JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1979, p. 903-909
0095-1137/79/12-0903/07$02.00/0

Vol. 10, No. 6

Immune Response and Prevalence of Antibody to Norwalk Enteritis Virus as Determined by Radioimmunoassay

NEIL R. BLACKLOW,[1]* GEORGE CUKOR,[1] MARY KAY BEDIGIAN,[1] PETER ECHEVERRIA,[2] HARRY B. GREENBERG,[3] DAVID S. SCHREIBER,[4] AND JERRY S. TRIER[4]

Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts 01605[1]; United States Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand[2]; Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205[3]; Gastroenterology Division, Peter Bent Brigham Hospital, Harvard Medical School, Boston, Massachusetts 02115[4]

Received for publication 11 September 1979

A solid-phase microtiter radioimmunoassay was established for the detection of Norwalk virus and its antibody, with clinical materials from human volunteers previously studied in Massachusetts as reagents. A study of 308 Massachusetts residents showed that serum antibody to Norwalk agent was rarely present during childhood but was detectable in approximately 50% of adults. All volunteers inoculated with Norwalk virus who developed illness seroconverted (10/10), whereas only one-third (5/15) of nonill volunteers seroconverted ($P = 0.0009$). The 10 nonill, nonseroconverting subjects had undetectable to low preexisting antibody levels. Paradoxically, 10/13 subjects with preexisting antibody became ill, whereas 17/25 lacking antibody did not ($P = 0.009$). All 3 subjects with preexisting anti- Norwalk radioimmunoassay blocking activity in duodenal intraluminal fluids became ill, whereas only 5/11 lacking such activity developed illness ($P = 0.15$). These data further support the unique concept that some individuals are susceptible to repeated infections with this agent, whereas others are incapable of developing infection.

Acute infectious nonbacterial gastroenteritis is a very common illness that frequently occurs in epidemics involving older children and adults (3, 7). Although an etiological agent has not been cultivated in vitro, the syndrome has been experimentally induced in human volunteers by the oral administration of bacteria-free stool filtrates derived from several well-defined gastroenteritis outbreaks (3, 25). Virus-like particles, 27 nm in size and possessing characteristics of parvoviruses, have been visualized by immune electron microscopic examination of these infectious fecal filtrates, and at least three distinct serotypes are recognized (2, 17, 23, 25).

The most thoroughly studied of these agents is that derived from a gastroenteritis outbreak in Norwalk, Ohio (8), and it is the prototype of these agents. By use of immune electron microscopy (IEM), it has been shown that volunteers shed 27-nm particles (22) and develop serum antibody responses after infection with Norwalk virus (17, 18). Although IEM has been an invaluable first assay for study of Norwalk virus in vitro, it is a slow, cumbersome, semiquantitative technique that is not amenable to extensive immunological, epidemiological, or viral characterization studies. Thus, there has been a great need for development of rapid and sensitive assays for the study of non-cultivatable Norwalk and Norwalk-like gastroenteritis viruses. A major advance has recently occurred through the development in one laboratory of a radioimmunoassay (RIA) technique for quantitation of Norwalk virus antigen and its antibody (14); the technique has been used to demonstrate the frequent role of Norwalk virus in outbreaks of nonbacterial gastroenteritis (13). The RIA test relies of necessity on human clinical materials for its critical reagents because it has not been possible to purify Norwalk antigen from stools sufficiently to permit preparation of useful hyperimmune animal sera. In this paper, we report the development of an RIA for the Norwalk agent that uses as reagents clinical materials derived from human volunteers previously studied in Massachusetts (18, 20, 24). We have been able to confirm the previously reported RIA technique (14) with different reagents and have employed the assay to examine age prevalence of serum antibody and to study anti-Norwalk activity in human milk and intestinal intraluminal fluids. The RIA test has also provided us with the opportunity to examine further the concept (18) that some individuals are suscepti-

ble to repeated infection with Norwalk virus whereas others appear to be incapable of developing infection.

