Immunity to Calicivirus Infection

Suzanne M. Matsui1,3 and Harry B. Greenberg1,2,3

1Department of Medicine, Division of Gastroenterology, and 2Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, and 3Medical and Research Services, VA Palo Alto Health Care System, Palo Alto, California

The evolution of our understanding of immunity to calicivirus infection, using Norwalk virus as the prototype, is discussed in three stages: (1) “ancient times” (1972–1978), when human volunteer studies prevailed, (2) the “middle ages” (1978–1990), which were characterized by the development and implementation of solid-phase immunoassays based on native viral antigens, and (3) “modern times” (1990 to present), which began with the cloning of the genome of the noncultivatable 8FIIa strain of Norwalk virus and resulted in a readily available source of recombinant virus-like particles that have revolutionized the study of caliciviruses. Throughout these stages, it has been shown repeatedly that short-term immunity develops to homologous virus. However, the search for determinants of long-term immunity continues. These studies will likely be facilitated by the newest reagents—the noninfectious recombinant virus-like particles—used in the setting of human volunteer studies and large epidemiologic studies.

Although studies of calicivirus infection have evolved substantially over the past 3 decades, a complete understanding of immunity to these viruses remains elusive. It is well established that infected individuals develop short-term immunity to homologous virus, but the determinants of longer-term immunity have not been elucidated. Molecular cloning and expression of the Norwalk-like viruses have made available reagents, such as recombinant (r) virus-like particles (VLPs), that are antigenically and morphologically indistinguishable from native virions. Clinical studies using these and other reagents will likely lead to a deeper understanding of long-term immunity to calicivirus infection in the next several years.

Work on immunity to calicivirus infection can be divided into three stages on the basis of the research tools available at the time. The first stage, “ancient times,” was from 1972 to 1978, the heyday of human volunteer studies. The second stage, the “middle ages,” was from 1978 to 1990, a time when solid-phase immunoassays that were dependent on limited native viral antigens predominated. The third and current stage, “modern times,” started in 1990 coincident with the development of renewable supplies of recombinant antigens and hyperimmune antisera, which can be used in diagnostic assays and studies of immune response.

This review focuses on Norwalk virus (NV), the most extensively studied strain of the human caliciviruses. It is assumed that immunity to other members of the Norwalk-like genogroups of caliciviruses (genogroups I and II) is similar. However, we know much less about immunity to Sapporo virus and related viruses in genogroup III, and it is possible that immunity to these agents differs from that to NV and related viruses.

Evolution of the Study of Calicivirus Infection

Ancient times. From the earliest studies of the NV, the finding that most adult volunteers became ill after exposure to the virus has intrigued investigators and stirred curiosity about immunity to this agent [1]. This finding implied either that natural immunity to this virus is not widespread in the general population or that the pathogen is facile in evading host immune defenses. Subsequent studies demonstrated that volunteers did not develop illness if they were rechallenged with the original homologous inoculum between 6 and 14 weeks of the initial exposure, suggesting that short-term immunity to homologous virus develops [2, 3].

Techniques used to characterize NV were applied to studies of other outbreaks of acute nonbacterial gastroenteritis that had a similar clinical course. Wyatt et al. [3] examined the relationships between NV and isolates from family outbreaks in Hawaii and in Montgomery County, Maryland. Human volunteers who were determined to have been “definitely ill” from a primary challenge with stool filtrates from these outbreaks were rechallenged with homologous or heterologous (derived from an outbreak different than primary challenge) virus. In each of the groups, none of the volunteers became ill when rechallenged with homologous virus 6–14 weeks after the initial disease-causing challenge.
Results from cross-challenge studies demonstrated the complex relationships among these viruses that caused clinically similar disease. NV and Hawaii virus (HV) appeared to be antigenically distinct, as recent illness with NV did not protect against disease with HV, and vice versa. Recent illness with NV protected volunteers from challenge with Montgomery County virus (MCV), but 1 of 3 volunteers initially infected with MCV became ill when challenged with NV, despite the fact that these strains appeared to be related serologically. Recent illness with HV protected all 4 volunteers from rechallenge with MCV, but the converse was not tested. These findings were consistent with data from studies of NV by immune electron microscopy [4].

No increase in antibody titer to the NV particle was found with HV convalescent serum, but a definite antibody response developed after NV rechallenge and illness. Convalescent serum from MCV-infected volunteers showed a moderate but significant rise in antibody titer to the NV particle and a further increase in titer after NV rechallenge and illness.

The question of long-term immunity was addressed by Parrino et al. [5] in homologous rechallenge studies with NV. Six of the 12 volunteers who were reinfected with the 8F1Ha NV inoculum became ill. When all 12 were rechallenged with the identical inoculum 27–42 months later, the 6 who developed symptomatic illness after the initial inoculum became ill once again, suggesting that long-term immunity was not conferred by a single infection with NV. Four of the 6 who became ill after the second challenge were given a third inoculum 4–8 weeks after the second was administered. Although 1 of the 4 volunteers became ill again, 3 did not. These findings are in agreement with those of earlier studies (discussed above) that suggested short-term immunity (up to 14 weeks) usually follows NV gastroenteritis. Parrino et al. [5] also found that the level of serum antibody before initial challenge, as determined by immune electron microscopy, could not be used to predict whether an individual would develop illness.

