JOURNAL OF CLINICAL MICROBIOLOGY, July 1997, p. 1909–1914 Vol. 35, No. 7
0095-1137/97/$04.00+0

Expression and Self-Assembly of Recombinant Capsid Protein from the Antigenically Distinct Hawaii Human Calicivirus

KIM Y. GREEN,[1]* ALBERT Z. KAPIKIAN,[1] JOSE VALDESUSO,[1] STANISLAV SOSNOVTSEV,[1] JOHN J. TREANOR,[2] AND JUDY F. LEW[1]

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland,[1] and University of Rochester Medical Center, Rochester, New York[2]

Received 11 November 1996/Returned for modification 13 January 1997/Accepted 11 April 1997

The Norwalk and Hawaii viruses are antigenically distinct members of the family *Caliciviridae* and are considered to be important etiologic agents of epidemic gastroenteritis, with most studies focusing on the role of Norwalk virus. To further investigate the importance of Hawaii virus, Hawaii virus-like particles (VLPs) were produced by expression of its capsid protein in the baculovirus system and these VLPs were used as the antigen in an enzyme-linked immunosorbent assay that was efficient in the detection of a serologic response to Hawaii virus. The ready availability of Hawaii VLPs should enable larger-scale epidemiological studies to further elucidate the importance of this agent.

Norwalk virus (NV) and NV-like viruses are small, noncultivatable viruses in the family *Caliciviridae* that are major etiologic agents of epidemic gastroenteritis in adults and older children (17). The NV-like human caliciviruses are comprised of at least four distinct serotypes represented by NV, Hawaii virus (HV), Snow Mountain virus (SMV), and Taunton virus, which have been defined by immune electron microscopy (IEM). HV was recovered from a family outbreak of gastroenteritis (29). Cross-challenge studies in adult volunteers, with NV and HV, as well as serologic studies by IEM, have established that these two viruses are antigenically distinct (5, 15, 32). In addition, such volunteer studies have generated important reference reagents for the NV and HV strains that have been used to establish the specificity of new recombinant-protein-based diagnostic assays in the absence of a cell culture system (8, 13, 14, 26, 30). Recent sequence comparisons of the RNA-dependent RNA polymerase (64% amino acid identity) and capsid-encoding (48% amino acid identity) regions of the genomes of NV and HV were consistent with the antigenic differences observed in earlier studies (22). In addition, by sequence analysis of various circulating strains, the human caliciviruses associated with epidemic gastroenteritis have been assigned to either genogroup I (NV related) or genogroup II (SMV or HV related) (reviewed in reference 20). A third genetically distinct group of human caliciviruses represented by the Sapporo and Manchester viruses has also been described (20). However, the full extent of the genetic and antigenic diversity among the human viruses in the family *Caliciviridae* has not been elucidated.

The epidemiology of NV is the most thoroughly investigated of the human caliciviruses (17). Early studies that established the association of NV with epidemic gastroenteritis employed diagnostic assays that relied on viral antigens present in stool specimens obtained from volunteers infected with NV (9, 10). The recent molecular characterization of NV (12) and other human calicivirus (4, 19, 25) genomes has facilitated the development of recombinant DNA-based diagnostic techniques (4, 13, 14, 24). Expression of the capsid protein of NV in the baculovirus system resulted in the production of recombinant NV (rNV) virus-like particles (VLPs) that were similar in efficiency, sensitivity, and specificity to native NV for the detection of serologic responses to NV by both enzyme-linked immunosorbent assay (ELISA) and IEM (8, 13, 26, 30). The availability of an unlimited supply of recombinant antigen for the detection of serological responses to NV has enabled recent large-scale epidemiologic studies that have confirmed the role of NV in gastrointestinal illness in various settings (7, 11, 28). The purpose of our present study was to develop HV-specific diagnostic assays to elucidate the role of HV or HV-related viruses in gastrointestinal disease.