MATERIALS AND METHODS

Serum specimens. Paired serum samples obtained before and from 2 to 6 weeks after challenge with the Norwalk agent were obtained from nine normal human volunteers previously studied in Massachusetts (18, 20). Two additional previously studied (20, 24) Norwalk-infected subjects provided serum samples that were used as reagents in the RIA test. Pre-Norwalk challenge sera were available from 13 additional volunteers (20, 24) for testing by RIA. In addition, preinfection and convalescent serum samples from 16 Norwalk-inoculated volunteers studied by the National Institutes of Health were provided under code by one of us (H.B.G.).

Control serum samples consisted of (i) acute and convalescent serum pairs from five children and four soldiers infected with rotavirus (9, 12); (ii) preinfection and convalescent serum samples from individual volunteers challenged with the Hawaii (25) and W (2) agents and with toxigenic *Escherichia coli* (provided under code by one of us [H.B.G.]); (iii) hyperimmune guinea pig sera prepared against the following parvoviruses: adenovirus-associated virus serotypes 1, 2, 3, and 4, densonucleosis virus, bovine parvovirus 1 (Haden), H-1 virus, minute virus of mice, and rat virus (15); (iv) hyperimmune guinea pig serum prepared against SA11 rotavirus (5) and hyperimmune monkey sera against poliovirus type 1 and coxsackie virus B5.

Three hundred and eight serum specimens were obtained throughout the year during the period of 1975 to 1979 from Massachusetts residents and used in a Norwalk antibody prevalence survey. Of these 308 samples, 173 were collected from patients hospitalized for reasons other than acute or chronic inflammatory gastrointestinal disease or hematological disease requiring transfusion (4). The remaining 135 sera were obtained from nonhospitalized, healthy individuals.

All sera were stored at −20 or −70°C until use.

Stool specimens. A diarrheal stool specimen collected from a volunteer experiencing Norwalk illness (20) was employed as antigen in the RIA test. Twenty-eight stool samples obtained from volunteers experimentally inoculated with Norwalk virus were supplied under code by one of us (H.B.G.). Control stool specimens consisted of (i) two stool samples obtained from different volunteers before challenge with Norwalk virus (both subjects subsequently developed illness and shed virus detectable by RIA) and (ii) 31 patients experiencing naturally occurring acute diarrheal disease. Of these 31 patients, 11 had documented infection with rotavirus (5 children and 6 adults) (5, 9, 10, 11), and 20 adults had illness of unknown cause but not associated with Norwalk agent by serology (10, 12). All stools were stored at −70°C until use.

Small intestinal intraluminal fluid specimens. After an overnight fast, duodenal intraluminal contents were collected (1, 18, 20, 24) by a tube placed under fluoroscopic control from 14 volunteers before administration of the Norwalk agent and again 3 to 7 days after challenge. Fluids were tested by single radial immunodiffusion (Calbiochem-Behring Corp., La-Jolla, Calif.) for levels of total immunoglobulin A (IgA) and IgG. Specimens were stored at −70°C until use.

Milk specimens. Milk and serum samples from 33 healthy mothers were collected within a week of parturition (6). Specimens were stored at −70°C until use.

RIA procedure. A solid-phase microtiter RIA (19) was adapted for the detection of Norwalk virus and its antibody by the previously reported procedure (14).

