HHS Public Access

Author manuscript

J Pediatric Infect Dis Soc. Author manuscript; available in PMC 2022 October 28.

Published in final edited form as:

J Pediatric Infect Dis Soc. 2022 October 25; 11(10): 459–462. doi:10.1093/jpids/piac063.

Preexisting Heterotypic Ligand-blocking Antibody Does Not Protect Against Genogroup II Norovirus Episodes in Young Children

Sylvia Becker-Dreps[1,2], **Paul D. Brewer-Jensen**[2], **Fredman González**[3], **Yaoska Reyes**[3,4], **Michael L. Mallory**[2], **Lester Gutiérrez**[3,4], **Nadja A. Vielot**[1], **Marta Diez-Valcarce**[4,5], **Jan Vinjé**[4], **Ralph S. Baric**[2], **Lisa C. Lindesmith**[2,a], **Filemon Bucardo**[3,a]

[1]Department of Family Medicine, University of North Carolina at Chapel Hill (UNC-Chapel Hill), Chapel Hill, North Carolina, USA,

[2]Department of Epidemiology, UNC-Chapel Hill, Chapel Hill, North Carolina, USA,

[3]Department of Microbiology, National Autonomous University of Nicaragua, León (UNAN-León), León, Nicaragua,

[4]Division of Viral Diseases, U.S. Centers for Disease Control and Prevention, Atlanta, Georgia, USA,

[5]Department of Epidemiology, Emory University Rollins School of Public Health, Atlanta, Georgia, USA

Abstract

A birth cohort design was used to understand whether heterotypic ligand-blocking norovirus antibodies provide cross-protection within the GII genogroup. We found that almost one-half of children who experienced a norovirus GII episode had preexisting antibodies heterotypic to the infecting genotype; therefore, these antibodies did not provide cross-protection.

Keywords

children; diarrhea; gastroenteritis; immunity; Nicaragua; norovirus

Noroviruses cause a substantial burden of disease in children. Despite this high disease burden, currently, there are no licensed vaccines against norovirus, although vaccine candidates targeting children are under development. To inform vaccine development, it

Corresponding Author: Sylvia Becker-Dreps, MD, MPH, Department of Family Medicine, UNC-Chapel Hill, 590 Manning Drive, Chapel Hill, NC 27599-7595, USA. sbd@unc.edu.
[a]Co-senior authors.

Potential conflicts of interest. L. C. L. and R. S. B. hold patents on norovirus vaccine design and ongoing collaborations with VaxArt and Takeda Vaccines that are unrelated and do not pose conflicts of interest with this report. S. B. D. has received investigator-initiated research awards from Takeda Vaccines unrelated to this report.
All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

is helpful to understand patterns of natural protection against norovirus, including whether there is cross-protective immunity between different norovirus genotypes.

Birth cohorts provide a unique opportunity to understand the development of immunity and patterns of protection; newborns can be followed for infectious outcomes alongside immunological assessments [1, 2]. We used a birth cohort platform to evaluate the role of preexisting homotypic and heterotypic immunity in protection against future norovirus episodes. We focused on genogroup II, which is associated with a higher disease burden than genogroup I. Specifically, we were interested in whether within-genogroup heterotypic blocking antibodies present at the time of exposure confer protection. Since noroviruses cannot easily be grown in cell culture, we used a surrogate neutralization assay measuring antibodies blocking attachment factors, histo-blood group antigens (HBGA). This assay has been shown to correlate with protection in norovirus challenge studies [3]. The results may provide insights into the breadth of immunity needed to be induced by future norovirus vaccines.

METHODS

Study Design

The study used samples from a birth cohort of 444 children in León, Nicaragua between 2017 and 2019 [4]. Cohort children were visited weekly in their homes to assess for acute gastroenteritis (AGE) episodes, defined as an increase in stool frequency to >3 stools in a 24-hour period or a substantial change in stool consistency and/or vomiting. Stool was collected during each AGE episode. Serum was obtained at the ages of 6 weeks, 5 months, 12 months, and every 6 months thereafter until the end of the study. Saliva was collected at 6 months of age to identify secretor and Lewis status of children, known to be associated with differences in susceptibility to norovirus based on host HBGA phenotypes [5]. Ethical approval was received from the Institutional Review Boards of the National Autonomous University of León and the University of North Carolina at Chapel Hill.