A cDNA copy of ORF2, encoding the capsid protein of HV (HCV/Hawaii/71/U.S.), was generated in a first-round reverse transcription-PCR using a viral RNA template extracted from the stool specimen of an adult volunteer as previously described (22). A second PCR that utilized primers 5′GGATCC GTTTTGTGAATGAAGATG3′ (the 5′ end of ORF2 and a *Bam*HI restriction enzyme site) and 5′GCGGCCGCATTAC TGCACTCTTCT3′ (complementary to the 3′ end of ORF2 with a *Not*I restriction enzyme site) was used to generate an approximately 1,600-bp fragment that was cloned into plasmid pCRII by using the TA system (Invitrogen). The resulting plasmid (designated pCRII-HVORF2) was digested with *Bam*HI and *Not*I, and the ORF2 DNA fragment was purified from an agarose gel and ligated into the compatible sites of the pVL1393 transfer plasmid (Invitrogen) downstream from the baculovirus polyhedrin promoter. Plasmid pVL1393-HVORF2 was cotransfected with linearized baculovirus DNA (BaculoGold; Pharmingen) into Sf9 cells. Recombinant viruses were amplified and screened for the production of VLPs by direct electron microscopy, and selected positive recombinant viruses were plaque purified twice. For production of recombinant HV (rHV) VLPs, recombinant baculoviruses were inoculated onto Sf9 monolayers at a multiplicity of infection of 5 to 10 and particles were purified in 10 to 50% sucrose gradients from the culture fluid of infected cells. VLPs obtained by expression of the HV capsid protein in the baculovirus system were morphologically similar to previously described NV VLPs (13) (Fig. 1). By negative-stain electron microscopy, numerous approximately 27- to 35-nm particles were observed, some of which appeared empty and others of which appeared full. Subviral-size (approximately 20-nm) particles (not shown) were also

* Corresponding author. Mailing address: National Institutes of Health, Building 7, Room 129, 9000 Rockville Pike, Bethesda, MD 20892. Phone: (301) 496-5811. Fax: (301) 496-8312. E-mail: kgreen@atlas.niaid.nih.gov.