To test for Norwalk antigen, a pair of pre- and postchallenge sera collected 1 month apart from a volunteer who developed experimental Norwalk illness was selected. Each serum was diluted 1:10,000 in phosphate-buffered saline, and 75 μl was used to coat the wells of microtiter plates (220-25, Cooke Engineering Co., Alexandria, Va.). All reagents used in the RIA contained 0.1% sodium azide. The plates were kept for 18 to 24 h at room temperature (about 21°C) and then washed twice with phosphate-buffered saline. The wells were then saturated with 1% bovine serum albumin in phosphate-buffered saline for 18 to 24 h at 4°C and again washed twice. Stool samples to be tested were prepared as 5 to 10% suspensions in sterile veal infusion broth supplemented with 0.5% bovine serum albumin (5). The suspensions were clarified by centrifugation at 2,000 × *g* for 15 min, and 25 μl of the supernatant fluid was placed into each of four wells, two of which were coated with the prechallenge serum and two of which were coated with the postchallenge serum. The plates were again incubated for 18 to 24 h at room temperature and then washed five times. Convalescent serum from a volunteer who became ill after challenge with Norwalk virus was used as the source of ^{125}I-labeled detection antibody. The source of this detection antibody was not the same volunteer who provided the coating antibodies. The preparation of immunoglobulin and the iodination procedure have been previously described (5). Each well received 25 μl of detection antibody containing 2×10^5 cpm. The plates were incubated at 37°C for 4 h, washed five times, and cut apart. The amount of radioactivity bound to each well was determined in a gamma spectrometer (PRIAS, Packard Instruments, Downers Grove, Ill.). The average of the counts bound to the wells coated with postchallenge serum was divided by the average of the counts bound to the wells coated with prechallenge serum to obtain a positive-to-negative ratio. Samples exhibiting positive-to-negative values greater than 2 were considered positive for Norwalk antigen.

A blocking test was used for detection and quantitation of anti-Norwalk activity in serum, small intestinal intraluminal fluid, and milk samples. Plates were coated as above but with the postchallenge serum only. Antigen for all blocking tests was derived from a single stool specimen collected from a volunteer experiencing experimental Norwalk illness. Serum from this individual was not used in any assay. Stool suspension was prepared as above and then extracted once with an equal volume of 1,1,2-trichloro-1,2,2-trifluoroethane (J. T. Baker Co., Phillipsburg, N.J.). A 25-μl amount of a 1:2 dilution of the extract was added to each well as antigen. This amount of antigen bound 800 to 1,200 cpm and represented 16 U. Serum, small

intestinal intraluminal fluid, and milk specimens were initially diluted 1:50 in phosphate-buffered saline with 1% bovine serum albumin and serial twofold dilutions were then prepared. A 50-μl quantity of a given dilution was added to each of two washed wells to which antigen had been bound. Diluent alone served as a control. Plates were incubated for 18 to 24 h at room temperature and washed five times before the addition of detection antibody and determination of bound radioactivity. A 1:50 dilution of a serum known to be negative for Norwalk antibody by IEM (18) did not alter the number of counts bound, whereas the same dilution of a known IEM-positive serum reduced the counts to 90 to 120 cpm. A given dilution of a test specimen was considered to be positive if it reduced the number of counts bound by at least 50% compared with the buffer control.

RESULTS

Specificity of RIA test. The specificity of the RIA test for Norwalk virus and its antibody was established in the following ways. First, pre- and postchallenge serum specimens from nine volunteers inoculated with the Norwalk agent were tested by RIA, and titers were compared with their previously reported IEM antibody ratings (18) as shown in Table 1. In every instance in which a seroconversion was indicated by IEM, it was also detected by RIA. Three of the nine volunteers were not ill and failed to show seroconversion by either technique. One ill volunteer (volunteer 5) showed seroconversion by RIA only. Second, 25 pairs of pre- and postchallenge serum specimens from volunteers inoculated with the Norwalk agent (including the nine serum pairs studied by IEM) were tested by RIA, and results were compared with RIA data obtained at National Institute of Allergy and Infectious Diseases, NIH. The NIH assay (14) has been established with human clinical materials different from those used as reagents in this report. Fifteen serum pairs exhibited a seroconversion (≥fourfold rise in antibody titer) by the present test, of which 13 were shown to seroconvert at NIH. Ten pairs of pre- and postchallenge serum specimens failed to demonstrate seroconversion by both assays. Third, paired serum samples from individuals experiencing diarrheal illness due to Hawaii agent, W agent, toxigenic *E. coli*, or rotavirus did not show seroconversion to the Norwalk agent. Fourth, 12 hyperimmune animal sera prepared against known parvoviruses, rotavirus, poliovirus, and coxsackievirus were negative for Norwalk antibody. Fifth, 28 diarrheal stool specimens from volunteers experiencing Norwalk illness were tested for Norwalk antigen, and results were compared with RIA data obtained at NIH. Thirteen specimens were positive in both assays, and an additional specimen was positive at NIH. The remaining specimens were negative in both assays. Finally, 31 control non-Norwalk diarrheal stool specimens and 2 control prechallenge Norwalk volunteer stool specimens were all negative for Norwalk antigen in the present RIA test.

