HUMAN VACCINES & IMMUNOTHERAPEUTICS
2016, VOL. 12, NO. 7, 1653–1669
http://dx.doi.org/10.1080/21645515.2015.1125054

Taylor & Francis
Taylor & Francis Group

REVIEW

Norovirus vaccines: Correlates of protection, challenges and limitations

Nada M. Melhem

Medical Laboratory Sciences Program, American University of Beirut, Beirut, Lebanon

ABSTRACT

Norovirus (NoV) is responsible for at least 50% of all gastroenteritis outbreaks worldwide. NoVs are classified into 6 different genogroups (GGI- GGVI) based on the viral capsid protein with NoV genogroup II genotype 4 (GII.4) being the predominant strain causing human diseases. Supportive therapy involving reversal of dehydration and electrolyte deficiency is the main treatment of NoV gastroenteritis. However, the worldwide increased recognition of NoV as an important agent of diarrheal gastroenteritis prompted researchers to focus on establishing preventive strategies conferring long-lasting immunity. This review describes the current status of animal and human vaccine models/studies targeting NoV and addresses the factors hampering the development of a broadly effective vaccine.

ARTICLE HISTORY
Received 27 July 2015
Revised 6 November 2015
Accepted 21 November 2015

KEYWORDS
diversity; genogroups; immunity; NoV; vaccine; virus-like particles

Introduction

Gastroenteritis caused by NoV has been recently reported to be responsible for at least 50% of all gastroenteritis outbreaks worldwide and a major cause of foodborne illness.[1,2] Data from the Centers for Disease Control and Prevention suggest that NoV is the leading cause of acute gastroenteritis across all age groups seeking medical care in emergency departments, outpatient clinics and the community.[1] Recent reviews of the literature on community, outpatient and hospital-based studies in developing and developed countries report that NoV gastroenteritis account for 10–15 % of severe cases in children less than 5 y of age and 9–15% of mild to moderate diarrhea among individuals of all ages.[3,4] NoV is the most common cause of acute gastroenteritis in the United States. Each year, it causes 19–21 million illnesses and results in 56,000–71,000 hospitalizations and 570–800 deaths. NoV is also the most common cause of foodborne-disease outbreaks in the United States (http://www.cdc.gov/NoV/about/overview.html.). Due to the widespread use of rotavirus (RV) vaccine, NoV is currently the most common cause of acute gastroenteritis requiring medical attention among children followed by rotavirus. NoVs are single-stranded RNA viruses of the family *Caliciviridae*[5] and thus undergo genetic drift due to an error-prone RNA polymerase as well as the ability to undergo recombination.

NoV: Transmission, pathogenesis and treatment

Fecal-oral spread is the primary mode of NoV transmission. The average incubation period is 24–48 hours. The symptoms include vomiting (≥50% of cases), diarrhea, nausea, abdominal cramps, malaise and low-grade fever. Illness usually resolves in 12–72 hours but can last longer in young children, elderly people, hospitalized and immunocompromised individuals. Asymptomatic infections with viral shedding have been documented. A number of factors contribute to the high communicability of NoV most importantly the low infectious dose of the virus (18–100 particles),[6] the high levels of virus shedding [7] ($> 10^9$ particles/ml of stool during the first days after infection) known to precede illness and to be prolonged in immunosuppressed persons, the stability of the virus at temperatures ranging between 0°C and 60°C, and finally the high rate of mutation and recombination leading to antigenic diversity.[8,9] Knowledge about the pathogenesis of NoVs emerged as a result of challenge studies showing a broadening and blunting of the intestinal villi, infiltration of the polymorphonuclear and mononuclear cells into the lamina propria, in addition to cytoplasmic vacuolization.[5,10] The molecular basis of the rapid illness burst remains not fully understood though. This has been hampered by the lack of *in vitro* or *in vivo* culture systems for NoV.

The susceptibility to NoV infection has been associated with the presence of a α1, 2-linked fucose on histo-blood group antigens (HBGAs). The gene Fucosyl transferase 2 (*FUT2*) is involved in the expression of HBGA (specifically the generation of the H antigen motif). The latter serve as binding receptors for human NoVs and thus predict susceptibility to the infection.[10-12] Secretor-positive individuals encoding FUT-2 express protein antigens on mucosal surfaces that were originally related to binding of GI.1-NoV virus like particles (VLPs). More recent data confirmed the association between expression of HBGA and susceptibility to GI.1-NoV in human challenge studies.[11] Despite the similarities in reported secretor in many regions of the world, reports suggest that the non-secretors, i.e. expressing inactive FUT2, are different and mainly affected by selective pressure in different populations.[13,14] Importantly, resistance to infection by few NoVs genogroups, specifically the most common circulating strain (GII.4) has been explained by the lack of or weak expression of the HBGA motifs. This

CONTACT Nada M. Melhem, PhD melhemn@aub.edu.lb 325 Van Dyck Hall, Medical Laboratory Sciences Program, Faculty of Health Sciences, American University of Beirut. P.O. Box 11-0236 Riad El Solh 1107-2020, Beirut, Lebanon.

association was compared to the well-established association between chemokine CCR5 receptor and resistance to human immunodeficiency type −1(HIV-1) infection. While the non-secretor phenotype was suggested to confer resistance to NoV infection, reports show that non-secretors are infected by a diverse group of NoVs including GII.4, GII.2 Snow Mountain and GI.3.[15-17] These viruses have unknown receptors. This data add to the incomplete understanding of the pathogenesis of NoV. Importantly, the inhibition of binding to HBGAs was suggested as an antiviral treatment option of NoV infection[18] despite the reported evidence describing the lack of association between symptomatic NoV infection and the expression of HBGAs.[16] Further studies are clearly needed to help elucidating the role of these receptors.

Supportive therapy involving reversal of dehydration and electrolyte deficiency is the main treatment of NoV gastroenteritis. The administration of nitazoxanide to children with viral gastroenteritis led to reduction in the duration of the illness.[15] Moreover, a higher frequency of resolution of diarrhea among immunosuppressed patients receiving orally administered human immunoglobulin is reported as compared to controls, yet without statistical significance.[19] Recently, NoV-specific monoclonal antibodies were developed in chimpanzees immunized with NoV-GI.1.[20] These neutralizing antibodies are a proof-of-concept for the potential generation of antibodies targeting the predominant NoV strains. This is especially promising with similar approaches in HIV,[21,22] HCV[23] and influenza.[24] The prophylactic administration of bovine lactoferrin or probiotic fermented milk failed at preventing NoV infection in children and elderly residents of a health care facility, respectively.[25,26] The development of an effective treatment for NoV infections among children and immunosuppressed patients is needed to reduce the burden of gastroenteritis.

Viral diversity

The NoV genome is a linear single-stranded, positive-sense RNA of approximately 7.6 kb in length. The genome is composed of 3 large open reading frames (ORFs) designated as ORF-1, ORF-2 and ORF-3. ORF-1 encodes 6 non-structural proteins including the protease and the RNA-dependent RNA-polymerase (RdRp). ORF-2 and ORF-3 encode the structural viral components viral protein 1 (VP1) (major capsid protein) and VP2 (minor capsid protein), respectively.[27] VP1, showing the highest degree of sequence variability in the viral genome, consists of a shell (S) responsible for VP1 assembly and a surface exposed P domain. The P domain is further subdivided into 2 protruding subdomains: P1 and P2. P1 is reported to enhance the stability of the virus. P2 is located at the outer surface of the capsid and contains a hypervariable region. The latter contains a binding cleft for HBGA attachment factors. P2 is also implicated in binding to neutralizing antibodies. The P domain is the most exposed region of the capsid protein. Attachment to host cells and antigenic determinants are likely present in this domain.[28,29] Expression of the VP1 capsid as a recombinant protein independently of other viral parts leads to self-assembly into a virus-like particle (VLP). The latter has structural and antigenic characteristics that cannot be distinguished from the virus.[15] VP2, the minor capsid protein, is thought to play a role in the production and the stability of VP1.[30]

Based on the amino-acid sequence of VP1, NoVs are divided into 6 genogroups (GI-GVI). GI, GII and GIV are known to infect humans.[5] Genogroups are further subdivided into genotypes based on the RdRp sequence or capsid sequence. At the genomic level, strains of the same genogroups are 51–56% similar whereas genotypes have 69–87% similarity.[11,31] At least 8 and 21 genotypes belong to GI and GII, respectively.[1] The genogroup II, genotype 4 NoVs, designated GII.4, are responsible for the majority of NoV outbreaks worldwide. GII.4 NoVs are continuously changing and viral variants emerge every couple of years.[32-35] The use of VLPs revealed the ability of the surface-exposed P2 subdomain (279–405) to interact with neutralizing antibodies and NoV-specific carbohydrate-binding ligands. The surface exposed, highly variable sites of the P2 subdomain (epitopes A to E) undergo changes that were associated with the emergence of new strains causing NoV outbreaks.[28,36,37] These sites are situated around the HBGA pocket. The binding of NoV to specific HBGA as well as antibody binding is affected by these putative epitopes as well as flanking binding sites.

