Studies of the Infectivity of Norwalk and Norwalk-Like Viruses

Project Scope

Norwalk virus (NV) is representative of a large group of related enteric viruses (human caliciviruses) that are the predominant cause of acute gastroenteritis outbreaks in the United States. They are typically transmitted through ingestion of feces-contaminated water and food, exposure to aerosolized feces and vomitus, contaminated surfaces, and through direct person-to-person contact. In previous taxonomic classifications, Norwalk-like viruses (NLVs) were one of four genera of the family Caliciviridae and included two genotypes. Genotype I of the NLVs included the prototype NV and related viruses and genotype II included Snow Mountain Virus (SMV), both of which were the focus of this research project.[1]

Genetic traits that may increase or decrease host susceptibility to enteric virus infection are being increasingly recognized. Host immunity to NV and NLVs, however, remains poorly characterized. The overall objective of this research project was to improve understanding of the risks associated with exposure to NV and SMV, as a function of dose and host susceptibility factors.

The research grant constitutes the second phase of a two-part strategy to define the dose-infectivity relationship of NV and a parallel study of the infectivity of SMV. The first phase was a pilot NV dose-ranging study that was supported by EPA and was continued in this phase of research. The specific objectives of this research were to:

Grant Title and Principal Investigator

Studies of the Infectivity of Norwalk and Norwalk-Like Viruses (EPA Grant #R826139)

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Key Findings and Implications

Analytical Accomplishments:

- Norwalk virus (NV) was found to be highly infectious, with clinical infection observed at <1 PCR detectable units. Although the dose-response analyses were not finalized, preliminary models indicated that Snow Mountain Virus (SMV) is not as infectious as NV.
- There was good correlation between viral shedding and seroconversion (≥ 4-fold increase in NV- and SMV-specific immunoglobulin G).
- Salivary IgG response to NV mirrors serum IgG response; thus saliva sampling may be viable, less invasive, alternative to collecting and testing serum or stool samples.

Implications of Research:

- Older adults were generally more susceptible to NV and SMV infection, indicating they should be considered a susceptible sub-population.
- The low infectious dose of NV, in conjunction with mild or asymptomatic infection and prolonged shedding facilitates its secondary transmission which has strong potential health consequences to immuno-compromised populations, including infants and the elderly.
- Provides support for increased consideration and research into host susceptibility in microbial risk assessments.

Publications include 8 peer reviewed articles and 26 conference/workshop presentations.

Project Period: January 1998 to January 2001 (extended through January 2003)

[1] While this research was being conducted, the genomes of NV and several related NLVs were fully characterized. As a result, the genetically diverse caliciviruses are currently classified as belonging to one of two distinct genera of Caliciviridae, Norovirus, and Sapovirus. However, the former classification of NV and NLVs is retained in this summary report.

Relevance to ORD's *Drinking Water Research Multi-Year Plan* (2003 Edition)

This project contributes directly to two of three Long-term Goals for drinking water research: (1) by 2010, develop scientifically sound data and approaches to assess and manage risks to human health posed by exposure to regulated waterborne pathogens and chemicals, including those addressed by the Arsenic, M/DPB, and Six-Year Review Rules; and (3) by 2009, provide data, tools, and technologies to support management decisions by the Office of Water, state, local authorities, and utilities to protect source water and the quality of water in the distribution system.

This research has several implications for improving microbial risk assessment, especially as related to the effectiveness of drinking water treatment methods (such as those required under EPA's Surface Water Treatment Rule) and exposure to drinking water contaminated with enteric viruses. It provides support for increased consideration of and research into host susceptibility in microbial risk assessments. This research has demonstrated that susceptibility to Norwalk virus is multifactorial and influenced by both acquired immunity and specific genetic traits.

1. Identify the infectious dose range (the minimum amount of an agent that must be consumed to give rise to a clinical infection) of NV and SMV in adult human volunteers with various levels of preexisting antibodies;
2. Determine host characteristics that affect susceptibility to infection; and
3. Evaluate the fit of several mathematical models of dose-infectivity to the collected data.