Middle ages. The observations of the ancient times were largely confirmed and extended during the middle ages, when more-sensitive serologic assays became available. The main investigative tool was the solid-phase immunoassay that relied on native viral antigens and paired human infection serum [6, 7].

Antibody acquisition was studied in sera from children in the United States, Yugoslavia, and Bangladesh and from 2 populations of Ecuadorian Indians [8]. In general, antibody prevalence was lowest in those 0–5 years old and increased with age. In the United States, NV antibody prevalence ranged from <20% in the 0–5-year-old age group to ~65% in the 11- to 15-year-old age group. The pattern of antibody acquisition in Yugoslavia paralleled that found in the United States; however, it started from a higher baseline (30%) and rose to >80% in the respective age groups. In Bangladesh and among rural Ecuadorian Indians (Tiwaeno, Tzapino, and Bai’s villages), antibody prevalence ranged from 75% to 100% in the first 5 years of life and remained in this high range throughout childhood. By contrast, antibody to NV was not detectable in a separate isolated group of Ecuadorian Indians (Gabaro village).

A more detailed analysis of antibody acquisition in children in Bangladesh was done by Black et al. [9]. In this longitudinal study, the prevalence of antibody to NV was lowest (7%) in the youngest children (2–7 months old). The rate of antibody acquisition, however, was quite dramatic over the next year or two, as documented by the rise in antibody prevalence to >60% among children 20–25 months old. By age 4 years, all children in this study had antibody to NV. It was estimated that 1%–2% of the 5.6 episodes of diarrhea experienced on average by these children was attributable to NV infection.

Immunity to NV infection among children from less developed countries was also studied on two remote islands off Panama’s Caribbean coast [10]. In that study, a trend toward higher rates of infection among younger children was observed: 71% of the NV infections occurred in children <3 years old, a group that accounted for 35% of the study population. Among children <5 years old, a higher baseline titer of NV antibody (>1:100) appeared to correlate with protection from subsequent infection with NV. This finding was clearly distinct from findings in the adult volunteer studies cited above. A similar positive correlation between antibody titer and protection was obtained in a study of NV infection due to classic human calicivirus (Otofuke-like, genogroup III virus), as opposed to Norwalk-like calicivirus, in a Japanese orphanage [11]. Among these infants, who were 0–21 months old, preexisting serum antibody to human Otofuke-like calicivirus was associated with protection against clinical illness after exposure to the virus.

To study the determinants of resistance to NV infection in adult volunteers, Greenberg et al. [12] analyzed serum and local jejunal antibody levels in volunteers before they received the NV inoculum. No correlation between these antibody levels and resistance to infection could be demonstrated. To the contrary, the geometric mean titer of Norwalk antibody in jejunal fluid of susceptible volunteers was significantly higher than that in resistant volunteers. A similar trend was noted for serum antibodies, suggesting that nonimmune mechanisms may play a role in determining response to NV challenge. A similar finding was made by Blacklow et al. [13], who analyzed the same specimens. One interpretation of this observation has been that in adults in developed countries, the antibody level to NV is an indicator of past exposure and susceptibility, while the absence or low level of antibody in adults signifies relative resistance to infection.

Madore et al. [14] reexamined the antigenic relatedness among Norwalk-like viruses that included NV, Snow Mountain virus (SMV), and HV. Most volunteers (75%–90%) who received one of these challenge viruses had rises in serum antibody titer to the homologous challenge virus. Serum antibody titer to heterologous viruses also rose in those who had had a significant seroresponses to the homologous virus. More specifi-
cally, 50% of the volunteers challenged with SMV had a seroresponse to HV, and 62% of those challenged with HV had a seroresonse to SMV. Of those challenged with NV, 40% had a seroresonse to SMV, but none had a seroresonse to HV. One of the volunteers challenged with HV developed a seroresonse to NV. The magnitude of the seroresonse to homologous virus was substantially greater than to heterologous virus, as observed previously. The presence of preexisting homologous serum antibody was not correlated with protection from or development of illness by any of the challenge viruses. Preexisting heterologous serum antibody to SMV appeared to be associated with susceptibility to illness by NV, but the significance of this correlation is not known. These results point out the complexities of the serologic relationships among the Norwalk-like caliciviruses and support the findings of previous studies (reviewed above) in which serum antibody response did not correlate directly with protective immunity.