Different populations may be susceptible to one NoV strain due to the ability of NoV to bind more than one type of the polymorphic HBGA. Moreover, one population could be susceptible to more than one NoV strain. Importantly, the antigenic variation of NoVs will generate escape mutants from the existing memory immune responses. The latter does not eliminate cross-reactive immune responses resulting from previous exposure to NoV infection. Data show that new NoV variants (specifically GII.4) appear every 2–7 y as a result of genetic drift, an observation compatible with the immune escape mechanism observed with influenza A virus.[32,38] The binding profile of GII.4 to HBGAs facilitates the infection of 80–95% of the population. This suggests that this interaction is subject to immune pressure and may partially explain the predominance of GII.4 NoV.[39,40] Bull et al.[41] reviewed the role of receptor switching, sequence space, duration of herd immunity and replication fidelity on the evolution of NoV. As previously described the sequence similarity of the NoV capsid protein residues is not a straightforward predictor of HBGA binding. This 'host receptor switching' is a clear outcome of the evolution of the capsid protein.[42] Despite the fact that HBGAs are referred to as 'restriction factors' of the NoV-HBGA interaction, viral strains binding to non-secretors clearly implicate other factors in susceptibility to and pathogenesis of NoV.[41] Sequence space is defined as the number of sites in the virus genome. RNA viruses are viruses with small genomes; non-synonymous substitutions are targeted by negative selection leading to genetic change; whereas structural genes undergo minimal positive selection (the pressure driving the maintenance of beneficial mutations). Negative selection is primarily the evolution pressure preventing lethal mutations from persisting in the virus. The mutations introduced in the GII.4 variant are thought to enhance its ability to bind diverse HBGAs leading to increased susceptibility to the virus and potential pandemic spread.[38] It is yet to determine whether genetic stasis leading to viral fitness as the case with Influenza viruses is also happening with NoVs.[41]

Low fidelity of the NoV RdRp has an inverse relationship with strain prevalence. Genotypes with low fidelity are suggested to provide these strains with a fitness enhancing the ability of the most prevalent viruses to avoid the immune system, i.e., GII.4, over less prevalent ones. This fitness allows these strains to escape immune recognition through alteration of genetic properties.[43] Recombination events are detected in the polymerase as well as the junction of the polymerase and the capsid. The combination of low fidelity and recombination events need further investigation especially due to similar processes affecting the influenza shift and drift. Immune recognition is altered by changes of the virus antigenic properties. The diversity displayed in the hypervariable region of P2 domain correlates with the epidemiologic fitness of the strain, i.e. the most prevalent strain. This is well described in analyses of the VP1 of GII.2, GII.4, GII.7 and recombinant GII.b/GII.3 strains.[38,43,44]

The genome of NoVs has a high mutation rate leading to continuous evolution of the virus into new variants. While a link between increased viral evolution and increased viral incidence has been reported in Influenza, this link has not been established for the emergence of new NoV variants to viral incidence and epidemiological fitness. Recent reports suggest that the major capsid proteins of GII.4 are rapidly evolving leading to new viral strains with altered antigenicity.[32,38,43,45] Antigenic variation of RNA viruses, including NoVs, is successfully associated with evasion of the host immunity. It is suggested that GI NoVs have a conserved network of amino-acids required for capsid integrity. Consequently, this genogroup can tolerate limited variation. On the other hand, the capsid of GII NoVs has a flexible structural variation. Novel antigenic epitopes are generated as a result of insertions and deletions in the P2 region leading to propagation of escape variants that are consequently missed by the immune system.[11] Residues of the P2 subdomain of GII.4 are described to be under selective pressure and consequently leading to antigenic drift resulting in escape of the immune responses.[28,29,38,40]

In summary, antigenic drift, strain recombination,[35] antigenic shift and the polymerase fidelity of NoVs [43] clearly contribute to the continuous propagation of GII.4. The evolution of GII.4, described as being epochal (long periods of status quo followed by outbursts of variation) over time generates escape mutants that are periodically selected for by herd immunity.[11]

Viral shedding, natural infection with NoV and seroprotection

Limited data are available on the impact of natural infection with NoV and the sustainability of protection following infection. The scarce number of seroprevalence studies reveal that antibodies to NoV are detected during the first 2 y of age.[3] It was suggested that children in the developing countries can exhibit higher prevalence of antibodies directed against NoV as compared to developed countries due to early exposure, even though inconsistently. Previous studies indicated that infection with enteric viruses including NoV is significantly associated with early age[46-48] and consequently early exposure leads to development of immunity against the virus. In a recent longitudinal study held in the Cameroon,[49] reports show that adults and children show similar rates of infections with the virus. The prevalence rates among children ranged between 1 and 15%.[50,51] The same study reported the possible cross-protection between genotypes of the same genogroup; however, the duration of the study extended for only one year consequently the duration of protection could not be exact. Importantly, exposure to the natural infection increases with time as reflected by the accumulation of antibodies.[52] Similar results are reported in other developing countries whereby constant exposure to NoV infections in India has been described across all age groups.[53] The authors suggested that NoV infections occur in children and infants with reinfections being more common as compared to other developed settings (specifically the United Kingdom). Lower levels of NoV-specific antibodies were detected among adults above 50 y of age in the UK, suggesting increased susceptibility to infection.

In recent years, more studies evaluated the prevalence of NoV-specific antibodies in sera of patients from different age groups in different countries. Antibodies to GII.4 NoV were detected in 70% of sera samples collected from Portuguese adults [54] whereas the seroprevalence of IgG against GI.4, GII.3, and GII.4 NoV genotypes was 84.1%, 76.3%, and 94.5%, respectively in a cohort of Koreans.[55] This study reported low levels of antibodies among <23-month age group. An increase in seroprevalence occurred in early childhood, reaching 60.5% for GI.4, 65.1% for GII.3, and 90.7% for GII.4 at age 2–5 years, and over 80% for all 3 genotypes in subjects aged 20 y or older. Similar results were described in a cohort of young Chinese children with acute diarrhea.[56] High and stable seroprevalence rates were reported for GII.4 and GII.3 for children under 1 y of age (70.9% for GII.3 and 88.7% for GII.4). The anti-GII.4-positive rates were statistically higher than GII.3 among children less than 5 y participating in this study.[56] More studies from Europe shows similar high seroprevalence of GII.4 antibodies among children and young adults.[57] In a study following a newborn for up to 2 y of age, 4 different NoV infections were reported and antibodies were specific and non-protective.[58] The same group reported the generation of anti-GII.4 antibodies following infection among children less than 2 y old targeting closely related genotypes.[59,60] More than 90% of individuals are seropositive to NoV by the time they reach adulthood; however, the role of pre-existing immunity is still not fully understood when related to protection or reducing the risk of acquiring new NoV infections.[15] The fact that asymptomatic infection occurs at an early age as reported by outbreak and human challenge studies[48,61-63] provides critical insight to the potential reservoir of transmission at an early age and its clear implication on the need for an effective vaccine.

Similarly, limited data are available on the excretion of the virus during the natural course of infection in healthy individuals.[64,65] Long-term shedding was described among children less than 6 months,[63] in elderly,[64] hospitalized and immunosuppressed patients.[62] Predictive modeling of viral shedding classifies shedding into regular shedding with a mean of 14–16 d and long shedding with a mean ranging between 105 and 136 d[66] Recent studies suggest the lack of a significant difference of virus shedding between asymptomatic and symptomatic NoV infections despite the individual variation in virus peak levels detected in stool.[67,68] This study reported different viral

shedding activities as compared to previous reports[7,69] whereby infected adults shed virus during a range of 15–56 d. The authors argued that the variable results in NoV shedding might be due to the use of different PCR techniques to detect the virus. Atmar et al.[7] used NoV GI.1 which might have different shedding characteristics than the GII.4. This variation in peak levels along with slightly longer duration of shedding among symptomatic patients could be associated with enhanced efficiency of spreading NoV infection. The nosocomial transmission of NoV has prompted shedding studies among health care workers to assess the potential transmission of virus (NoV GII.4) in the hospital setting following recovery.[70] The results of the study concluded the ability of symptomatic health care workers to shed and transmit the virus. Further cohort studies are clearly needed to determine the impact of viral shedding and prevalence of antibodies on transmission and susceptibility to NoV, respectively.