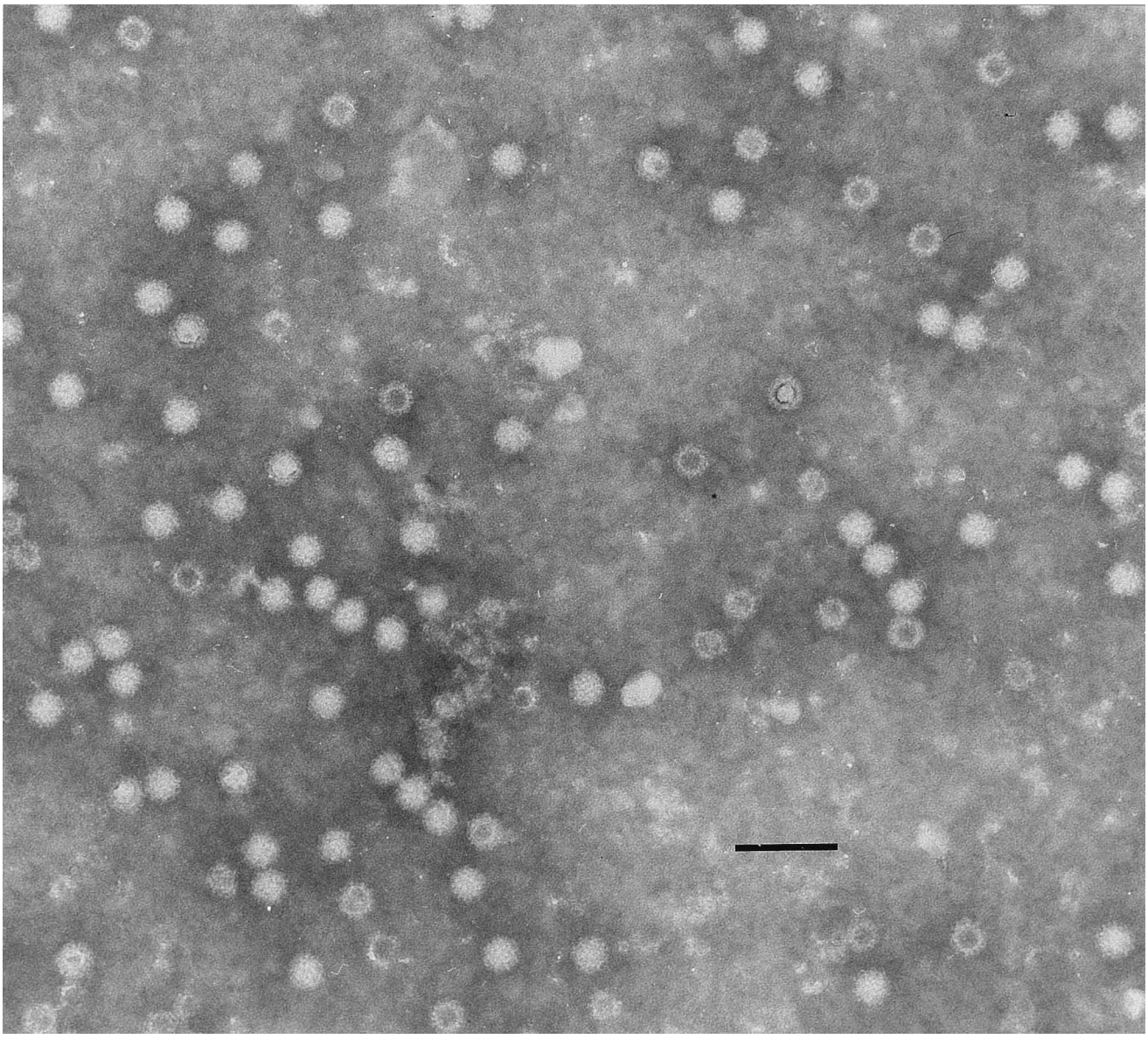

FIG. 1. Purified HV-like particles expressed in the baculovirus system and visualized by negative staining with 3% phosphotungstic acid, pH 7.2. Bar, 100 nm.

observed variably. Hyperimmune sera specific for the rHV VLPs were generated by immunization of guinea pigs with purified VLPs by using standard protocols, and these sera reacted with the rHV protein by ELISA and Western blotting (data not shown).

The rHV VLPs were used as an antigen in the development of an ELISA that was performed in a format similar to that previously described by Jiang et al. for rNV (13). Briefly, purified rHV VLPs were diluted in phosphate-buffered saline and adsorbed to the wells (1 μg/well) of a polyvinyl microtiter plate by incubation overnight at 4°C. Following blocking of nonspecific binding sites on the plate with 5% BLOTTO (nonfat dry milk in phosphate-buffered saline), serial twofold dilutions of each serum sample were made at a starting dilution of 1:50. The binding of antibodies to the rHV antigen was detected with peroxidase-conjugated goat anti-human antibody (Cappel) and 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS) substrate. Titers were reported as the reciprocal of the highest dilution that met the definition for a positive reaction with the rHV antigen (an optical density at 405 nm of $\geq$0.200). A significant serologic response was defined as a fourfold or greater increase in the titer of the antibody to the recombinant antigen between preinfection or acute-phase and postinfection sera. Paired sera from an individual were tested simultaneously in duplicate wells. A rNV ELISA (13) was used to detect antibodies to NV by using reagents kindly provided by Mary Estes, Baylor College of Medicine, Houston, Tex.