To carry out these objectives, the researchers conducted two major human feeding studies using healthy adult male and female volunteers aged 18 to 51 years selected from the University of North Carolina and the surrounding area. The first study was continued from phase I and included 76 volunteers challenged with NV. The second study included 15 volunteers challenged with SMV. In both studies, eligible volunteers were admitted to UNC's General Clinical Research Center for oral dosing and stayed five days for periodic monitoring for gastrointestinal and related symptoms, including diarrhea, vomiting, abdominal pain, fatigue, and chills. The researchers prepared inoculums for both studies using reverse transcriptase-polymerase chain reaction (RT-PCR) and electron microscopy. In the NV study the inoculum was estimated to contain approximately $1x10^8$ "PCR detectable units" (PDU; used to measure human caliciviruses in both clinical and environmental samples) of NV per ml. A total of eight different doses using this inoculum, ranging from $1x10^{-6}$ µL to 100 µL, were used in the NV study. The inoculum for the SMV study was estimated to contain about $1x10^6$ PDU of SMV per mL and three doses ranging from 0.01 µL to 100 µL were used. Stool, serum, and saliva specimens were collected before dosing, for up to seven consecutive days after dosing, and at (approximately) Days 8, 14, and 21 postchallenge. In addition, all vomitus passed by the subjects during the first seven days after dosing were collected for subsequent analysis. All sera from the NV-dosed volunteers were tested for anti-NV immunoglobulin G (IgG) by enzyme immunoassay (EIA) using recombinant NV antigen. Sera from the SMV-challenged volunteers were tested for anti-SMV IgG by EIA using recombinant SMV antigen. The salivary antibody response using IgG and IgA antibody titers was also evaluated using EIA in both studies. Infection was defined as viral shedding (detection of NV RNA or SMV RNA in stool by RT-PCR) or seroconversion (4-fold or greater rise in NV or SMV-specific IgG). Collected vomitus specimens were also tested for NV RNA or SMV RNA.

Previous research has shown that genetic polymorphisms at the α1,2 fucosyltransferase (*FUT2*) gene locus that affects the production or secretion of ABH histo-blood group and related (H) antigens from the digestive tract and mucosal tissues are associated with varying degrees of susceptibility to NV and other pathogens. Because H antigens are important receptors for NV docking and entry, individuals homozygous for *FUT2* polymorphisms that prevent H antigen expression may be resistant to infection. The investigators measured H antigen expression was measured using immunological methods for all subjects in the NV study. Lastly, to characterize the cellular immune (T-cell) response in NV-challenged

volunteers, peripheral blood mononuclear cells (PBMCs) from whole blood samples were collected and analyzed.

Project Results and Implications

Norwalk Virus Study: The researchers demonstrated that NV is highly infectious, with clinical infection observed at <1 PDU. A total of 33 percent of volunteers became infected with NV. Infected subjects were generally older than uninfected subjects and were twice as likely to have NV-specific IgG in their baseline serum specimen. Thus, the presence of anti-NV serum IgG was not protective against infection. Most infections (72 percent) were associated with mild illness (nausea and vomiting were most common) with the remainder being asymptomatic. Symptoms typically were first observed on the second day after inoculation and resolved within 24-48 hours of onset. Most infected subjects (70 percent) shed NV in their stool by Day 2 post-challenge. At the first follow-up visit (Day 8 postchallenge), 85 percent of the infected subjects continued to shed NV in stool, compared to 30 percent at Day 14 postchallenge. NV virus RNA could still be detected in stool specimens collected from 3 subjects at the final follow-up visit (18-23 days postchallenge). There was correlation between viral shedding and seroconversion ($\geq$ 4-fold increase in NV-specific IgG).