Immune correlates of protection

The worldwide increased recognition of NoV as an important agent of diarrheal gastroenteritis prompted researchers to establish preventive strategies conferring long-lasting immunity. Previous attempts at generating vaccines have been impaired by the fact that NoV cannot be grown in cell culture systems. NoV VLPs, morphologically and antigenically similar to NoVs, are studied extensively and tested as promising vaccine candidates. VLPs are non-replicating particles devoid of the NoV genome.[15] They consist of recombinantly-expressed VP1 capsid protein. With the previous lack of a culture-system to produce live-attenuated or inactivated NoV, the use of VLPs has been an alternative approach. Recent developments have however established evidence demonstrating the ability of MuNoVs to persistently infect mouse B cell lines *in vitro*.[71] VLPs are immunogenic and able to enhance uptake by antigen-presenting cells (APCs) and to stimulate effector cells.[72] VLPs are similar to the wild virus with strain- and group-specific antigenic determinants.[11] The expression of VP1 as a recombinant protein results in self-assembly into particles that are structurally and antigenically similar to the virus.[72] There are 2 well described recombinant expression systems: the baculovirus replicon system[73] and the Venezuelan equine encephalitis replicon system.[74] These systems are used to amplify the capsid protein *in vitro* which then self-assembles to generate enough VLP quantities to be used in vaccine studies. Due to the high interest in developing a NoV vaccine, VLPs have been used as surrogates for studying the immunological and antigenic correlates of protection in animal and human immunization and/or challenge studies.

VLPs have also been instrumental in understanding the function of HGBAs as receptors or attachment factors mediating NoV infection whereby the first studied correlates of protection following experimental infection are the serum antibodies blocking the binding of NoV VLPs to HGBAs.[75] Human volunteers experimentally challenged with recombinant NoV VLP mounted blocking titers that peaked 28 d post challenge and were sustained at 180 d. These titers correlated with protection from disease and led to lower virus shedding. On the other hand, other studies did not associate these titers with clinical disease.[76-78]

The serum hemagglutinin inhibition assay (HAI) assay was also assessed as an alternative method to measure immune responses following challenge[79] and vaccination.[80] HAI titer was reported to increase with a concurrent increase in serum anti-NoV responses (measured by ELISA) following HuNoV challenge of healthy adults. Moreover, HAI titers were reported to significantly correlate with HBGA blocking antibody (ab) titers. Importantly, this study showed that infected human volunteers who did not manifest NoV gastroenteritis following challenge had a higher HAI titer as compared to those developing viral infection.[79] Following vaccination of healthy adults with GI.1 VLP, El Kamary et al.[80] used both HAI and HBGA blocking assay; this group demonstrated that IgG responses and HAI were in agreement in 72% to 75% of the cases and at the highest doses of the tested vaccine. Serum HAI, described as an easy assay measuring immune responses, is suggested as an alternative correlate of protection. However, its use is still outshined by the HBGA blocking assay.

While the functionality of the ab neutralization assay remains incomplete,[81] recent data demonstrated that high levels of HGBA blocking abs are detected in NoV –vaccinated individuals and were capable of reducing the likelihood of developing moderate to severe diarrhea in these individuals as compared to placebo recipients.[81,82] The same group suggested GII.4 virus-specific serum IgA as a possible correlate of protection due to their association with lower frequency of infection and illness. However, previous studies reported the inability of serum-specific IgA levels to protect against GI.1 challenge while prechallenge levels of salivary IgA and NoV-specific memory IgG correlated with protection against NoV.[83] These studies are performed on adults and it is yet to determine whether similar pattern of immune responses would be detected among children. While recent reports demonstrated cytokine responses following GI.I infection as compared to uninfected individuals;[84] the functional role of these cytokines as a correlate of protection is yet to be investigated.[84,85]

The lack of a well-established correlate of protection led to the use of the most commonly tested surrogate, i.e., HGBA blocking antibodies. A confounding factor related to protection from NoV infection is the debate around the duration of immunity, either following natural infection or vaccination. Simmons et al.[86] estimated a duration of 4.1 y to 8.7 y. This estimate was based on mathematical modeling taking into account the age-specific incidence of HuNoV, population immunity and the seasonality of the infection. Different NoV genotypes and viruses in a genotype bind different HBGA. Moreover, many NoVs bind more than one type of HBGA. This differential HBGA binding is a complicating factor allowing a virus neutralized in one population to infect another one.[11] While the understanding of immunologic correlates of protection against noroviruses has grown, it is yet to identify consistently reliable correlate(s) of immunity and protection from disease. This is especially critical for the progress of vaccine studies targeting an evolving RNA virus.

Below is a summary of the most recent developments in animal and human vaccine/challenge studies and their impact on the immune responses and the future progress of a NoV vaccine.

Animal studies

Original studies testing VLPs in the mice model showed the stimulation of NoV-specific IgG and mucosal IgA responses. These responses were more efficiently mounted through the intranasal administration as compared to the oral route.[72,87] Similarly, the intranasal delivery of VLPs in BALB/c mice induced humoral and cellular specific responses.[88] These studies provided the proof of concept that mucosal administration of VLP-based vaccines can generate protective responses in animal models. Moreover, these studies experimented with the inclusion of different adjuvants to assess their effect on the generated immune responses. The use of adjuvants was reported to induce higher IgA-secreting cells in Peyers patches and Th2 cytokines.[72] Originally, NoV infection, NoV challenge studies, and studies assessing immunity to infection and re-infection were performed on human volunteers. Recent reports have established the chimpanzee model as an animal model to study NoV infection and immunity.[89] Chimpanzees vaccinated with GI VLPs were protected against homologous challenge with NoV infection and mounted a long-lasting serum antibody response (up to 24 months). These responses correlated with resistance to viral re-infection. The shedding of the virus in this model was also similar to the one observed in humans. While results showed promise in the evaluation of vaccine efficacy using the chimpanzee model, these studies have been banned due to ethical restrictions on the use of non-human primates.[90] Similarly, cellular immunity (central memory $CD4^+$ T cell phenotypes as well as an antigen-specific $CD4^+$ T cell response) and high titers of NoV-specific IgG were detected following intranasal immunization to NoV P particle and full length VLPs in the mice model.[91] These results confirmed the ability of different platforms to induce humoral and cellular immunity and an important advancement toward the development of a NoV vaccine in face of its increasing association with diarrheal outbreaks among children.

Table 1 includes a summary of the animal studies and their ability to stimulate immune responses. Gnotobiotic pigs[92] and calves[93] were found to be successfully permissive for infection with human NoV GII.4. Low-to-moderate levels of serum-specific IgG and IgA were induced,[93] moreover, gnotobiotic pigs and calves inoculated orally/intranasally with a VLP expressing human NoV GII.4 mounted Th1 (IFN-γ, IL-12) and/or Th2 responses (IL-4).[93,94] Following homologous challenge, this model was able to mount systemic immune responses (Table 1). The same model was used further to compare the efficacy of P particles and VLPs derived from GII.4 against NoV infection.[95] Briefly, pigs were divided into 3 groups: group 1 received P particle via the intranasal route, group II received VLP via the intranasal route and group 3 received the NoV GII.4 variant orally (i.e. simulation of a primary infection). The animals were then challenged with a homologous strain of NoV and T cell responses studied. The results of this study show that previous infection with NoV led to substantial protection, which was higher than that provided by the P or the VLP particles. NoV primary infection induced an increase in $CD4^+$ T cell IFN-γ T cells in the duodenum. The latter is in agreement with observations detected in humans.[96] Moreover, this study showed an inverse correlation between the expansion of T cells regardless of the subset and protection post challenge with NoV infection. P particles and VLPs induced similar protection rates as vaccine candidates with higher numbers of activated $CD4^+$ T cells in different tissues, $CD8^+$ T cells in the duodenum, T regs in peripheral blood and $CD4^+$ $CD25^-$ $FoxP3^+$ T regs producing TGF-β in the spleen following vaccination with the former. While this study is important since it compares the protective efficacy of VLP- or P particle-based vaccine candidates against NoV infection, it also demonstrates/evaluates the role of different T cell subsets following primary infection, vaccination with VLP or P particles before and post-challenge. Conflicting results have been reported while comparing P particles to VLPs as vaccine candidates in the mice model whereby either similar responses were described or a P particle Th2-skewed response compared to a balanced Th1/Th2 response detected following use of VLPs.[91,97]

An important challenge hampering the formulation of NoV vaccines is the diversity of the virus. Consequently, it is yet to demonstrate the ability of any vaccine to induce durable cross-strain protection. Vaccination of chimpanzees with GII VLP protected animals against homologous viral challenge but not following heterologous challenge with GI.1.[89] The lack of broadly cross-reactive responses between NoV genogroups is a limiting factor hampering the generation of an effective vaccine. This has pushed the field toward extensive investigation of vaccine candidates stimulating immune responses against homologous and heterologous viral variants. Murine NoV (MNV-1) and human NoVs share many biochemical and genetic characteristics which led to the use of the former to understand the relationship between basic mechanisms of NoV replication in tissue culture, pathogenesis in a natural host and the role of innate and adaptive immune responses in the control of NoV.[98] The use of 2 subcutaneous doses of live MNV or Venezuelan Equine Encephalitis replicon particles (VRPs) expressing the MNV VP1 capsid protein induced long-term protection against oral infection with MNV.[99] The use of this live vaccine allowed for a sustained protection of 6 months and was found to be more protective than VRP –induced protection. In addition, the depletion of $CD4^+$ and/or $CD8^+$ T cells was associated with significant increase of viral titer in the intestines. The functionality of T cells was also tested and authors demonstrated that perforin but not IFN-γ is important for the clearance of MNV infection. This study highlighted the importance of the adaptive immunity to animal NoVs, even though in a homologous system of vaccination and challenge. The results of this study suggest that the generated long lasting immunity following oral exposure to MNV infection is a shared responsibility between the humoral and the cellular immune responses especially since mice lacking anti-MNV antibodies were protected.