Paired preinfection or acute-phase and postinfection sera from selected HV, NV, or NV-related virus studies were evaluated for antibodies to rHV to establish the specificity of the rHV ELISA as follows: (i) five adult volunteers who had developed gastrointestinal illness following oral administration of

TABLE 1. Serologic responses of adult volunteers orally challenged with HV to rHV or rNV antigen as measured by ELISA[a]

Individual	Serum sample	Reciprocal titer of antibody to:	
		rHV	rNV[a]
1	Prechallenge	800	3,200
	Postchallenge	**51,200**[b]	6,400
2	Prechallenge	800	800
	Postchallenge	**3,200**	800
3	Prechallenge	800	3,200
	Postchallenge	**25,600**	3,200
4	Prechallenge	<400	1,600
	Postchallenge	**1,600**	800
5	Prechallenge	50	400
	Postchallenge	**25,600**	400

[a] rNV data are from Green et al. (8). Volunteers challenged with HV stool filtrate or HV passaged in volunteers also failed to develop a serologic response to native NV by IEM (5, 15).

[b] Values in boldface indicate serologic response as defined in the text.

HV (32) (Table 1); (ii) three chimpanzees that were challenged with NV by the alimentary route and developed antibody responses to native NV by IEM and to rNV as previously reported (8, 33), (no. 1 to 3 in Table 2); (iii) two adult volunteers challenged orally with the Montgomery County agent, a virus antigenically related to NV (15, 29) (no. 4 and 5 in Table 2); and (iv) a member of the U.S. military infected under natural conditions with the Desert Shield strain (HCV-DSV395/90/Saudi Arabia), a virus belonging to NV-related genogroup I (21) (no. 6 in Table 2). Thus, the rHV ELISA detected evidence of infection with native HV efficiently but did not detect evidence of infection with NV or NV-related virus. These findings were consistent with previous reports that the rNV ELISA did not efficiently detect serologic evidence of infection in volunteers challenged with HV (8, 30).

The specificity of the rHV ELISA was also evaluated by using paired sera from adult volunteers who underwent oral challenge with SMV strain HCV/SMV/76/U.S., a reference strain that has been identified as antigenically distinct from NV and HV by IEM and belonging to genogroup II (as does HV) by sequence analysis of the polymerase region (6, 31). The polymerase region of the SMV genome shares 59 and 92% amino acid identity with NV and HV, respectively (22, 31). Four of five adult volunteers challenged with SMV and previously shown to develop a serologic response to this virus in a blocking antibody immunoassay (BAI) (26) failed to develop a serologic response to either rHV or rNV by ELISA (no. 7 to 10 in Table 2). The characteristic inefficiency of the rHV ELISA in detecting significant antibody responses to SMV was consistent with the identification of HV and SMV as distinct serotypes by IEM and suggests that a strong genetic relatedness in the polymerase region does not necessarily reflect antigenic relatedness in the capsid protein. However, one individual (no. 11 in Table 2) infected with SMV developed a serologic response to both rHV and rNV. In addition, a pediatric patient in a Canadian hospital undergoing natural infection with the Toronto 19 strain (HCV/TV19/90/CAN), belonging to genogroup II (23), showed a serologic response to both rNV and rHV (no. 12 in Table 2). The cross-reactive responses to both NV and HV observed in individuals 11 and 12 in Table 2 are of interest because each of them underwent infection with viruses that have been genetically characterized as belonging to genogroup II. It is not known whether these cross-reactive responses reflect evidence of prior calicivirus infection in these individuals or the current infection. These occasional broad antibody responses indicate that the analysis of paired sera from individuals undergoing natural infection with circulating viruses must be interpreted with caution in regard to the antigenic specificity of the infecting strain.

The relationship of the rHV was examined with paired sera derived from three other well-characterized gastroenteritis outbreaks that were not associated with NV in previous studies. Four adult volunteers who were orally administered the Wollan or W agent (16), which was associated with a gastroenteritis outbreak in an English boarding school (3), and who developed illness following the challenge were examined for serologic responses to the rHV. Two of the four developed a serologic response to rHV by ELISA, whereas none of them developed a response to rNV or to NV in each of the three tested by IEM (no. 13 to 16 in Table 2) (16). Paired sera from four individuals involved in the Henryton II and Morgantown, W.Va., outbreaks (1, 2) were examined (no. 17 to 20 in Table 2). An agent(s) associated with the Morgantown, W.Va., outbreak could not be identified in this serologic analysis. However, it is possible that the Wollan and Henryton II outbreaks were caused by a human calicivirus. Further characterization of these viruses is needed to address this possibility.