TABLE 1. *Comparison of RIA and IEM tests for measurement of antibody responses in nine volunteers inoculated with Norwalk agent*

Volunteer	Illness	Serum	RIA titer[a]	IEM rating[b]
1	Yes	Prechallenge	<50	0–1+
		Postchallenge	800	2+
2	Yes	Prechallenge	200	0–1+
		Postchallenge	≥6,400	3–4+
3	Yes	Prechallenge	100	1 → 1–2+
		Postchallenge	≥6,400	4+
4	Yes	Prechallenge	200	1–2 → 2+
		Postchallenge	≥6,400	3+
5	Yes	Prechallenge	3,200	3–4+
		Postchallenge	12,800	4+
6	Yes	Prechallenge	50	1–2+
		Postchallenge	3,200	4+
7	No	Prechallenge	<50	1+
		Postchallenge	<50	1–2+
8	No	Prechallenge	50	1+
		Postchallenge	100	1+
9	No	Prechallenge	<50	1–2+
		Postchallenge	<50	1–2+

[a] Titer expressed as reciprocal.

[b] Antibody rating system as previously reported (18). An antibody response is a rise of 1+ in this assay.

Age distribution of human antibody to Norwalk agent. Prevalence of human antibody to the Norwalk agent in different age groups in Massachusetts was examined as shown in Fig. 1. Three hundred and eight serum specimens were tested at a 1:50 dilution, of which 105 (34%) were positive. Antibody present in 20% of infants in the 0- to 3-month-old age group presumably was of maternal origin. The percentage of sera with antibody rapidly diminished during the next several months and remained at a low level (5%) throughout childhood. The curve of antibody frequency showed a steep rise during adolescence and early adulthood, indicating that approximately one-half of individuals over the age of 18 years possess antibody to the Norwalk agent. Antibody prevalence levels were maintained in approximately 50% of middle-aged and elderly adults.

Correlation of clinical illness with serum antibody to Norwalk agent. Of the 25 pairs of pre- and postchallenge serum samples from

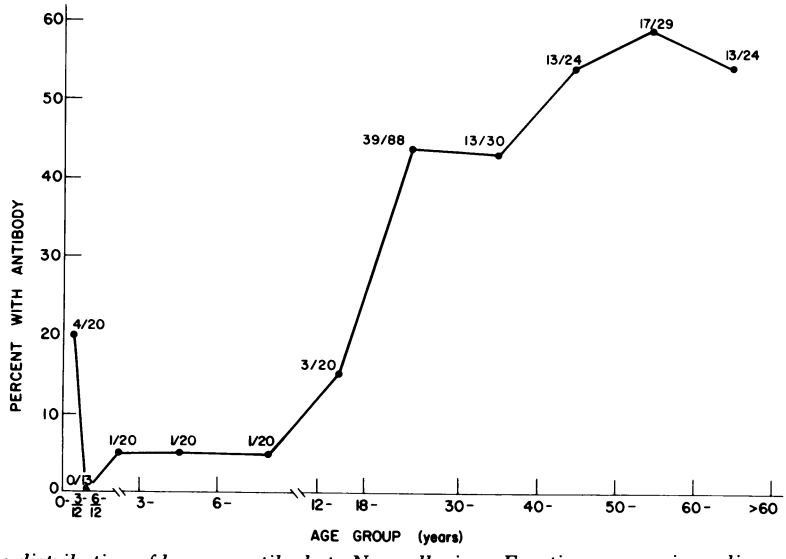

FIG. 1. *Age distribution of human antibody to Norwalk virus. Fractions appearing adjacent to each point represent number with antibody/number tested for each age group.*