Recently, the use of MNV-3 elicited stronger systemic and mucosal antibody responses when compared to those induced by MNV-1 following initial infection with either virus followed by challenge with a homologous strain.[100] The resulting humoral and cellular responses were cross-reactive toward MNV-1 challenge following primary infection with MNV-1. MHC class $II^{-/-}$ genetically deficient mice were not able to mount tissue-specific immune responses following infection with MNV-3 followed by

Table 1. Norovirus vaccine studies: Animal studies.

Vaccine Formulation	Model and Mode of immunization	Challenge	Immune Responses Post Vaccination	Protection Post Challenge	Reference
GI or GII VLPs	Chimpanzee; Intramuscular	Homologous and heterologous challenge (GI, GII)	Serum antibody response	Protected against infection post challenge with GI or 18 months post vaccination.	Bok et al., 2011
GII.4 VLPs and P particles	Mice; Intranasal	Challenge study	Serum IgG responses and $CD4^+$ T cell responses to VLPs and P particles	NA	Fang et al., 2013
GII.4 VLP	Pigs; Oral/ Intranasal	Homologous challenge	Low-to-moderate titers of NoV specific serum IgG	Not tested	Cheetham et al., 2006
GII.4 VLP	Calves/ Oral/ Intranasal	Homologous challenge	Low titers of serum antibodies; systemic and intestinal ASC; Th1and Th2 activation	Not tested	Souza et al., 2008
GII.4 VLP	Pigs; Oral/ Intranasal	Homologous challenge	Systemic and intestinal ab immune responses; IgM, IgA and IgG ASC; Th1 and Th2 cytokines	Increased protection rate against diarrhea and viral shedding following immunization and challenge	Souza et al., 2007
GII.4 VLP/P particles	Pigs; Intranasal	Homologous challenge	T cell expansion in tissues (stronger responses by P particles)	Substantial cross-protection against diarrhea	Kocher et al., 2014
GII.4 VLP/P particles	Mice; Intramuscular/ Intradermal	No challenge	High avidity antibodies induced by VLPs and IFN-γ production by T cells	Not tested	Tamminen et al., 2012
Live MNV and VRPs expressing VP1 capsid protein	Mice; Subcutaneous	Homologous challenge	B and T cell responses	Long term protection against oral infection with MNV (up to 6 months)	Chachu et al., 2008
MNV-3	Mice; Intraperitoneal	Homologous challenge	Systemic and mucosal virus-specific antibody and $CD4^+$ T cell responses	Cross-protection upon challenge /secondary infection	Zhu et al., 2013
GI.1–1968 or GII.4–2002 VLPs	Mice; Oral	No challenge	Cross-reactive IFN-γ production	Not tested	LoBue et al., 2010
Monovalent VLP GI/GII or multivalent	Mice; Footpad inoculation	Heterologous challenge (oral)	Cross-reactive antibody immune responses by multivalent vaccines	Decreased viral load following MNV challenge correlated with reduction in clinical diseases but not protection against infection	LoBue et al., 2009
rVSV-VP1 (NoV)	Mice; Intranasal and Oral (combination)	No challenge	High serum–specific IgG; mucosal responses (fecal and vaginal IgA)	Not tested	Ma et al., 2014
Recombinant adenovirus vector expressing capsid of NoV GII.4	Mice; Intranasal (prime-boost regimen)	No challenge	NoV-specific serum IgA, IgG and IgM ; mucosal responses (fecal IgG and IgA); NoV-specific IFN-γ T cell responses; Th1 and Th2 responses	Not tested	Guo et al., 2008 and 2009
P particle-VP8 chimeric vaccine (bivalent)	Mice; Intranasal	NA for NoV (RV challenge)	Antibodies blocking the binding of NoV VLPs to HBGA	Not tested for NoV	Tan et al., 2011
P particle and M2e influenza epitope (bivalent)	Mice; Intranasal or subcutaneous (with adjuvant)	NA for NoV	Antibodies blocking the binding of NoV VLPs to HBGA	Not tested for NoV	Xia et al., 2011
rVP6 of RV and NoV GII.4 VLPs (bivalent)	Mice; Intradermal or Intramuscular	No challenge	NoV-specific IgG; cross-reactive Nov specific antibody responses; antibodies blocking the binding of NoV VLPs to HBGA	Not tested	Blazevic et al., 2011
rVP6 of RV, NoV GII.4 VLPs and GI-3 VLPs (trivalent combination)	Mice; Intramuscular	No challenge	Trivalent: Cross-reactive serum IgG antibodies against heterologous NoV-VLPs; antibodies blocking the binding of NoV VLPs to HBGA	Not tested	Tamminen et al., 2013

(*Continued*)

Table 1. (*Continued*)

Vaccine Formulation	Model and Mode of immunization	Challenge	Immune Responses Post Vaccination	Protection Post Challenge	Reference
GI and GII.4 consensus VLPs (bivalent)	Rabbits; Intramuscular	No challenge	(similar for single and trivalent) High serum antibody titers as compared to monovalent vaccines	Not tested	Parra et al., 2012

NA, Not applicable; ASC, antibody-secreting cell.

homologous challenge. Importantly, this study demonstrated the ability of MNV-3-specific $CD4^+$ T cells to confer partial tissue protection (ileum but not colon) to $RAG1^{-/-}$ mice. Different cytokines profiles were induced by MNV-1 and MNV-3; further knowledge is needed to determine the impact of these cytokines on protective immunity. The P domain (P Polypeptide) was detected in the stool of NoV-infected patients; however, the role of this truncated protein without a C-terminal arginine cluster is unknown. Consequently, a number of studies compared the ability of these domains to VLPs in stimulating a cell-mediated immune response in the BALB/c mice model. The ability of the P domain complexes to induce a polyfunctional $CD4^+$ T cell memory was studied[91] following the intranasal immunization of mice with 3 doses of the P particle, P dimer or NoV VLP. Sera were collected and splenocytes of immunized mice were used *in vitro* in intracellular cytokine staining assays. The P domain and VLP complexes induced strong antibody responses in immunized mice. Moreover, P domain complexes and VLPs induced $CD4^+$ central memory responses. The lower immunogenicity of the P dimer as compared to the P domain and VLP is suggested to be due to its small size and the lower valence of antigenic structures. Moreover, P domain complexes and VLP complexes were able to induce the maturation of bone marrow-derived dendritic cells (DCs), a finding supporting the existence of T cell epitopes presented efficiently by DCs. It remains unknown whether these particles are able to stimulate a cytotoxic T cell response. Similar studies elucidated further the role of DCs in controlling MNV infection by facilitating the generation anti-MNV antibodies whereby DC- depleted mice suffered from increased viral titers in the intestinal tract and a significant decrease in the generation of antibody responses 2 weeks following infection.[101] This finding confirmed the role of DCs in the generation of an adaptive immune response against NoVs in this model. While histological data from patients infected with NoV showed the expansion of perforin-positive cytotoxic T cells,[102] the cellular immune responses mounted against NoVs were further studied in the animal model. Mice were immunized with Venezuelan equine encephalitis (VEE) virus replicon particles expressing NoV VLPs derived from GI.1–1968 or GII.4–2002.[103] Splenocytes from mice immunized with live MNV were stimulated *in vitro* with a panel of VLPs containing overlapping 15-mers peptides spanning the NoV GI.1 and GII.4 capsids. $CD4^+$ T cells were able to secrete IFN- γ following homotypic but not heterologous *in vitro* stimulation with human NoV VLPs. The use of cell suspensions depleted of $CD4^+$ and /or $CD8^+$ T cells showed that significantly lower levels of IFN-γ were produced by splenocytes stimulated *in vitro* as compared to CD8-depleted splenocytes. The role of $CD4^+$ Th1 T cell responses against NoVs has been previously addressed.[96,104] The same group previously demonstrated that multivalent vaccination could be used as a tool to generate cross-reactive antibody immune responses to heterologous strains of NoV.[105] Decreased viral load following MNV challenge correlated with reduction in clinical disease but not protection.

Other vectors have been tested as well in animal models for possible stimulation of immune responses against NoVs. A recent recombinant vesicular stomatitis virus (rVSV) expressing human NoV capsid protein (rVSV-VP1) was administered intranasally and orally to BalB/c mice; this model induced in BalB/c mice NoV-specific mucosal and T cell immune responses.[106] High NoV-specific serum IgG response was detected; this response was described to be stronger than the one induced by VLP-based candidate vaccines. Specifically, fecal and vaginal IgA was detected following inoculation of mice with this vaccine candidate. T cell proliferative responses to norovirus antigens were also stimulated by this vaccine candidate. This study introduced VSV as a possible live vector for the delivery of a NoV vaccine.