Serologic responses to rHV were examined in 223 individuals involved in 23 outbreaks of nonbacterial gastroenteritis that were not associated with NV in a previous study (27). Twenty-three (10.3%) of the individuals examined showed a fourfold or greater increase in antibody to HV (Table 3), and as previously reported (27), only 3 (1.4%) of 207 developed a serologic response to NV in the NV BAI ELISA. Two of the outbreaks met the definition for an HV outbreak established previously, i.e., 50% or more of the affected individuals showed a serologic response to the assayed agent (9, 18). Furthermore, 14 (61%) of these 23 outbreaks included one or more individuals who developed a serologic response to rHV, suggesting that a human calicivirus distinct from both NV and HV may have been the primary cause of illness in these outbreaks. As assays become available for other antigenically distinct human caliciviruses, the identification of caliciviruses as etiologic agents of epidemic gastroenteritis will undoubtedly increase.

NV has been established as a cause of nonbacterial epidemic gastroenteritis, but few studies have examined the role of HV. Expression of ORF2 of HV in the baculovirus system resulted in the production of VLPs that were antigenically indistinguishable from native HV, and these VLPs were used in the development of an ELISA for the detection of HV-specific antibodies. Moreover, a dissociation of serologic responses to NV and HV was observed when paired sera from challenge studies were analyzed, consistent with the major antigenic differences between these two viruses that had been established in cross-challenge studies and by IEM. The extent of antigenic variation among circulating human calicivirus strains associated with epidemic gastroenteritis is an important characteristic of these viruses that is relevant for an understanding of their epidemiology and natural history. Several classification systems for human calicivirus serotypes have been proposed, but the limited quantities of reference stool specimens containing the native virus have impeded the development of standardized reagents. The availability of recombinant VLPs for major reference strains such as NV and HV, the first two distinct serotypes described, should facilitate the development of such reagents.

TABLE 2. Comparison of serologic responses to rHV and rNV in adults or chimpanzees infected with various agents of gastroenteritis

Human or chimpanzee	Source of serum	Serum sample	Reciprocal titer of antibody to:	
			rHV	rNV[a]
1	NV challenge (chimpanzee 14G)	Prechallenge	400	1,600
		Postchallenge	200	**≥102,400**[b]
2	NV challenge (chimpanzee 15G)	Prechallenge	400	400
		Postchallenge	200	**409,600**
3	NV challenge (chimpanzee 16G)	Prechallenge	<50	<50
		Postchallenge	<50	**6,400**
4	Montgomery County agent challenge	Prechallenge	100	400
		Postchallenge	100	**3,200**
5	Montgomery County agent challenge	Prechallenge	200	1,600
		Postchallenge	200	800
6	Desert Shield virus (DSV395), U.S. military	Preinfection	3,200	400
		Postinfection	1,600	**6,400**
7	SMV challenge (86-23)	Prechallenge	6,400	400
		Postchallenge	12,800	400
8	SMV challenge (86-24)	Prechallenge	1,600	1,600
		Postchallenge	3,200	1,600
9	SMV challenge (86-26)	Prechallenge	400	400
		Postchallenge	400	200
10	SMV challenge (86-27)	Prechallenge	800	800
		Postchallenge	1,600	1,600
11	SMV challenge (86-25)	Prechallenge	400	≤50
		Postchallenge	**12,800**	**200**
12	Toronto virus (TV19) nosocomial outbreak	Acute phase	<50	<50
		Postinfection	**800**	**200**
13	Wollan agent challenge	Prechallenge	6,400	400
		Postchallenge	3,200	200
14	Wollan agent challenge	Prechallenge	1,600	1,600
		Postchallenge	**6,400**	1,600
15	Wollan agent challenge	Prechallenge	400	200
		Postchallenge	800	200
16	Wollan agent challenge	Prechallenge	400	50
		Postchallenge	**1,600**	100
17	Henryton II outbreak	Acute phase	3,200	3,200
		Postinfection	3,200	3,200
18	Henryton II outbreak	Acute phase	3,200	3,200
		Postinfection	**12,800**	3,200
19	Morgantown, W. Va., outbreak	Acute phase	400	800
		Postinfection	400	800
20	Morgantown, W. Va., outbreak	Acute phase	400	3,200
		Postinfection	400	1,600