volunteers inoculated with the Norwalk agent, 10 were from individuals who developed clinical illness. As shown in Table 2, all 10 ill subjects demonstrated a seroconversion to the Norwalk agent, whereas only one-third (5/15) of challenged volunteers who did not develop overt illness seroconverted. ($P = 0.0009$ by Fisher exact test). The 10 nonill challenged volunteers who failed to seroconvert had nondetectable preexisting antibody titers (9 subjects) or a low prechallenge titer of 1:50 (1 subject).

Correlation of preexisting serum antibody to the Norwalk agent with subsequent development of clinical illness is depicted in Table 3. Of 18 volunteers who developed illness, 10 had preexisting serum antibody titers of ≥1:50. By contrast, it was striking that only 3 of 20 nonill subjects possessed preexisting serum antibody. Interestingly, all three of these individuals, even though they failed to develop overt illness, developed a seroconversion after challenge. Of individuals with preexisting antibody, 77% (10/13) developed illness, whereas only 32% (8/25) of initially seronegative subjects became ill ($P = 0.009$ by Fisher exact test).

Anti-Norwalk agent activity in human milk and small intestinal intraluminal fluids. Serum and milk samples collected from 33 healthy women within a week of parturition were tested by RIA. Fifteen women (45%) possessed serum Norwalk antibody titers of ≥1:50. Of these 15 mothers, 6 had anti-Norwalk agent RIA blocking activity in their milk samples at a titer of ≥1:50. One had a titer of 1:200, and the remaining five had titers of 1:50. No woman had blocking activity in her milk in the absence of serum antibody.

Serum and duodenal intraluminal fluids collected from 14 volunteers before challenge with

TABLE 2. *Correlation of seroconversion to Norwalk agent by RIA with development of clinical illness in 25 inoculated volunteers*

Clinical illness	Seroconversion[a] by RIA	
	Yes	No
Yes	10	0
No	5	10

[a] Fourfold or greater rise in titer of antibody to Norwalk agent between pre- and postchallenge serum specimens. Data are numbers of volunteers. $P = 0.0009$ (10/10 seroconversions with illness versus 5/15 without).

TABLE 3. *Correlation of preexisting serum antibody to Norwalk agent with development of clinical illness in 38 inoculated volunteers*

Clinical illness	Pre-existing serum RIA antibody[a]	
	Yes	No
Yes	10	8
No	3	17

[a] RIA antibody titer of ≥1:50 to Norwalk agent. Data are numbers of volunteers. $P = 0.009$ (10/13 with antibody became ill versus 8/25 without).

the Norwalk agent were tested in RIA. Two of seven seropositive subjects had anti-Norwalk agent RIA blocking activity in their duodenal fluids, whereas one of seven seronegative individuals showed blocking activity in his duodenal fluid. As shown in Table 4, it was striking that the three individuals with anti-Norwalk agent RIA blocking activity in their intestinal fluids all developed illness after challenge with the Norwalk agent. In contrast, of the 11 subjects lacking blocking activity in their prechallenge intestinal fluids, 6 failed to develop clinical illness ($P = 0.15$ by Fisher exact test). The group of volunteers with blocking activity possessed levels of IgA and IgG in their intestinal fluids comparable to levels found in the volunteers lacking blocking activity. The three individuals whose intestinal fluids had blocking activity at a 1:50 dilution had levels of 1.8, 1.4, and 1.1 mg of IgA per 100 mg of protein in their specimens, and values of 26, 26, and 37 mg of IgG per 100 mg of protein. No individual, well or ill, developed a fourfold rise in intestinal fluid blocking activity 3 to 7 days after challenge with Norwalk virus.