Guo et al. demonstrated the ability of an adenovirus vector expressing the capsid of human NoV to induce humoral, mucosal and T cell responses in the mouse model.[107,108] NoV-specific IgG, IgA and IgM were detected in sera along with fecal IgG and IgA. IFN-γ, TNF-α, IL-4 and IL-2 increased as well. However this vaccine strategy is hampered by the preexisting immunity to adenovirus in the human population and to our knowledge was not taken further during the last few years.

As previously noted, the use of NoV P particle in preclinical animal trials revealed the ability of this system to induce robust immune responses and protection in the murine model. NoV P particle was used in a dual vaccine combination with the VP8 of rotavirus, the major neutralizing antigen[109] and the peptide M2e of influenza.[110] The P particle-VP8 chimeric vaccine stimulated high titers of specific anti-NoV antibodies as well as higher VP8-specific antibodies when compared to the titers induced following vaccination with the RV VP8 particle alone. Mice were immunized via the intranasal route. This bivalent vaccine blocked the binding of NoV VLP to HBGA attachment factors.[109] Similarly, a bivalent vaccine containing NoV P particle and the M2e influenza epitope induced a strong and protective Th2 response against lethal challenge of mice with mouse-adapted influenza virus H1N1. Similarly, this vaccine candidate stimulated strong anti-NoV antibodies inhibiting the binding

of NoV VLPs to the corresponding receptors (vaccine and challenge were administered via the intranasal route and the subcutaneous route with adjuvant).[110] While the use of the NoV P particle as a candidate platform for vaccine development against RV and Influenza virus is tested, it also showed a substantial stimulation of NoV-specific responses. The role of these dual vaccines is to be evaluated in humans.

More work on a potential vaccine candidate containing NoV and RV combination was recently tested.[111,112] Blazevic et al.[111] used rVP6 of RV and NoV GII.4 VLPs to immunize BALB/c mice parenterally. This combination vaccine induced cross-reactive NoV- (GII.4, GII-12, and GI-3 VLPs as heterologous antigens) and RV-specific antibody responses. Importantly, sera from immunized mice (with the single or bivalent form) were found to block the binding of GII.4 VLPs to HBGA H-type 3 of mice. This blocking activity was retained for up to 27 weeks. Similarly, the VLP GII.4 single vaccine form stimulated a cross-reactive antibody response within the same GII genogroup and with GI. Tammineen et al.[112] extended the ingredients of the vaccine above to include GI.3 VLPs, the most common GI genotype among pediatric patients. This was in an attempt to induce neutralizing antibodies against both GI and GII NoVs. In this study, a trivalent combination vaccine containing GI.3 and GII.4 VP1 derived VLPs as well as recombinant VP6 of RV (the most conserved and abundant RV protein) were administered via the intramuscular (IM) route.[112] IM administration of the trivalent vaccine was performed and compared to single administration of each of GI.3 and GII.4 VP1 derived VLPs as well as recombinant VP6 of rotavirus, separately. The results showed the generation of cross-reactive IgG antibodies against heterologous NoV VLPs, specifically GII.4, GII.12 and GI.1. These responses lasted for 6 months. The administration of single monovalent vaccines induced stronger homologous responses. Importantly, the combination vaccine stimulated T-cell responses with robust IFN-γ specific responses. These responses were raised against both NoV and RV 15-mer peptides representing the capsid P domain of homologous and heterologous NoV (GII). This response was also durable and lasted for 24 weeks.

Interestingly, IM immunization of rabbits with GII.4 consensus VLP generated high serum antibody titers against VLP derived from different wild-type GII.4 viruses. Low levels of cross reactivity were detected against GI NoV strains using these sequences.[113] In an attempt to investigate the ability of this vaccine model to induce better responses against heterologous viral strains, a bivalent vaccine containing GI.1 and GII.4 consensus VLPs was tested. These forms were also administered intramuscularly. The bivalent form of the vaccine induced the highest homologous and heterologous antibody titers. This suggests that the use of consensus protein can stimulate a broadly cross-reactive antibody response against NoV genotypes. An earlier study using the murine model reported that a trivalent vaccine (GI.1, GII.1, and GII.2) was able to induce high level of antibodies against homologous and heterologous VLPs, including GII.4. The same group reported however that a tetravalent form of the vaccine containing GI.1, GII.1, GII.2 and GII.4 was less efficient at stimulating a broad antibody response.[103]

While many experimental vaccine designs have used the murine model, a number of concerns remain in place in the results presented above: 1) the different disease biology between mice and humans whereby only immunocompromised mice clinically manifest diarrhea as demonstrated by severe illness following MNV infection among innate-immunity-deficient mice (IFN-α/β and IFN-γ receptor-deficient)[114]; 2) the difficulty in extrapolating the clearance of MNV from the intestines to the possible physiological results in humans; 3) MNV does not use HBGA for attachment and is characterized by little genetic and antigenic variation.[115] Nevertheless, the mouse model is still a convenient and a cost effective platform for potential NoV vaccine studies.[98] Recently, the use of the Tulane virus (TV), a recovirus strain (ReCV), isolated from the stool of rhesus macaques [116] has been advanced as a possible surrogate model for human NoV gastroenteritis. Anti-NoV and anti-TV neutralizing antibodies have been reported in macaques;[117] moreover, TV-neutralizing antibodies were detected in sera of primate caretakers[118] as well as the general population yet at lower levels.[119] The role of HBGA was also tested in TV infection whereby type A and type B were found to be involved in the latter. Due to all the above, this model is thought to reflect the diversity of human NoVs, the reproducibility of the disease symptoms, the feasibility of testing the susceptibility to infection and attachment to receptors as a result of the availability of susceptible and non-susceptible cell lines.[115,120] The non-human primate model is genetically and immunologically close to humans and consequently could be the next-generation model to characterize and investigate NoV infection and potential vaccines.

In summary, the variability of the presented results from the animal models may be related to a number of factors: the antigen dose, the mode of delivery of the vaccine, the lack of data on previous correlates of immunity (previous NoV exposure) and the effect of previous exposure on protection or decrease in severity of illness following exposure to homologous or heterologous viral variants. The experimental work performed on candidate NoV vaccines in animal models provided the proof-of-concept for the feasibility of using VLP-based vaccines and other platforms and their ability to stimulate humoral and cellular immune responses. Most of the evidence in the field of NoV originates from generated data from animal models;[121] the promising results from animal models enhanced the interest in human challenge studies and vaccine trials further in an attempt to prevent and control human NoV (HuNoV) infection. Moreover, HuNoV was experimentally used to infect chimps, gnotobiotic pigs or calves.[89,92,93] Recently, a human NoV mouse model has been advanced.[122] The main advantage of using this model is the use of human viruses in an easy manipulated small animal model suggested to provide a useful resource for evaluating anti-NoV therapies. MNV on the other hand is the only NoV with an existing efficient culture system in the small mouse model. Since its identification, this model has been the most frequently used model as a surrogate for human NoV.[98]

The diversity of NoV has challenged/hampered the design of an efficient vaccine. Monovalent NoV vaccine studies performed in animals have proved the ability of these platforms to induce sometimes a protective and other times non-protective immune responses (Table 1). Vaccines have been engineered to include components representing different variants/and or strains in an attempt to generate broadly reactive immune

responses against different NoV strains. Bivalent[113] and multivalent vaccines with NoV components[76,103,105] were tested along with bivalent [109-111] and trivalent forms[112] with non-NoV representation (Table 1). The main results of these studies show the stimulation of cross-reactive humoral immune responses in the animal model. Comparing these results is not an easy task due to the lack of standardized route of administration of the vaccines, dosages, and the lack of complete understanding of a correlate of protection; nevertheless these trials have paved the way for intensifying NoV vaccine research in human trials.

Human trials

During the past decade, a number of human trials testing NoV vaccines have been advanced (Table 2). Many of these trials use the VLP system that proved safe and immunogenic in animals and humans.[123] The safety and the immunogenicity of NoV GI.1 VLP were studied by Tacket et al.[104] Healthy adult volunteers between 18 and 40 y of age received increasing doses of NoV VLP on day 1 and day 21. The vaccine was administered orally. Following vaccination and regardless of the dosages, volunteers developed significant increases in serum IgA secreting cells with transient rise in IFN-γ produced by peripheral blood mononuclear cells (PBMCs) in response to HuNoV. The latter observation was recorded at lower VLP dosages. Moreover, mucosal IgA was detected (fecal, vaginal and salivary) albeit in only 40% of the vaccinees. This study marked the beginning of a whole new series of vaccine studies in an attempt to elucidate the correlates of protection following infection with HuNoV.

