* 1 to be highly specific. Because 10.4% of the control population in our study exhibited titers of 1:40 and higher, we considered 1:40 and 1:80 reactions in single specimens as "borderline positives." These titers were considered significant of infection when clinical symptoms suggested Shiga dysentery. This diagnosis is strongly indicated when IHA titers are $\geq 1:160$.

Seventy-four percent of the 50 cases of dysentery due to *S. dysenteriae* 1 demonstrated antibody titers of $\geq 1:40$. These titers were evident as early as 13 days after onset of symptoms and in 1 case as early as 6 days after onset. Mean

FIG. 3. Geometric mean titers to *S. sonnei* of passengers and boatmen on raft trips.

FIG. 4. Geometric mean titer to *S. flexneri* 6 between paired sera of ill and non-ill crew abroad cruise ship.

titers peaked between 31 and 50 days after which they decreased to a mean level of 1:121 in specimens collected more than 70 days after onset of illness. An early serological response was reported by Cáceres and Mata (4), who demonstrated that in 281 individuals with *S. dysenteriae* 1 or 2, more than 80% had significant titers ($\geq 1:40$) after day 9 and maximal titers after 1 month. In the Shiga dysentery patients there was a progressive decline in titer after that. By month 3, about one-half of the specimens had significant titers. Evidence suggested that this serological response was due to antibody belonging to the immunoglobulin M (IgM) fraction.

When the hemagglutination test was applied to three epidemiological investigations, it was found to be useful in two *S. sonnei* outbreaks and one *S. flexneri* 6 outbreak. Confirmation of the cause of the outbreak was obtained in the ill group in all three outbreaks, but significant titers in individual cases could not be determined. Serological confirmation of *S. sonnei* was achieved in the ill group either when paired sera were collected several days (17 to 26) after the outbreak (supertanker study) or when single specimens were obtained several weeks (7 to 12) after the episode (Colorado raft trips). Detectable titers in the supertanker outbreak were at low levels, but greater than 50% of the ill crewmen demonstrated measurable titers within 3 weeks after symptoms occurred. A decrease in titer noted in the second sample of the pair suggests the antibody probably belongs to the IgM fraction. In studying the immunoglobulin classes after *S. sonnei* infection, Hasenson et al. (11) indicated that specific IgM antibodies were detected early after onset (2 to 5 days), rose to a maximum, and began declining after 15 days.

Even though only one specimen was obtained from each person on the raft trips, meaningful data were obtained when the group was analyzed as a whole. The geometric mean titer was significantly higher in ill passengers than in well passengers. The low levels of antibodies detected 7 to 12 weeks after onset of illness in this study may suggest specific IgM antibodies in the declining phase.

In an extensive gastrointestinal outbreak aboard a cruise ship where all available clinical, epidemiological, and bacteriological data strongly indicated *S. flexneri* 6 as the pathogen responsible for the outbreak, serological data from the hemagglutination test was also of value in implicating this organism. A significantly greater difference between paired sera was demonstrated for ill crewmen than for non-ill crewmen.

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