[a] Data for rNV titers are from reference 8 (chimpanzees 1 to 3 and humans 4, 5, 13 to 15, and 17 to 20) or references 21 and 23 (humans 6 and 12, respectively).
[b] Data in boldface indicate a serologic response as defined in the text.

TABLE 3. Summary of serologic responses to rHV in individuals involved in 23 outbreaks of gastroenteritis not associated with NV

Location of outbreak	Setting	Date of outbreak (mo/yr)	No. of individuals with ≥4-fold response to indicated virus (assay)/no. (%) tested	
			HV (rHV ELISA)	NV (BAI)[a]
At sea	Cruise ship	9/76	0/4	0/4
New York	Hospital	11/76	5/18 (28)	0/19
North Carolina	College	3/77	1/7 (14)	0/8
Wyoming	Recreational area	7/77	0/7	1/7 (14)
Vermont	Nursing home	1/78	**3/6 (50)**[b]	1/3 (33)
Florida	Nursing home	2/78	1/25 (4)	0/25
Missouri	Community	5/78	1/6 (17)	0/6
At sea	Cruise ship	1/79	1/8 (13)	0/10
At sea	Cruise ship	2/79	2/10 (20)	0/12
Florida	Hospital	5/79	0/15	0/5
Alaska	Family	6/79	1/13 (8)	0/13
Virginia	Swimming pool	7/79	1/11 (9)	0/7
Hawaii	Restaurant	1/80	1/14 (7)	0/9
Florida	Nursing home	3/80	1/6 (17)	0/4
California	Restaurant	5/80	0/9	0/8
Illinois	College	5/80	**3/5 (60)**	1/7 (14)
Illinois	Restaurant	5/80	1/12 (8)	0/12
Washington	Hospital	5/80	0/7	0/7
Colorado	Nursing home	6/80	0/1	0/6
New York	Camp	7/80	1/14 (7)	0/4
Florida	Hospital	7/80	0/9	0/10
New York	Restaurant	4/82	0/7	0/7
Wisconsin	Hospital	6/82	0/9	0/14
Total			23/223 (10.3)	3/207 (1.4)

[a] Data are from Midthun et al. (27), who used the NV BAI, an assay that employed NV antigen in stool and is similar in efficiency and specificity to the rNV ELISA (8).

[b] Data in boldface indicate an outbreak in which ≥50% of the individuals developed a serologic response to the assayed virus.

We thank Mariam Watson, Ali Salimi, and Ernest Williams for excellent technical assistance. We extend our appreciation to Robert M. Chanock, Chief of the Laboratory of Infectious Diseases, for his support.