The ability of NoV GI.1 VLP expressing a TLR4 receptor agonist as an adjuvant was also tested in human trials.[80] Healthy volunteers (18–49 years) tolerated increasing doses of the vaccine. The vaccine was administered via the intranasal route as an alternative mucosal delivery to the oral one. IgA and IgG ASCs were detected among all study participants following the administration of 2 different doses (50 and 100 μg) of the vaccine. IgA ASC highly expressed the gut homing receptors $CD19^+$ $CD27^+$ integrin $\alpha_4/\beta_7{}^+$ $CD62L^-$ and thus homing to peripheral and mucosal tissues whereas IgG ASC were $CD19^+$ $CD27^+$ $CD62L^+$ supporting homing to peripheral lymphoid tissues only. Moreover, the HAI assay was performed on sera of volunteers; the results of this functional assay show that vaccinees mounted a high HAI titer at higher doses of the vaccine with 72% and 75% agreement between the HAI and the IgG and IgA recorded data, respectively. The ability of this vaccine to induce mucosal and systemic immunity is noteworthy; however, its cross-reactive capabilities were not tested nor its ability to prevent infection with HuNoV. The same vaccine construct was used to further evaluate the ability of GI.1 VLP to induce B cell memory responses.[124] B memory (B_M) cells, essential for reactivation upon antigenic re-exposure, were detected in circulation for up to 6 months following primary immunization. A dose-dependent functional B_M response was detected following the intranasal administration of the vaccine to healthy adults. Moreover, the frequencies of these cells in blood correlated with the level of antibodies in sera. 62% of vaccine recipients were protected following oral challenge as compared to 81% in the placebo group. High HBGA blocking ab titers was associated with a decrease in the rate of infection. This study is important due to the demonstration the maintenance of NoV-specific antibody response ensuring a rapid and strong anamnestic humoral response. It is yet to demonstrate of the ability of any vaccine targeting HuNoV to induce a similar impact on T cell immune responses. These reports proved the ability of this VLP-based vaccine to generate a strong humoral immune response with possible long-term protection through the maintenance of antibodies.[80,124] The same results toward other HuNoV strains would be an important step forward in the development of this vaccine candidate.

A randomized, double-blind, placebo-controlled, multicenter trial was conducted to assess the safety, immunogenicity, and efficacy of GI.1 VLP (baculovirus expression system) vaccine in preventing gastroenteritis.[125] Two doses of vaccine or placebo were administered intranasally to healthy 18–50 y adults followed by homologous inoculation with HuNoV. The participants enrolled in this study were likely to develop NoV –associated gastroenteritis due to the expression of O or A blood groups and a functional FUT2 gene. The results of this study confirm that the vaccine recipients were less likely to develop illness as compared to placebo-recipients. 70% of the vaccinees mounted NoV-specific IgA response similar to previous reports.[80] This study, like previously mentioned ones, did not test the vaccine immunogenicity and protective efficacy among young children and elderly. Nevertheless, it proved the ability of a 2-dose vaccine to stimulate homologous protection against NoV and to prevent infection.

While humoral immune responses are important in protection against enteric viruses including NoV, little is known about the ability of VLP particles to stimulate T cell functions. A group of volunteers were infected with snow mountain virus (SMV) and immune responses determined. Serum IgG and salivary IgA were detected post-challenge. These immune responses were cross-reactive within genogroups. VLPs of 3 NoV strains (GI.1, GII.1, and GII.2) were used to stimulate PBMCs *in vitro* pre- and post-challenge of these volunteers with SMV in an attempt to determine the ability of SMV challenge to stimulate T cell responses.[96] While this study does not address protective immunity, it highlighted the possible impact of previous NoV infection whereby PBMCs from many infected and uninfected participants mounted an *in vitro* IFN-γ response and produced other cytokines including TNF-α and IL-2.

The predominance of GII.4 as the circulating strain has been attributed to the evolution of P2 domain of these strains associated with receptor switching and antigenic drift.[38] As a result, evasion of protective immunity as well as resistant populations arises. Nevertheless, GI strains were used in human studies. VLPs of 5 GI strains were used *in vitro* to assess the heterotypic humoral and cellular immune responses following HuNoV infection.[77] Sera from healthy adults infected with GI.1 1968 strains were used. This study demonstrated the generation of cross-reactive IgG antibodies to a panel of GI VLPs using ORF2 genes of G1.1 1968, GI.2–1999, GI.3–2000 and GI.4–2000 as well as ORF-2 of GI.1–2001. Authors concluded that the high degree of reactivity observed could be explained by 1) the ability of GI.1 to induce high affinity antibodies; 2) the pre-existing memory B cell responses with high affinity to other GI

strains or 3) the conserved capsid protein acting as a cross-reacting ab binding site. IFN-γ specific T cell responses were also detected *ex-vivo* in PBMCs of most volunteers targeting GI VLP strain other than the infection one. This bias of humoral and T cell responses to GI VLPs was explained as a potential mechanism of deceptive imprinting or original antigenic sin (OAS). OAS explains the ability of the host immune responses to stimulate memory cells to produce specific cellular responses to a similar antigen rather than inducing a primary response to a virus variant slightly different from the founder or infecting strain.[126]

The majority of challenge studies were performed using GI.1 NoV strains. However, GII.4 is the predominant HuNoV genotype worldwide. Frenck et al.[127] performed the first challenge study using the GII.4 strain; the latter was administered orally to healthy adults 18–49 y old. This study recruited secretors and non-secretors in order to assess the association of HBGA type and susceptibility to HuNoV infection. Low levels of pre-existing anti-GII.4 antibodies were required prior to recruitment of participants to reduce the impact of previous immunity. The authors reported the strong correlation between the secretor status, i.e., expression of FUT-2 allele, and symptomatic disease.

The ability of a diverse panel of GI VLPs to induce high IgG levels with cross-reactive affinities was demonstrated and discussed above.[77] The authors suggested that this could be due to the ability of GI.1 NoV to stimulate high-affinity antibodies to heterologous strain, and that a common capsid protein may provide a common epitope recognized by cross-reactive antibodies. This argues for the need to establish a panel of monoclonal antibodies for epitope mapping. Moreover, this study confirmed previous reports supporting the ability of GI NoV strains to induce *in vitro* an IFN-γ response to homologous and heterologous GI strains. This study was taken further and study participants, 18–49 y old, were immunized intramuscularly with 2 doses of GI.I and GII.4C VLPs.[82] Sera samples from vaccinated participants and those receiving placebo were tested for VLP-specific antibodies responses to a variety of HuNoV strains (GI, GII.4 and non-GII.4). Moreover, the potential of cross-protection provided by this vaccine was tested via an antibody blockade assay to test for prevention of HuNoV infection. This vaccine induced IgG responses to NoV strains that were not included in the vaccine. The results of the blockade assay positively correlated with the vaccine-induced immune responses in vaccinated adults. An interesting finding is the range of cross-protection exerted by the vaccine candidate whereby the production of cross-blocking antibodies and protection were detected against a newly emergent GII.4 strain (not a constituent of the vaccine) to which pre-existing immunity was reported to be unlikely. The authors suggested that the activation of pre-existing cross-reactive memory B cells contributed to antibody responses directed against GII.4C, a vaccine component. This study supports the use of a multivalent NoV vaccine; however, the evolution of the described antibody responses following virus challenge was not assessed. Similarly, a bivalent VLP formulation with GI.1 and GII.4 was designed and administered intramuscularly to healthy adults (18–49 years old).[128]A seroresponse was detected to both VLPs. This study differs from others in that IM administration of this bivalent vaccine resulted in higher serum ab responses as compared to the oral or intranasal route.[80,104] HBGA blocking titer was also high and suggested by authors to protect from HuNoV gastroenteritis.

Recently, a randomized, double-blind, placebo-controlled trial was performed at 5 sites in the United States. This trial was performed to evaluate the safety and tolerability of 2 doses of NoV VLPs produced in a baculovirus expression system containing GI.1 and GII.4.[129] The vaccine and placebo were administered intramuscularly. Eligible participants were of 18–50 y of age with functional FUT2 gene. Sera were collected prior to vaccine administration and 4 weeks post administration of the second dose. The data from this study reveals the ability of this construct to induce robust antibody responses following a single dose of the vaccine to both strains with a clear skew toward GI.1. Undetectable-to-little increase in these responses were recorded following the second dose. The vaccines and the placebo received a heterologous GII.4 orally as the challenge strain. This is the first efficacy evaluation of bivalent vaccines; vaccination appeared to decrease the incidence of acute illness, i.e. diarrhea and/or vomiting following challenge with GII.4. This study confirmed HuNoV infection however at a lower rate than those previously reported in other human challenge studies.[125,127]

The most recent published data are the results of the phase I clinical trial using VLPs containing GI.1 and GII.4 administered intramuscularly to 18–49 y healthy adults.[130] The results show the ability of this bivalent vaccine to induce serum antibody responses to GI.1 and GII.4 antigens albeit higher in the former. The specificity and the magnitude of the ASC response were also assessed. IgA-specific ASC was predominantly detected to GI.1 VLPs. One week following the first immunization, the vaccine recipients mounted high ASC; higher VLP doses did not correlate with higher frequencies of ASC. Following the second immunization, vaccine recipients showed lower ASC frequencies to both genogroups within the bivalent vaccine as compared to week one following the first vaccination. This is suggestive of little boosting effect following second vaccination while a rapid and robust B cell response resulted following the first vaccination. The authors suggested the activation of preexisting vaccine-specific memory B cells, i.e., a recall response rather than activation of naïve B cells. This is indicative of a previous exposure to HuNoV infection. A mucosal homing phenotype was detected on the surface of activated B cells following IM vaccination, suggesting the ability of a non-mucosal route of vaccine delivery to stimulate ASCs with mucosal homing characteristics. This study would need to be reproduced among participants that have not been previously exposed to HuNoV.