Virus Detection and Genotyping

Viral RNA was extracted from stools using QIAamp Viral RNA Mini Kits (Hilden, Germany) and tested by reverse-transcriptase quantitative PCR (RT-qPCR) for GI and GII noroviruses, rotavirus, sapovirus, and astrovirus, as described previously [4]. Norovirus GII-positive samples with Ct values <33 underwent sequencing [6] and were genotyped using an online typing tool [7].

Blockade of Virus-like Particle Ligand-binding Assays

Sera collected before each AGE episode with 1 of the 3 most commonly detected GII genotypes, GII.4, GII.12, and GII.17, were tested in a surrogate neutralization assay for blockade of virus-like particle (VLP) binding to HBGA, as described previously [8]. HBGA ligand substrates were human B saliva for GII.12 VLPs and porcine gastric mucus (PGM) for GII.4 and GII.17 VLPs. The percentage of VLP binding in the presence of serum was compared to binding in the absence of serum. Mean ID_{50} titers and 95% confidence intervals (CI) were determined from normalized dose-response sigmoidal curves fit in GraphPad 9.1

(San Diego, CA, USA). Sera that did not block at least 50% of VLP binding to ligand at the lowest dilution tested were assigned a titer equal to 0.5 times the limit of detection for statistical analysis.

Statistical Analysis

We compared the difference in mean age at the time of AGE episodes using a one-way ANOVA test. We used the Tukey-Kramer test to make pairwise comparisons between groups of unequal size. We estimated the percentage and 95% CI of children who had either homotypic or heterotypic antibodies to the infecting norovirus genotype. Geometric mean titers (GMT) of ID_{50} blockade antibodies were compared between the groups of children experiencing GII.4 vs non-GII.4 norovirus episodes using the Mann-Whitney *U* test for non-parametric data. In the analysis of preexisting non-GII.4 antibodies among children experiencing a GII.4 norovirus episode, the antibody level to the heterotypic VLP with the highest ID_{50} value (either GII.12 or GII.17) was used.

RESULTS

The 444 cohort children experienced 1312 AGE episodes between June 12, 2017 and November 6, 2019. All children were breastfed for some amount of time (median duration of any breastfeeding: 9.9 months). For 88% of AGE episodes (n = 1151), a stool sample was collected and screened by RT-qPCR (Figure 1). Ten percent (n = 113) of the AGE stools tested positive for GII norovirus; of these, 77 were genotyped. The most common GII genotypes identified were GII.4 Sydney [P16 or P31] (n = 45), GII.12 [P16] (n = 14), and GII.17 [P13 or P17] (n = 8). Each child contributed 1 stool sample. Of the 67 children experiencing a norovirus episode with 1 of these 3 most common GII genotypes, 41 [GII.4 (n = 31), GII.12 (n = 4), GII.17 (n = 6)] had a serum collection before the norovirus AGE episode with sufficient volume for analysis. These sera were collected a mean of 3.8 months before the norovirus AGE episode.

The 41 children had a mean age of 11.6 months (range: 2.6–28.8 months) at the time of their norovirus AGE episode and 63% were males. GII.17 episodes occurred at an older age (mean 18.6 months) than GII.4 or GII.12 episodes (mean 10.9 months, $P = .0011$ and 6.0 months, $P = .0002$, respectively). The children were similar in terms of host HBGA phenotypes: all children were secretors, 98% were Lewis B positive and 2% were Lewis negative. Thirty-four children received 2 doses of oral monova-lent rotavirus vaccine, while 7 received 1 dose. Two norovirus-positive stools also tested positive for rotavirus; however, the Ct values for norovirus (21, 22) were lower than for rotavirus (34, 34), and both children had received rotavirus vaccine.

The ID_{50} values of HBGA-blocking antibodies by genotype of the norovirus episode are shown in Figure 2. Seventeen children [41% (95% CI: 26%, 57%)] had preexisting antibodies to a genotype heterotypic to the infecting genotype and 6 children [15% (95% CI: 3%, 26%)] had preexisting antibodies to the same genotype as the infecting genotype. The children who developed GII.4 episodes had lower GMT of preexisting GII.4-specific antibodies [GMT = 13.3 (95% CI: 9.5, 18.8)] as compared to children who developed either GII.12 or GII.17 episodes [GMT = 173.4 (95% CI: 32.6, 921.1), $P = .0001$]. Children who

developed GII.12 or GII.17 episodes had a similar GMT of preexisting homotypic antibodies [GMT = 16.3 (95% CI: 9.3, 28.7)], as compared to preexisting GII.12 and GII.17 antibodies among children who developed GII.4 episodes [GMT = 31.1 (95% CI: 15.3, 63.3), $P = .68$].