DISCUSSION

We have established an RIA test for the detection of Norwalk antigen and antibody that is specific, sensitive, rapid, and quantitative. The assay uses as reagents clinical materials derived from human volunteers previously studied in Massachusetts. Our data confirm the validity of the recently reported RIA test for Norwalk virus and its antibody (14), which uses a different battery of human volunteer clinical materials as reagents. We have used the RIA test to study the age distribution of human antibody to the Norwalk agent and to examine features of the immune response to the virus.

The specificity of this RIA test has been demonstrated by correlation with (i) IEM data, (ii) an independent RIA test that uses different reagents, (iii) clinical findings in volunteers, and (iv) studies of control materials pertaining to other viruses and diarrheal infections.

This RIA test, as presently designed, does not permit determination of antibody class or other precise definition of the anti-Norwalk agent blocking activity observed in milk and intestinal intraluminal fluids; future experiments will be needed to clarify the nature of this blocking activity. The anti-Norwalk blocking activity is, however, strongly suggestive of antibody because of the close correlation that we observed between antibody in serum specimens and blocking activity in body secretions collected simultaneously. Of the 47 tested milk and intestinal secretion specimens, 9 were RIA positive, and 8 of these 9 specimen donors simultaneously possessed serum RIA antibody. Conversely, 25 of these 47 subjects lacked serum antibody, and 24 of these 25 seronegative volunteers lacked RIA blocking activity in their milk and intestinal fluid specimens.

In an extensive age-related antibody prevalence study, we have shown that acquisition of antibody to the Norwalk agent is minimal during childhood and that the frequency of antibody does not rise rapidly until adolescence and early adulthood. Approximately 50% of adults in our population possessed antibody at a 1:50 dilution; this dilution of serum was tested since it has been the lowest commonly reported dilution used for measurement of antibody to gastroenteritis viruses by immunoassay (13, 26). This distribution of antibody to Norwalk virus is in striking contrast to the prevalence of antibody to rotavirus, for which we have shown a majority of children develop antibody by the age of 2 years (4). Indeed, many of the serum samples that we examined for Norwalk antibody were the same specimens that we previously studied for rotavirus antibody prevalence (4). Preliminary examination of a limited number of sera by RIA in the Washington, D.C., area (14) has produced findings consistent with our Norwalk antibody prevalence data, and data similar to ours have been generated by use of a less sensitive immune adherence assay (16).

Our previously reported Norwalk agent volunteer rechallenge studies (18) indicated the presence of two cohorts of individuals with different clinical forms of immunity. One cohort possessed long-term immunity, as evidenced by resistance to illness on challenge and late homologous rechallenge. The other cohort became ill on first challenge and again when rechallenged 27 to 42 months later, but three of four volunteers remained well after a third challenge 4 to 8 weeks after the second, indicating short-term immunity. We also showed that the first

TABLE 4. *Correlation of preexisting anti-Norwalk agent RIA blocking activity in duodenal secretions with development of clinical illness in 14 inoculated volunteers*

Clinical illness	Preexisting blocking activity in duodenal secretions[a]	
	Yes	No
Yes	3	5
No	0	6

[a] Anti-Norwalk agent RIA blocking activity titer of ≥1:50. Data are numbers of volunteers. $P = 0.15$ (3/3 with blocking activity became ill versus 5/11 without).

cohort persistently lacked serum IEM antibody to Norwalk virus (before and after challenge) and that the second (ill) cohort generated IEM antibody responses and could develop illness in the presence of serum antibody. Our present data obtained with the RIA test support and extend our earlier rechallenge study observations, providing additional evidence that clinical immunity to Norwalk virus is complex and fails to fit immunological concepts traditionally associated with common human viral illnesses. First, we have made the paradoxical observation that presence of preexisting serum RIA antibody is associated with development of illness, since 10 of 13 subjects with antibody developed illness, whereas only 8 of 25 lacking antibody became ill. Second, all 3 subjects with preexisting anti-Norwalk RIA blocking activity in duodenal fluids developed illness, whereas 5 of 11 volunteers lacking blocking activity became clinically ill. Third, 10 volunteers who failed to develop illness also possessed nondetectable (9 subjects) to low (1 subject) prechallenge serum RIA antibody titers and did not seroconvert. Fourth, it is also clear from our studies that some nonill subjects seroconvert (5 of 15 volunteers) and that some do not (10 of 15 volunteers), providing further evidence that disease-resistant individuals may comprise two subsets: one partially resistant with subclinical infection (nonill seroconverters), and the other completely resistant. This finding is supported by our previous observations (20, 21) that volunteers challenged with the Norwalk or Hawaii agents may in some cases develop histological lesions of the small intestine in the absence of clinical illness.

