# One More Step Toward Understanding the Immune Response to Norovirus

**Bonita E. Lee[1] and Xiao-Li Pang[2]**

[1]Department of Pediatrics, and [2]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada

**(See the major article by Malm et al on pages 1755–62.)**

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Forty-two years have passed since norovirus (NoV) was first identified by Dr Albert Z. Kapikian as the etiological agent of a gastroenteritis outbreak, and this virus is now well known as the cause of at least 5 global gastroenteritis outbreak epidemics since 1990s [1]. With widespread use of molecular diagnostic assays, the role of NoV in sporadic gastroenteritis is also increasingly being recognized. A systematic review of 31 studies in both developed and developing countries estimated that NoV accounted for 10%–15% of severe gastroenteritis cases in children aged <5 years and 9%–15% of mild and moderate diarrhea cases among persons of all ages, resulting in an estimated 1.7–1.9 million outpatient visits and 19–21 million total illnesses per year in the United States [2]. NoV is becoming the predominant cause of sporadic gastroenteritis in young children in areas with rotavirus vaccine programs [3, 4].

Understanding the pathogenicity of and host response to NoV has been markedly hampered by the absence of an in vitro cell culture system or an in vivo small animal model for human NoV. Historical and recent challenge studies [5] using classic Norwalk virus demonstrated a low infectious dose and an average incubation period of 24–48 hours. Asymptomatic infection occurred and those with symptoms had diarrhea, nausea, vomiting (in >50% of cases), abdominal cramps, and malaise that usually resolved in 12–72 hours, with prolonged symptoms observed in young individuals, elderly individuals, and immunocompromised individuals. Infections were traditionally thought to result in only short-term immunity; this, coupled with the high genetic and antigenic diversity of NoV through mutations and recombination, would lead to frequent reexposure, repeat infections throughout life, and a large human reservoir [6]. Studies using recombinant virus-like particles (VLPs) or P-particles created by in vitro expression of NoV capsid proteins suggested that human histo-blood group antigens (HBGAs), a group of heterogeneous and complex carbohydrates on red blood cells, that are expressed on mucosal epithelia and intestines in 80% of the population (secretors) are important determining factors for host susceptibility to NoV infection [7].

Most human infections are caused by NoV genogroup I (GI; with 9 different genotypes) or GII (with 22 different genotypes). Since the 1990s, up to 85% of global epidemics of NoV infection have been dominated by various GII.4 (GII genotype 4) variants that emerge every 2–3 years. Study of epitope changes among the GII.4 variants supported the hypothesis that protective herd immunity drives the emergence of new GII.4 variants every 2–4 years [8–10]. A recent challenge study showed that, unlike other strains, GII.4 could infect secretors of all ABO blood groups, which represent a majority of the human population [11]. Moreover, NoV GII.4 was found to have a higher mutation rate and a faster pace of evolution than other NoV strains, enhancing its epidemiological fitness [12]. In addition to outbreak epidemics, GII.4 variants play an important role in sporadic gastroenteritis, but a higher heterogeneity of NoV strains has been observed in NoV infections of children [13, 14]. It, therefore, seems possible that previous exposures result in some degree of long-lasting and cross-protective immunity to some, but not all, infecting NoV strains.

Efforts are being made to develop a vaccine for NoV, which necessitates a more sophisticated understanding of the immunological response to infection. In this issue of the *Journal*, Malm et al studied baseline antibodies and serological responses in children presenting with acute gastroenteritis caused by NoV. [15] The

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Correspondence: Bonita E. Lee, MD, MSc, Room 3-588B Edmonton Clinic Health Academy (ECHA), 11405–87 Avenue, Edmonton, Canada T6G 1C9 (bonita.lee@albertahealthservices.ca).

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authors collected serum from 43 patients within 1 week of symptom onset. These specimens provided data for baseline serology in infected children and there was no healthy cohort for comparison. The results showed lower baseline NoV genotype–specific total immunoglobulin G (IgG) against antigens derived from 2 GII.4 variants (GII.4-1999 and GII.4-2010) in children who were infected by GII.4, compared with those infected by non-GII.4 genotypes. Blocking antibody assay, which measured antibodies that block the binding of VLPs to HBGAs, is a better assessment of protective immunity than total IgG. This study observed good correlatiion between genotype-specific total IgG and blocking antibodies for NoV, supporting the role of total IgG as a marker of protective immunity in children. It would be interesting to do a household transmission study and measure baseline serological titers in family members with very recent or ongoing NoV exposure and look at the relationship between genotype-specific antibodies levels and development of symptomatic infection.

Although the number of patients with paired acute- and convalescent-phase sera was small in this study (n = 6), the data are illuminating, as they show rising titers of cross-reacting total genotype-specific IgG against both GII.4-1999 and GII.4-2010 but an increase in blocking antibodies only for GII.4-2010, supporting the hypothesis of antigenic variations in emerging GII.4 variants that allow escape from herd immunity. An infrastructure similar to that used in global surveillance leading to updates of the strains that are included in the annual influenza vaccines would potentially also be required for NoV vaccines, unless a vaccine producing long-term cross-protective immune response can be developed. This study also provided a glimpse into antibody kinetics after NoV infection, but the durability of the immune response to NoV, which has important implications for vaccine design, was not studied.

This study focused on immune response to GII.4 variants. In a recent study using a murine NoV model, different immune responses were elicited by genetically related murine NoV strains [16]. With the observation that there is a higher diversity of NoV genotypes in infected children, in contrast to the overall predominance of GII.4 variants in outbreaks, are the immune response against GII.4 variants fundamentally different from responses against other GII genotypes or NoV GI? What are the differences in viral pathogenicity among the various human NoV strains?

There are still many NoV puzzles to be solved. Debbrink et al provide an excellent overview of NoV vaccine development and status [17]. The combined efforts of experimental studies on molecular aspects, observational and cohort studies, and ongoing surveillance data will lead us toward solving these puzzles.

## Notes

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