REFERENCES

1. **Anonymous.** 1971. Gastroenteritis—Morgantown, West Virginia. Morbid. Mortal. Weekly Rep. **20:**223–224.
2. **Buscho, R. F., R. G. Wyatt, R. Dolin, N. R. Blacklow, H. L. DuPont, and R. M. Chanock.** 1973. Recurrent institutional outbreaks of acute infectious nonbacterial gastroenteritis: epidemiology and etiology. Am. J. Epidemiol. **98:**192–198.
3. **Clarke, S. D. R., G. T. Cook, S. I. Egglestone, T. S. Hall, D. L. Miller, S. E. Reed, D. Rubenstein, A. J. Smith, and D. A. Tyrell.** 1972. A virus from epidemic vomiting disease. Br. Med. J. **3:**86–89.
4. **Dingle, K. E., P. R. Lambden, E. O. Caul, and I. N. Clarke.** 1995. Human enteric Caliciviridae: the complete genome sequence and expression of virus-like particles from a genetic II small round structured virus. J. Gen. Virol. **76:**2349–2355.
5. **Dolin, R., A. G. Levy, R. G. Wyatt, T. S. Thornhill, J. D., and J. D. Gardner.** 1975. Viral gastroenteritis induced by the Hawaii agent: jejunal histopathology and seroresponse. Am. J. Med. **59:**761–769.
6. **Dolin, R., R. C. Reichman, K. D. Roessner, T. S. Tralka, R. T. Schooley, W. Gary, and D. Morens.** 1982. Detection by immune electron microscopy of the Snow Mountain agent of acute viral gastroenteritis. J. Infect. Dis. **146:**184–189.
7. **Gray, J. J., X. Jiang, P. Morgan-Capner, U. Desselberger, and M. K. Estes.** 1993. Prevalence of antibodies to Norwalk virus in England: detection by enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. J. Clin. Microbiol. **31:**1022–1025.
8. **Green, K. Y., J. F. Lew, X. Jiang, A. Z. Kapikian, and M. K. Estes.** 1993. Comparison of the reactivities of baculovirus-expressed recombinant Norwalk virus capsid antigen with those of the native Norwalk virus antigen in serologic assays and some epidemiologic observations. J. Clin. Microbiol. **31:**2185–2191.
9. **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 nonbacterial gastroenteritis. J. Infect. Dis. **139:**564–568.
10. **Greenberg, H. B., R. G. Wyatt, A. R. Kalica, R. H. Yolken, R. Black, A. Z. Kapikian, and R. M. Chanock.** 1981. New insights in viral gastroenteritis. Perspect. Virol. **11:**163–187.
11. **Hyams, K. C., J. D. Malone, A. Z. Kapikian, M. K. Estes, X. Jiang, A. L. Bourgeios, S. Paparello, R. D. Hawkins, and K. Y. Green.** 1993. Norwalk virus infection among Desert Storm troops. J. Infect. Dis. **167:**986–987.
12. **Jiang, X., D. Y. Graham, K. Wang, and M. K. Estes.** 1990. Norwalk virus genome: cloning and characterization. Science **250:**1580–1583.
13. **Jiang, X., M. Wang, D. Y. Graham, and M. K. Estes.** 1992. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J. Virol. **66:**6527–6532.
14. **Jiang, X., D. O. Matson, G. M. Ruiz-Palacios, J. Hu, J. Treanor, and L. K. Pickering.** 1995. Expression, self-assembly, and antigenicity of a Snow Mountain agent-like calicivirus capsid protein. J. Clin. Microbiol. **33:**1452–1455.
15. **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.
16. **Kapikian, A. Z., S. M. Feinstone, R. H. Purcell, R. G. Wyatt, T. S. Thornhill, A. R. Kalica, and R. M. Chanock.** 1975. Detection and identification by immune electron microscopy of fastidious agents associated with respiratory illness, acute nonbacterial gastroenteritis, and hepatitis A. Perspect. Virol. **9:**9–47.
17. **Kapikian, A. Z., M. K. Estes, and R. M. Chanock.** 1996. Norwalk group of viruses, p. 783–810. *In* B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), Virology. Lippincott-Raven, Philadelphia, Pa.
18. **Kaplan, J. E., G. W. Gary, R. C. Baron, N. Singh, L. B. Schonberger, R. Feldman, and H. B. Greenberg.** 1982. Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann. Intern. Med. **96:**756–761.
19. **Lambden, P. R., E. O. Caul, C. R. Ashley, and I. N. Clark.** 1993. Sequence and genome organization of a human small round-structured (Norwalk-like) virus. Science **259:**516–519.
20. **Lambden, P. R., and I. N. Clark.** 1995. Genome organization in the Caliciviridae. Trends Microbiol. **3:**261–265.
21. **Lew, J. F., A. Z. Kapikian, X. Jiang, M. Estes, and K. Y. Green.** 1994. Molecular characterization and expression of the capsid protein of a Norwalk-like virus recovered from a Desert Shield troop with gastroenteritis. Virology **200:**319–325.
22. **Lew, J. F., A. Z. Kapikian, J. Valdesuso, and K. Y. Green.** 1994. Molecular characterization of Hawaii virus and other Norwalk-like viruses: evidence for