DISCUSSION

Almost one-half of young children who experienced a GII norovirus episode had preexisting heterotypic blocking antibodies to other GII genotypes tested, showing that these heterotypic antibodies did not provide cross-protection against other GII episodes. Prior adult challenge studies showed no cross-protective immunity between genogroups [9], but little is known about cross-protective immunity within the same genogroup. Our findings complement a prior study by Malm, et al, which found that preexisting GII.4 binding was lower in children infected with GII.4 compared to those infected with other genotypes, and binding activity correlated with blockade activity [10]. This is the first study to provide information on preexisting blockade antibody levels to other genotypes within the same genogroup alongside weekly surveillance with PCR detection of norovirus AGE episodes.

Our immunological findings agree with 2 epidemiological studies from Peru, which found that repeat norovirus infections by different genotypes within the same genogroup were common [11] and that prior infections with GII.4 and GII.17 noroviruses did not protect against future GII.17 and GII.4 infections, respectively [12]. In contrast to the Peruvian studies, the current study based its findings on blockade responses to norovirus VLPs in serum. Patterns of protection may be different in adults; a clinical trial of the candidate GI.1/GII.4c norovirus vaccine in adults showed that the vaccine protected against GII.2 norovirus episodes [13]. It is possible that the vaccine boosted existing GII.2 immunity, or that antibody specificity in adults with multiple prior exposures is broader than found in naïve children.

We also detected homotypic antibodies in 6 of the 41 infants before their norovirus episode. In a post hoc analysis, we examined titers of maternal serum antibodies in the 5 infants with pre-episode serum collection at 5 months of age or younger, and found that all but one of the mothers had higher antibody levels than their corresponding infant, suggesting that antibodies detected were likely maternal in origin, and would wane in the time between serum collection and the norovirus episode.

A limitation of the study is that there were 3.8 months on average between serum collection and the AGE episode. It is possible that additional infections could have been acquired during the intervening period. Also, we did not measure norovirus-specific antibody levels in breastmilk, which could have played a role in protection against norovirus. Strengths of the study include a population-based sample of children, AGE surveillance with PCR detection of norovirus episodes, and the measurement of antibodies to a panel of GII VLPs using a surrogate neutralization assay found to correlate with protection against disease [1].

In summary, heterotypic norovirus GII HBGA-blocking serum antibodies were commonly found in young children before GII episodes. Our findings have implications for pediatric

norovirus vaccines, suggesting that vaccines may need to induce blockade antibodies to multiple GII genotypes to provide broad GII protection.

Financial support:

This work was supported by the National Institute of Allergy and Infectious Diseases [K24AI141744, R01AI127845, and R01AI148260]. F. G., Y. R., and L. G. were supported by an international research capacity-building award from the NIH-Fogarty International Center [D43TW010923].