In summary, human clinical trials have proved to date that NoV VLP vaccines are safe and immunogenic. Both oral and intranasal modes of immunization elicited ab responses post vaccination (Table 2).[80,104,123-125] Moreover, the intramuscular administration of candidate vaccines containing GI and GII strains stimulated strong immune responses against the vaccine genotype.[129,130] Human challenge studies[77,96] as well as vaccine studies (Table 2) tested the ability of different vaccine formulas

Table 2. Norovirus vaccine studies: Human Studies.

Vaccine Formulation	Mode of Immunization	Challenge	Immune Responses Post Vaccination	Protection Post Challenge	Reference
r GI.1 VLP	Oral	None	IgG and IgA antibody responses	NA	Ball et al., 1999
GI.1 VLP	Oral	None	serum IgA and IgG responses; increased IgA ASCs;low-to-moderate mucosal IgA; transient rise in IFN-γ by PBMCS	Not tested	Tacket et al., 2003
GI.1 VLP expressing TLR4 receptor	Intranasal	None	Serum NoV-specific IgA and IgG antibodies; IgA and IgG ASCs; HAI titers detected	Not tested	El-Kamary et al., 2010
GI.1 VLP expressing TLR4 receptor	Intranasal	GI.1 virus (oral)	Anamnestic humoral response (B memory cells), NoV-specific IgA and IgG; and mucosal ASCs; High HGBA blocking titers	62% of recipients protected against infection	Ramirez et al., 2012
GI.1 VLP (baculovirus expression system)	Intranasal	Homologous challenge (GI.1)	Serum NoV-specific IgA response; presence of HBGA blocking antibodies	Protection and prevention of infection (70%). Delay of infection onset without a decrease in duration of illness	Atmar et al., 2011
VLPs of 3 NoV strains (GI.1, GII.2, GII.1)	NA	Challenge study	Serum IgG and salivary IgA, Th1 cytokines (IFN-γ, TNF-α, IL-2)	NA	Lindesmith et al., 2005
GI.1 1968	NA	Challenge study	Cross-reactive IgG antibodies against a panel of GI VLPs; IFN-γ specific T cells	NA	Lindesmith et al., 2010
GI.I/GII.4C VLPs	Intramuscular	No challenge	Cross-reactive serum IgG and cross blocking antibodies against non-vaccine strains	NA	Lindesmith et al., 2015
GI.1 and GII.4 VLPs (bivalent)	Intramuscular	NA (phase 1 trial)	Serum antibodies response to GI.1 and GII.4, high HBGA blocking titers	Not tested	Treanor et al., 2014
GI.1 and GII.4 VLPs (bivalent)	Intramuscular	Heterologous GII.4 virus (oral)	Robust antibody responses following first dose against GI.1 and GII.4; high HBGA blocking titers	Decrease in the incidence of acute illness	Atmar et al., 2015; Bernstein et al., 2015
VLPs containing GI.1/GII.4	Intramuscular	No challenge	Serum antibody response to GI.1 and GII.4 antigens following immunization (high frequencies of IgA-specific ASC against GI.1 VLPs)	NA	Sundararajan et el., 2015

NA, not applicable; ASC, antibody-secreting cells; HAI, hemagglutination inhibition assay; HBGA, histo-blood group antigens.

to generate cross-reactive immune responses to account for the diversity of the circulating HuNoV strains. While the majority of these studies recruited healthy adults, HuNoV mainly affects children with acute gastroenteritis requiring hospitalizations. Consequently, many fundamental questions remain unanswered: how well these vaccines will extrapolate to children, the duration of protection among different age groups, the effect of previous infection on the frequency and the magnitude of the immune responses and protection, the impact of genetic variation and escape mutants on the efficacy of these vaccines. The breadth of the immune response, the impact of pre-existing immunity, the relationship between these responses and prevention of NoV infection are all understudied and require further attention. The results presented above indicate the feasible potential of a vaccine. The ability to formulate different VLPs and their ability to stimulate humoral and mucosal responses and cellular responses as well as stimulating HBGA blocking ab titers used as surrogates for protection are all suggestive of the potential success of a protective vaccine. While the route of immunization is a main factor affecting the efficacy of the vaccine, the nasal inoculation of VLPs expressing GI.1 followed by homologous challenge resulted in 47% decrease in gastroenteritis among vaccinated healthy volunteers with concurrent generation of robust NoV-specific IgA abs.[125] The intramuscular administration of bivalent vaccine has also been associated with a reduction in acute gastroenteritis.[129]

Recent developments have established evidence demonstrating the ability of MuNoVs to persistently infect mouse B cell lines *in vitro*.[71] The authors also tested the ability of GII.4 isolate to infect human B cell line; the former replicated efficiently in B cells and produced new infectious virus particles. The tropism of human and murine NoV to B cells is a breakthrough for the development of human NoV infection and vaccine model. The potential of this model is to be further investigated and might enhance our knowledge in the field of NoV research as well as correlates of protection in humans.

Challenges and limitations

A number of factors hamper the generation of an efficient and protective vaccine against NoV. The incomplete understanding of the virus shedding dynamics and its heterogeneity, the complications of diversity, evolution and selective pressure of the virus, and the debatable estimated immunity contribute to

delaying the development of a successful vaccine.[11] The generated viral variants and their ability to bind different HBGAs add to the complexity of generated immune responses targeting NoV. Moreover, a clear assessment of the impact of pre-existing immunity and protection against NoV infection is yet to be achieved.[11,75] While a number of animal and human studies are being studied for a potential NoV vaccine, the problem of cross-reactivity being skewed by previous exposure to NoV and its variability among individuals remains a major obstacle. In addition, the current state of studies on NoV vaccine in animal and human models is still faced with many imperfections: 1) the immunocompetent mouse model does not produce similar diarrheal disease as human NoV;[114] 2) the murine model does not allow for strain heterogeneity as the case among human NoVs; 3) the failed attempts to culture NoV in macrophages, DC and other cells lines of human and animal tissues[131,132] result in the inability to generate live attenuated or killed virus and consequently disrupting the possibility of analyzing a strong and long-term protection; 4) while the ReCV model has demonstrated its feasibility as a surrogate model for human disease and vaccine especially due to the similarities between the clinical disease manipulation in humans and the non-human primate macaques and more importantly the diversity observed in both,[115] this model is yet to be explored further; 5) many of the human studies listed above are performed in young healthy adults, leaving a burning question unanswered as to the extension of the findings to other age groups. This remains an impediment to vaccinologists trying to assess the impact of pre-existing immunity on successful immunization strategies; 6) finally, the duration of the vaccine-induced immunity and its toll on the NoV diversity and antigenic variability. All of these are critical to guide the further development of a broadly effective NoV vaccine. Defining and identifying specific T cell epitopes of NoV capsids are important to understand the antigenic and immunogenic relationships of different viral strains. This has been demonstrated to be critical in the mice model[103] whereby cross-reactive NoV $CD4^+$ T cell epitopes were identified. Vaccine studies might need to characterize major NoV ab-binding epitopes, a critical factor to prevent the emergence of antigenic variants. As a result of the lack of understanding the immune correlates of protection along with the continuous drifting of the virus, it was suggested that an annual process of strain selection, as the case with influenza virus, might be a possible solution targeting the predominant circulating strains.[10]

Lessons from acute infections: Influenza and rotavirus

Influenza vaccines are designed on annual basis due to the rapid evolution of the virus leading to the generation of escape variants going unrecognized by the existing antibody response. The currently available seasonal vaccines protect against matching influenza A virus (IAV) through the stimulation of humoral immune responses. The dilemma of Influenza control is currently addressing the immune pressures selecting for new variants that are no longer recognized by the influenza-specific antibodies.[133] While T cells are known to recognize conserved IAV epitopes and being potentially cross-protective,[134] recent studies suggest that mutations at the level of both $CD8^+$ and $CD4^+$ T cell epitopes can occur.[133] Consequently, the exclusion of antigens subject to immune selection might be worth capitalizing on.