genetic polymorphism among human caliciviruses. J. Infect. Dis. **170:**535–542.
23. **Lew, J. F., M. Petric, A. Z. Kapikian, X. Jiang, M. Estes, and K. Y. Green.** 1994. Identification of minireovirus as a Norwalk-like virus in pediatric patients with gastroenteritis. J. Virol. **68:**3391–3396.
24. **Liete, J. P. G., T. Ando, J. S. Noel, B. Jiang, C. Humphrey, J. F. Lew, K. Y. Green, R. I. Glass, and S. S. Monroe.** 1996. Characterization of Toronto virus capsid protein expressed in baculovirus. Arch. Virol. **141:**865–875.
25. **Liu, B. L., I. N. Clarke, E. O. Caul, and P. R. Lambden.** 1995. Human enteric caliciviruses have a unique genome structure and are distinct from the Norwalk-like viruses. Arch. Virol. **140:**1345–1356.
26. **Madore, H. P., J. J. Treanor, R. Buja, and R. Dolin.** 1990. Antigenic relatedness among the Norwalk-like agents by serum antibody rises. J. Med. Virol. **32:**96–101.
27. **Midthun, K., H. B. Greenberg, J. B. Kurtz, G. W. Gary, F. Y. Lin, and A. Z. Kapikian.** 1993. Characterization and seroepidemiology of a type 5 astrovirus associated with an outbreak of gastroenteritis in Marin County, California. J. Clin. Microbiol. **31:**955–962.
28. **Numata, K., S. Nakata, X. Jiang, M. K. Estes, and S. Chiba.** 1994. Epidemiological study of Norwalk virus infections in Japan and Southeast Asia by enzyme-linked immunosorbent assays with Norwalk virus capsid protein produced by the baculovirus expression system. J. Clin. Microbiol. **32:**121–126.
29. **Thornhill, T. S., R. G. Wyatt, A. R. Kalica, R. Dolin, R. M. Chanock, and A. Z. Kapikian.** 1977. Detection by immune electron microscopy of 26-27 nm virus-like particles associated with two family outbreaks of gastroenteritis. J. Infect. Dis. **135:**20–27.
30. **Treanor, J. J., X. Jiang, H. P. Madore, and M. K. Estes.** 1993. Subclass-specific serum antibody responses to recombinant Norwalk virus capsid antigen (rNV) in adults infected with Norwalk, Snow Mountain, or Hawaii virus. J. Clin. Microbiol. **31:**1630–1634.
31. **Wang, J., X. Jiang, P. Madore, J. Gray, U. Desselberger, T. Ando, Y. Seto, I. Oishi, J. Lew, K. Green, and M. Estes.** 1994. Sequence diversity of small, round-structured viruses in the Norwalk virus group. J. Virol. **68:**5982–5990.
32. **Wyatt, R. G., R. Dolin, N. R. Blacklow, H. L. DuPont, R. F. Buscho, T. S. Thornhill, A. Z. Kapikian, and R. M. Chanock.** 1974. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. J. Infect. Dis. **129:**709–714.
33. **Wyatt, R. G., H. B. Greenberg, D. W. Dalgard, W. P. Allen, D. L. Sly, T. S. Thornhill, R. M. Chanock, and A. Z. Kapikian.** 1978. Experimental infection of chimpanzees with the Norwalk agent of epidemic viral gastroenteritis. J. Med. Virol. **2:**89–96.