References

1. Atmar RL, Ramani S. Birth Cohort studies: toward understanding protective immunity to human noroviruses. Clin Infect Dis 2021; 72:230–2. [PubMed: 33501954]
2. Cannon JL, Lopman BA, Payne DC, Vinjé J. Birth cohort studies assessing norovirus infection and immunity in young children: a review. Clin Infect Dis 2019; 69:357–65. [PubMed: 30753367]
3. Reeck A, Kavanagh O, Estes MK, et al. Serologic correlate of protection against norovirus-induced gastroenteritis. J Infect Dis 2010; 202:1212–8. [PubMed: 20815703]
4. Vielot NA, González F, Reyes Y, et al. Risk factors and clinical profile of sapovirus-associated acute gastroenteritis in early childhood: a Nicaraguan birth cohort study. Pediatr Infect Dis J 2021; 40:220–6. [PubMed: 33464013]
5. Nordgren J, Sharma S, Bucardo F, et al. Both Lewis and secretor status mediate susceptibility to rotavirus infections in a rotavirus genotype-dependent manner. Clin Infect Dis 2014; 59:1567–73. [PubMed: 25097083]
6. Chhabra P, Browne H, Huynh T, et al. Single-step RT-PCR assay for dual genotyping of GI and GII norovirus strains. J Clin Virol 2021; 134:104689.
7. Tatusov RL, Chhabra P, Diez-Valcarce M, Barclay L, Cannon JL, Vinjé J. Human Calicivirus Typing tool: a web-based tool for genotyping human norovirus and sapovirus sequences. J Clin Virol 2021; 134:104718.
8. Lindesmith LC, McDaniel JR, Changela A, et al. Sera antibody repertoire analyses reveal mechanisms of broad and pandemic strain neutralizing responses after human norovirus vaccination. Immunity 2019; 50:1530–41.e8. [PubMed: 31216462]
9. Wyatt RG, Dolin R, Blacklow NR, et al. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. J Infect Dis 1974; 129:709–14. [PubMed: 4209723]
10. Malm M, Uusi-Kerttula H, Vesikari T, Blazevic V. High serum levels of norovirus genotype-specific blocking antibodies correlate with protection from infection in children. J Infect Dis 2014; 210:1755–62. [PubMed: 24970849]
11. Saito M, Goel-Apaza S, Espetia S, et al. Multiple norovirus infections in a birth cohort in a Peruvian Periurban community. Clin Infect Dis 2014; 58:483–91. [PubMed: 24300042]
12. Chhabra P, Rouhani S, Browne H, et al. Homotypic and heterotypic protection and risk of reinfection following natural norovirus infection in a highly endemic setting. Clin Infect Dis 2021; 72:222–9. [PubMed: 33501947]
13. Sherwood J, Mendelman PM, Lloyd E, et al. Efficacy of an intramuscular bivalent norovirus GI.1/GII.4 virus-like particle vaccine candidate in healthy US adults. Vaccine 2020; 38:6442–9. [PubMed: 32878708]

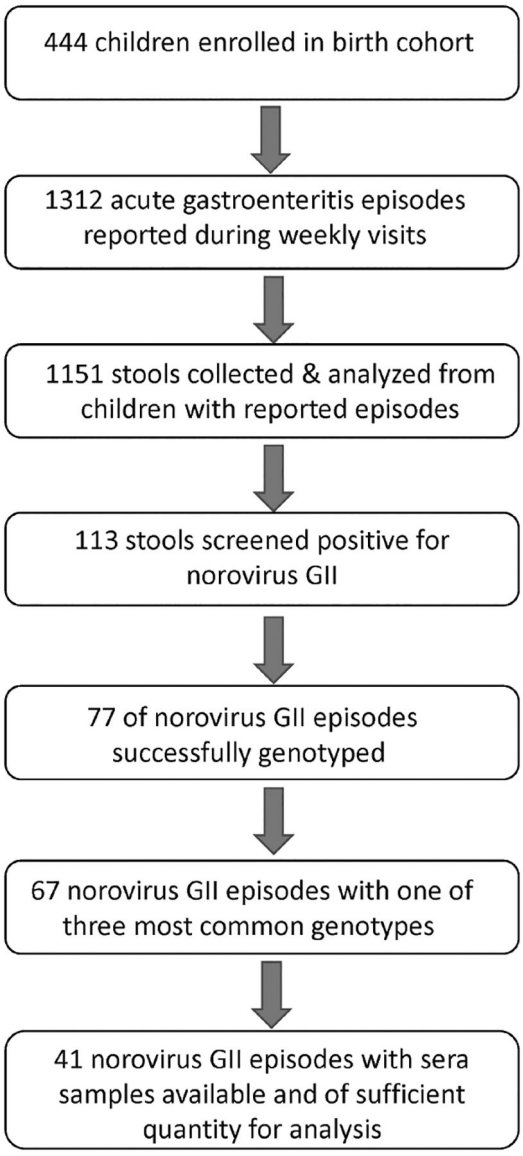

Figure 1.
Selection of norovirus GII episodes for serum blocking antibody analysis.