ACKNOWLEDGMENTS

We thank Frank Capozza for excellent technical assistance.

This work was supported by grant R806591010 from the U. S. Environmental Protection Agency, contract DAMD 17-76-C-6052 from the U. S. Army Medical Research and Development Command, and Public Health Service grants AMDD17537 and RR00888 from the National Institutes of Health.

LITERATURE CITED

1. **Albright, D. J., R. A. Whalen, and N. R. Blacklow.** 1974. Sensitivity of human foetal intestine to interferon. Nature (London) **247**:218–220.
2. **Appleton, H., M. Buckley, B. Thorn, J. Cotton, and S. Henderson.** 1977. Virus-like particles in winter vomiting disease. Lancet **i**:409–411.
3. **Blacklow, N. R., R. Dolin, D. S. Fedson, H. Dupont, R. S. Northrop, R. B. Hornick, and R. M. Chanock.** 1972. Acute infectious nonbacterial gastroenteritis: etiology and pathogenesis. Ann. Intern. Med. **76**:993–1008.
4. **Blacklow, N. R., P. Echeverria, and D. H. Smith.** 1976. Serological studies with reovirus-like enteritis agent. Infect. Immun. **13**:1563–1566.
5. **Cukor, G., M. K. Berry, and N. R. Blacklow.** 1978. Simplified radioimmunoassay for detection of human rotavirus in stools. J. Infect. Dis. **138**:906–910.
6. **Cukor, G., N. R. Blacklow, F. E. Capozza, Z. F. K. Panjvani, and F. Bednarek.** 1979. Persistence of antibodies to rotavirus in human milk. J. Clin. Microbiol. **9**:93–96.
7. **Dingle, J. H., G. F. Badger, A. E. Feller, R. G. Hodges, W. S. Jordan, and C. H. Rammelkamp.** 1953. A study of illness in a group of Cleveland families. I. Plan of study and certain general observations. Am. J. Hyg. **58**:16–30.
8. **Dolin, R., N. R. Blacklow, H. Dupont, S. Formal, R. F. Buscho, J. A. Kasel, R. P. Chames, R. Hornick, and R. M. Chanock.** 1971. Transmission of acute infectious nonbacterial gastroenteritis to volunteers by oral administration of stool filtrates. J. Infect. Dis. **123**: 307–312.
9. **Echeverria, P., N. R. Blacklow, and D. H. Smith.** 1976. Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agent in diarrhea in Boston children. Lancet **ii**:1113–1116.
10. **Echeverria, P., N. R. Blacklow, C. Zipkin, J. J. Vollet, J. A. Olson, H. L. Dupont, and J. H. Cross.** 1979. Etiology of gastroenteritis among Americans living in the Philippines. Am. J. Epidemiol. **109**:493–501.
11. **Echeverria, P., F. A. Hodge, N. R. Blacklow, J. L. Vollett, G. Cukor, H. L. Dupont, and J. H. Cross.** 1978. Travelers' diarrhea among United States marines in South Korea. Am. J. Epidemiol. **108**:68–73.
12. **Echeverria, P., G. Ramirez, N. R. Blacklow, T. Ksiazek, G. Cukor, and J. H. Cross.** 1979. Travelers' diarrhea among U. S. Army troops in South Korea. J. Infect. Dis. **139**:215–219.
13. **Greenberg, H. B., J. Valdesuso, R. H. Yolken, E. Gangarosa, W. Gary, R. G. Wyatt, T. Konno, H. Suzuki, R. M. Chanock, and A. Z. Kapikian.** 1979. Role of Norwalk virus in outbreaks of non-bacterial gastroenteritis. J. Infect. Dis. **139**:564–568.
14. **Greenberg, H. B., R. G. Wyatt, J. Valdesuso, A. R. Kalica, W. T. London, R. M. Chanock, and A. Z. Kapikian.** 1978. Solid-phase microtiter radioimmunoassay for detection of the Norwalk strain of acute nonbacterial, epidemic gastroenteritis virus and its antibodies. J. Med. Virol. **2**:97–108.
15. **Johnson, F. B., N. R. Blacklow, and M. D. Hoggan.** 1972. Immunological reactivity of antisera prepared against the sodium dodecyl sulfate-treated structural polypeptides of adenovirus-associated virus. J. Virol. **9**: 1017–1026.
16. **Kapikian, A. Z., H. B. Greenberg, W. L. Cline, A. R. Kalica, R. G. Wyatt, H. D. James, Jr., N. L. Lloyd, R. M. Chanock, R. W. Ryder, and H. W. Kim.** 1978. Prevalence of antibody to the Norwalk agent by a newly developed immune adherence hemagglutination assay. J. Med. Virol. **2**:281–294.
17. **Kapikian, A. Z., R. G. Wyatt, R. Dolin, T. S. Thornhill, A. R. Kalica, and R. M. Chanock.** 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J. Virol. **10**:1075–1081.
18. **Parrino, T. A., D. S. Schreiber, J. S. Trier, A. Z. Kapikian, and N. R. Blacklow.** 1977. Clinical immunity in acute gastroenteritis caused by Norwalk agent. N. Engl. J. Med. **297**:86–89.
19. **Purcell, R. H., D. C. Wong, H. J. Alter, and P. V. Holland.** 1973. Microtiter solid-phase radioimmunoassay for hepatitis B antigen. Appl. Microbiol. **26**:478–484.
20. **Schreiber, D. S., N. R. Blacklow, and J. S. Trier.** 1973. The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. N. Engl. J. Med. **288**:1318–1323.