There were also strong correlations between IgG seroconversion and $\geq$ 4-fold increases in NV-specific salivary IgA and IgG antibody titers. The researchers concluded that salivary IgG response mirrors serum IgG response, and provides an attractive alternative to collecting and testing serum or stool samples. Preliminary analyses of the phenotype data for the NV study subjects indicated that 54 percent were secretor positive (Se+) and presumably susceptible to infection. At least 17 subjects appeared to be secretor negative (Se-) and may have been resistant to infection, and further analysis was planned. T-cell responses were not evident in Se- volunteers' PBMCs at Day 8 postchallenge, which is consistent with the hypothesis that these individuals were resistant to NV infection. However, further analyses of the T-cell response in NV subjects were planned.

Snow Mountain Virus Study: A total of 60 percent of volunteers became infected with SMV, with infected subjects being generally older than uninfected subjects. Sixty-seven percent of the subjects developing infections had SMV-specific IgG in their baseline serum compared to 83 percent of the uninfected subjects. Seventy-eight percent of the infected subjects reported typical gastroenteritis symptoms with two having asymptomatic infections. Further analyses of the symptoms, onset and duration times were planned. All infected subjects with SMV RNA detected in their stool by RT-PCR also seroconverted. Virus shedding was detected as early as Day 2 postchallenge, and as late as Day 8 postchallenge. Further analyses of the shedding patterns were planned as well as the analysis of salivary immune response data. As in the NV study, subjects were tested for their H antigen secretor status, with preliminary results indicating that all the subjects who became infected were secretor-positive. This suggests that secretor status may be a marker for susceptibility to SMV infection as well as for NV infection.

Dose-Infectivity Model Development: Initial analysis of the dose-infectivity relationship for NV using a simple beta-Poisson model revealed that the model could not adequately account for variation in infectivity among the subjects. Thus, the researchers planned to develop and use other models that account for the presence of anti-NV serum IgG at baseline and secretor phenotype status of the volunteer. The relationship between NV dose and illness will be the subject of future research. Although the dose-response analyses were not finalized in the SMV study, preliminary models indicated that it is not as infectious as NV.

Summary: The completed, ongoing, and planned research on the infectivity of NV and NLVs has several implications for the broader field of microbial risk assessment. It provides support for the consideration of host susceptibility and demonstrates how to collect data on individuals and populations that provide

insight into host susceptibility. For example, this research confirmed that susceptibility to NV is multifactorial and influenced by both acquired immunity and genetic traits, such as whether exposed persons are secretor-positive. In this research, about 45 percent of the secretor-positives appeared to have acquired mucosal (salivary) immunity to NV and did not develop NV infection after exposure. Further, the combination of low infectious dose, mild or asymptomatic infection, and prolonged shedding may facilitate secondary transmission of NV and NLVs, and this finding has important implications for protecting immuno-compromised populations, including infants and the elderly. With increased understanding of susceptibility to human calicivirus infection, such factors may be included in microbial risk assessment models to more accurately estimate risks of NV and NLV infection and/or illness in different populations.

Investigators

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For More Information

NCER Project Abstract and Reports:

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/192/report/0

Peer Reviewed Publications

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Harrington, P.R., Yount, B., Johnston, R.E., Davis, N., Moe C. and Baric, R.S. 2002. Systemic, mucosal and heterotypic immune induction in mice inoculated with venezuelan equine encephalitis replicons expressing Norwalk virus-like particles. Journal of Virology 76(2):730-742.

Greene, S.R., Moe, C.L., Jaykus, L., Cronin, M., Grosso, L. and van Aarle, P. 2003. Evaluation of the nuclisens basic kit assay for detection of Norwalk virus RNA in stool specimens. Journal of Virological Methods 108: 123-131.

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Lindesmith, L, Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindblad, J., Stewart, P., LePendu J., and Baric, R. 2003. Determinants of susceptibility and protective immunity to Norwalk virus infection in humans. Nature Medicine 9(5):548-553.

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