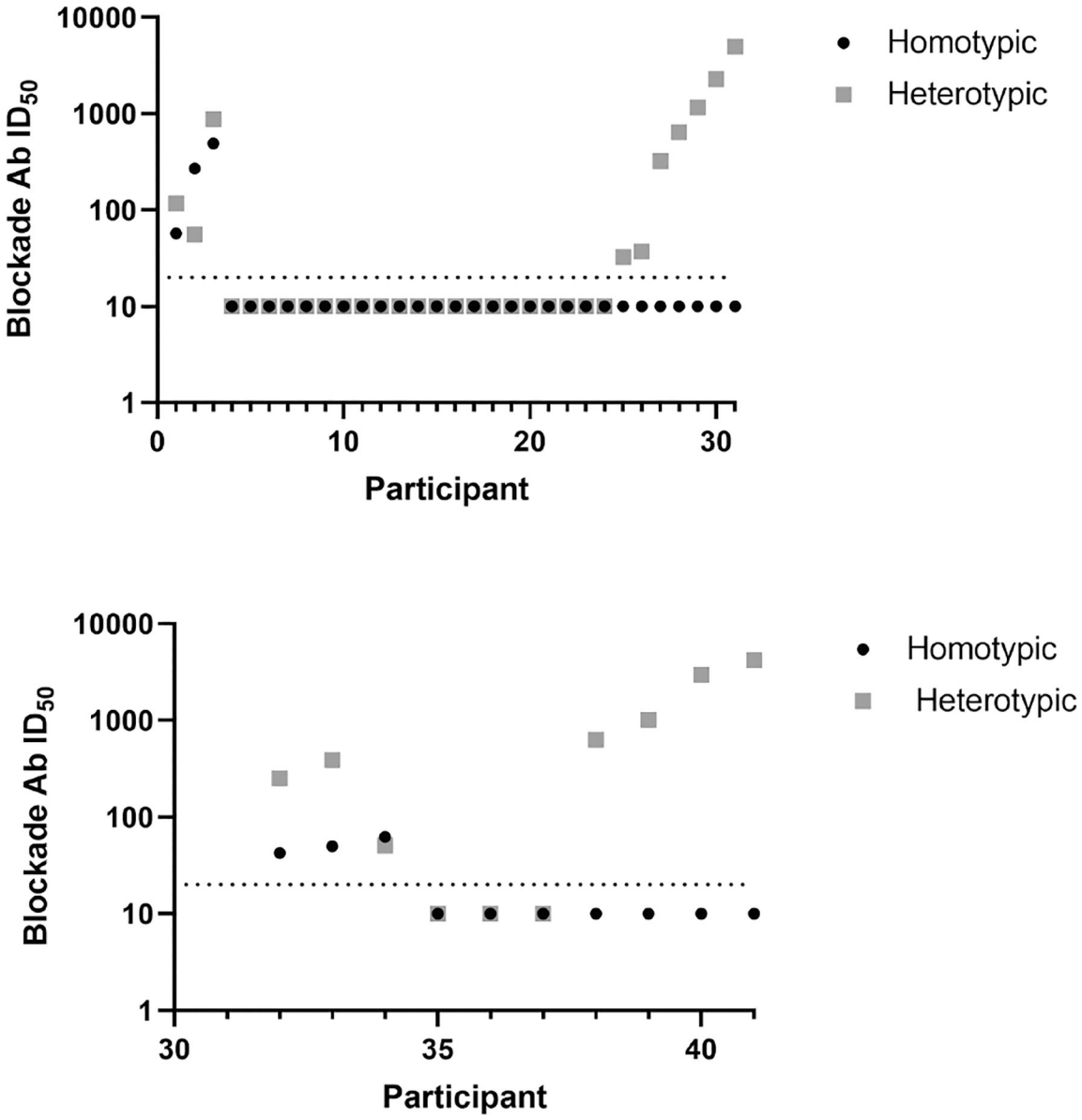

Figure 2.

Preexisting homotypic and heterotypic GII histo-blood group antigen (HBGA)-blocking antibody levels in children experiencing norovirus GII episodes. Top panel—Preexisting homotypic and heterotypic GII HBGA-blocking antibody levels in 31 children experiencing GII.4 episodes (homotypic = GII.4, heterotypic = either GII.12 or GII.17; if antibodies to both genotypes were detected, the genotype with higher titers is shown in the figure). Bottom panel—Preexisting homotypic and heterotypic GII HBGA-blocking antibody levels in 10 children experiencing GII.12 or GII.17 episodes (homotypic = GII.12 or GII.17, respectively, heterotypic = GII.4 or either GII.17 or GII.12, respectively; if more than 1 heterotypic antibody was detected, the genotype with higher titers is shown in the figure). ID_{50} was determined by dose-response curve fit of non-linear data, normalized to the mean percentage control binding. For heterotypic antibody levels, the antibody level to the

heterotypic virus-like particle (VLP) with the highest ID_{50} value is included in the figure. The marker on the *x*-axis indicates 1 participant. The dashed line indicates the lower limit of detection.