Modern times. The cloning of the 8FIIa strain of NV in the early 1990s [15–17] and the production of large quantities of VLPs [18] revitalized the field of NV research. Large quantities of rVLPs, which are comparable in size, structure, and antigenic properties to native virions, are produced in a baculovirus expression system. Having a readily renewable source of uniform recombinant particles has enhanced the development of newer, more sensitive and highly specific solid-phase immunoassays (e.g., rNV ELISA) that are now widely available for a variety of applications [19].

When used in epidemiologic studies, these assays have helped to determine that the age of antibody acquisition and illness with NV are lower than suspected previously. Evidence for high rates of NV infection and reinfection were found in stored serum that had been collected longitudinally from infants and toddlers in a residential facility in the United States [19]. Nearly half of 154 Finnish infants and children involved in a rotavirus vaccine study were found to have had at least one infection with NV over the 2-year study interval [20].

In a separate study that examined seroprevalence rates in children in several populations throughout the world, several patterns were observed. In the United Kingdom, NV-specific serum IgG gradually increased from being virtually undetectable in 5- to 11-month-old infants to ~70% in 11- to 16-year-old children [21]. At one extreme, the prevalence of NV antibodies was comparatively low among Japanese and Israeli children, and at the other end of the spectrum, seroprevalence was high among Australian aborigine children, reaching 100% in those 6–19 years old. This trend resembled that previously found in Bangladesh and among Ecuadorian Indians [8].

Lew et al. [20] also concluded that low preexisting NV IgG titer in children was associated with susceptibility to developing NV infection, as had been observed in studies from the middle ages of calicivirus study [10]. By contrast, in studies in which the newer, more sensitive serologic assays were used, adults with preexisting antibody to NV were not protected against infection in either the volunteer study or natural outbreak setting [22–24], a finding that is in agreement with the earlier study by Greenberg et al. [12]. The apparent incongruity between findings in children and those in adults may be explained in part if it is accepted that the direct correlation between serum antibody level and protection observed in young children reflects short-term, recent exposure. In adults in developed countries, serum antibody level is probably an indicator of past exposure rather than recent infection. The frequency with which children in certain populations (e.g., underdeveloped countries) are exposed to NV also may influence the development of immunity to the virus, since exposure may be so frequent that long-term immunity is not required.

The rVLPs, which are now produced successfully for genogroup I NV [18] and several genogroup II viruses, including Mexico virus [25], HV [26], Toronto virus [27], and Lordsdale virus [28], should facilitate the study of antigenic epitopes and determinants of immunity to this unique group of viruses. It is tempting to consider developing mucosal vaccines that are based on these rVLPs. Early studies toward this end have been published recently. Ball et al. [29] have completed a phase I trial of rNV particles given to adult volunteers with preexisting serum antibodies to the virus. They found that the VLPs are safe and immunogenic when ingested, but the level of the immune response to rNV particles was far lower than that seen in volunteers infected with wild-type virus. It has not been determined whether the moderate level of immune response observed is sufficient to prevent infection even in the short term; nor has it been determined whether this nonreplicating vaccine can induce a comparable immune response in volunteers who lack preexisting antibody. Future work using this system should include challenge studies to determine whether the rNV candidate oral vaccine can protect individuals from challenge with homologous (and heterologous) Norwalk-like viruses.

An innovative approach to immunization, the edible vaccine, is also being explored for NV. rNV capsid protein has been expressed in transgenic tobacco plants and potatoes [30]. In transgenic potatoes, about one-half of the rNV self-assembles into particles. When fed to mice, rNV derived from plants stimulates humoral and mucosal immune responses without requiring an adjuvant. Since there is no animal model of NV infection, the efficacy of this type of vaccination will require human volunteer feeding trials and challenge studies. Expression of rNV in more palatable raw transgenic plants (e.g., banana) is being investigated.

Conclusion

In summary, epidemiologic studies have repeatedly shown that NV and related viruses are widespread in the general population [31, 32]. Exposure to these viruses occurs in early childhood, as reflected in the antibody prevalence data discussed above. The earliest ages of exposure to and infection with NV
tend to cluster in certain subgroups in developed countries and are found more extensively in developing countries. Outbreaks of infection with viruses of genogroup I and II are common among adults in developed countries. Short-term immunity has been demonstrated consistently (in studies from what we termed ancient to modern times) and correlated directly with the development of serum and mucosal immune responses. In limited studies of genogroup III viral infections in young children, short-term immunity clearly correlated with serum antibody level. The relative paucity of epidemics of illness caused by genogroup III strains in adults implies that long-term immunity to these viruses may be generated more readily. Elucidating the determinants and correlates of long-term immunity to genogroup I and II caliciviruses will continue to challenge investigators in the next phase of studies on NV immunity. Recent tools derived from advancements achieved in molecular virology will certainly help researchers to delve further into this area, and the human volunteer will continue to be a most important model system for these studies.

Acknowledgment

We thank Mary K. Estes for providing a preprint of reference 29 for use in our oral presentation and in the preparation of this manuscript.

References

30. Mason HS, Ball JM, Shi JJ, Jiang X, Estes MK, Arntzen CJ. Expression of