21. **Schreiber, D. S., N. R. Blacklow, and J. S. Trier.** 1974. The small intestinal lesion induced by Hawaii agent acute infectious nonbacterial gastroenteritis. J. Infect. Dis. **129**:705–708.
22. **Thornhill, T. S., A. R. Kalica, R. G. Wyatt, A. Z. Kapikian, and R. M. Chanock.** 1975. Patterns of shedding of the Norwalk particle in stools during experimentally induced gastroenteritis in volunteers as determined by immune electron microscopy. J. Infect. Dis. **132**:28–34.
23. **Thornhill, T. S., R. G. Wyatt, A. R. Kalica, R. Dolin, R. M. Chanock, and A. Z. Kapikian.** 1977. Detection by immune electronmicroscopy of 26- to 27 nm virus-like particles associated with two family outbreaks of gastroenteritis. J. Infect. Dis. **135**:20–27.
24. **Widerlite, L., J. S. Trier, N. R. Blacklow, and D. S. Schreiber.** 1975. Structure of the gastric mucosa in acute infectious nonbacterial gastroenteritis. Gastroenterology **68**:425–430.
25. **Wyatt, R. G., R. Dolin, N. R. Blacklow, H. Dupont, R. Buscho, T. S. Thornhill, A. Z. Kapikian, and R. M. Chanock.** 1974. Comparison of three agents of acute infectious nonbacteial gastroenteritis by cross-challenge in volunteers. J. Infect. Dis. **129**:709–714.
26. **Yolken, R. H., R. G. Wyatt, G. Zissis, C. D. Brandt, W. J. Rodriguez, H. W. Kim, R. H. Parrott, J. J. Urrutia, L. Mata, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1978. Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. **299**:1156–1161.