Consequently, a new form of the vaccine is needed. An ideal vaccine should be able to induce both arms of the immune response: the humoral and cell-mediated. While strong and long-lived T cell responses are urgently needed, care should be given to the ability of this vaccine to induce cross-reactive immune responses targeting circulating strains and their variants. The role of preexisting $CD8^+$ T cell immunity to the 2009 H1N1 pandemic provided evidence that these cells are crucial and correlated with milder forms of the disease in the absence of specific antibodies.[135] A significant inverse correlation was also demonstrated between the severity of the disease and the levels of preexisting $CD4^+$ T cells.[136] It is clear that further understanding of the impact of the role of $CD8^+$ and $CD4^+$ T cells in the control of Influenza A viruses is critical. Drawing lessons from Influenza A and its corresponding vaccines is important to consider during the design of NoV vaccine models.

An efficacious immune response would have to induce long-term protection against NoV homologous and heterologous viral strains. In addition to cross-protection between distantly related NoVs, a vaccine should stimulate both humoral and T cell mediated immune responses. Defining the correlates of protection against NoV gastroenteritis through vaccine studies is crucial to predict and estimate the immunogenicity and effectiveness of these potential vaccines. Large-scale studies are needed and specifically in limited-resource countries. This is to preemptively prevent the hardships observed with RV vaccines. RV vaccines have been successful and effective in high income countries while partially protective in low income countries.[137] A number of determinants for variable levels of protection have been suggested to be associated with poor responses to RV vaccines. These include environmental enteropathy, genetic and epigenetic determinants, nutritional deficiencies, gastrointestinal normal flora and microbial infections other than RV, and RV type. Vaccine effectiveness studies in resource-limited settings are important to define immunological determinants of protection.

Conclusion

NoV remains an understudied causative agent of acute gastroenteritis in the developing countries. According to the 2013 Global Burden of Disease study (GBD), 11·8 million deaths were caused by communicable, maternal, neonatal, and nutritional disorders. Even though deaths caused by diarrhea fell by 51% between 1990 and 2013, 1·3 million are still reported (1·2–1·4) diseases during the year 2013.[138,139] RV was described as the leading cause of diarrhea among children less than 5 y old. NoV is not mentioned in the GBD among the infectious agents causing gastroenteritis and diarrhea. This is a significant indication to the lack of clinical diagnosis and reporting of NoV as an important cause of disease. Vigilant molecular surveillance is key to preventing future NoV epidemics and developing vaccines. Efforts should be made to introduce the clinical diagnosis of the virus due to its impact on the community as well as health care institutions. This will help direct infection control and treatment. A vaccine form with NoV and RV

representations could be ideal to control the 2 leading causes of viral acute gastroenteritis, especially due to the ability of the recently formulated candidates to induce protective immune responses.[109,111,112] Consequently, when designing a NoV vaccine, understanding the potential factors that might affect its efficacy in resource-limited countries will be important. Data from mathematical modeling suggest a 4–8.7 y protection from natural protection.[86] The duration of the protection following vaccination is to be determined and large field efficacy studies on children, adults, elderly and immunocompromised patients are needed. The innate responses and susceptibility to NoV, the role of herd immunity, a clear assessment of the impact of pre-existing immunity and duration of protection, genetic determinants of susceptibility, the role of cellular immunity and cytokine responses, comparison of these factors in different age groups from different socio-economic settings should be the focus of intervention studies.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

I thank Ms. Rana Charide and Ms. Maha Abul-Naja for their technical assistance.

References

[1] Updated norovirus outbreak management and disease prevention guidelines. MMWR Recomm Rep 2011; 60:1-18.
[2] Lanata CF. Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE, Child Health Epidemiology Reference Group of the World Health O, Unicef. Global causes of diarrheal disease mortality in children. PLoS One 2013; 8:e72788; PMID:24023773; http://dx.doi.org/10.1371/journal.pone.0072788
[3] Patel MM, Hall AJ, Vinje J, Parashar UD. Noroviruses: a comprehensive review. J Clin Virol 2009; 44:1-8; PMID:19084472; http://dx.doi.org/10.1016/j.jcv.2008.10.009
[4] Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 2008; 14:1224-31; PMID:18680645; http://dx.doi.org/10.3201/eid1408.071114
[5] Green KY. Caliciviridae: the noroviruses. In Knipe DM, Howley P, eds. Fields Virology. Fields Virology. 6th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2013.
[6] Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL. Norwalk virus: how infectious is it? J Med Virol 2008; 80:1468-76; PMID:18551613; http://dx.doi.org/10.1002/jmv.21237
[7] Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, Graham DY. Norwalk virus shedding after experimental human infection. Emerg Infect Dis 2008; 14:1553-7; PMID:18826818; http://dx.doi.org/10.3201/eid1410.080117
[8] Lee BE, Pang XL. New strains of norovirus and the mystery of viral gastroenteritis epidemics. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne 2013; 185:1381-2; PMID:24003105; http://dx.doi.org/10.1503/cmaj.130426
[9] Barclay L, Park GW, Vega E, Hall A, Parashar U, Vinje J, Lopman B. Infection control for norovirus. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2014; 20:731-40; PMID:24813073; http://dx.doi.org/10.1111/1469-0691.12674
[10] Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Engl J Med 2009; 361:1776-85; PMID:19864676; http://dx.doi.org/10.1056/NEJMra0804575
[11] Donaldson EF, Lindesmith LC, LoBue AD, Baric RS. Viral shape-shifting: norovirus evasion of the human immune system. Nat Rev Micro 2010; 8:231-41; http://dx.doi.org/10.1038/nrmicro2296
[12] Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R. Human susceptibility and resistance to Norwalk virus infection. Nat Med 2003; 9:548-53; PMID:12692541; http://dx.doi.org/10.1038/nm860
[13] Le Pendu J, Nystrom K, Ruvoen-Clouet N. Host-pathogen co-evolution and glycan interactions. Current opinion in virology 2014; 7:88-94; PMID:25000207; http://dx.doi.org/10.1016/j.coviro.2014.06.001
[14] Ferrer-Admetlla A, Sikora M, Laayouni H, Esteve A, Roubinet F, Blancher A, Calafell F, Bertranpetit J, Casals F. A natural history of FUT2 polymorphism in humans. Molecular biology and evolution 2009; 26:1993-2003; PMID:19487333; http://dx.doi.org/10.1093/molbev/msp108
[15] Robilotti E, Deresinski S, Pinsky BA. Norovirus. Clinical microbiology reviews 2015; 28:134-64; PMID:25567225; http://dx.doi.org/10.1128/CMR.00075-14
[16] Nordgren J, Kindberg E, Lindgren PE, Matussek A, Svensson L. Norovirus gastroenteritis outbreak with a secretor-independent susceptibility pattern, Sweden. Emerg Infect Dis 2010; 16:81-7; PMID:20031047; http://dx.doi.org/10.3201/eid1601.090633
[17] Rockx BH, Vennema H, Hoebe CJ, Duizer E, Koopmans MP. Association of histo-blood group antigens and susceptibility to norovirus infections. J Infect Dis 2005; 191:749-54; PMID:15688291; http://dx.doi.org/10.1086/427779
[18] Zhang XF, Tan M, Chhabra M, Dai YC, Meller J, Jiang X. Inhibition of histo-blood group antigen binding as a novel strategy to block norovirus infections. PLoS One 2013; 8:e69379; PMID:23894462; http://dx.doi.org/10.1371/journal.pone.0069379
[19] Florescu DF, Hermsen ED, Kwon JY, Gumeel D, Grant WJ, Mercer DF, Kalil AC. Is there a role for oral human immunoglobulin in the treatment for norovirus enteritis in immunocompromised patients? Pediatr Tansplant 2011; 15:718-21; PMID:21883746; http://dx.doi.org/10.1111/j.1399-3046.2011.01556.x
[20] Chen Z, Sosnovtsev SV, Bok K, Parra GI, Makiya M, Agulto L, Green KY, Purcell RH. Development of Norwalk virus-specific monoclonal antibodies with therapeutic potential for the treatment of Norwalk virus gastroenteritis. J Virol 2013; 87:9547-57; PMID:23785216; http://dx.doi.org/10.1128/JVI.01376-13
[21] Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, Zhou T, Schmidt SD, Wu L, Xu L, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 2010; 329:856-61; PMID:20616233; http://dx.doi.org/10.1126/science.1187659
[22] Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A, Kwon YD, Scheid JF, Shi W, Xu L, et al. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 2010; 329:811-7; PMID:20616231; http://dx.doi.org/10.1126/science.1192819
[23] Law M, Maruyama T, Lewis J, Giang E, Tarr AW, Stamataki Z, Gastaminza P, Chisari FV, Jones IM, Fox RI, et al. Broadly neutralizing antibodies protect against hepatitis C virus quasispecies challenge. Nat Med 2008; 14:25-7; PMID:18064037; http://dx.doi.org/10.1038/nm1698
[24] Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, Vachieri SG, Pinna D, Minola A, Vanzetta F, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science 2011; 333:850-6; PMID:21798894; http://dx.doi.org/10.1126/science.1205669
[25] Ochoa TJ, Chea-Woo E, Baiocchi N, Pecho I, Campos M, Prada A, Valdiviezo G, Lluque A, Lai D, Cleary TG. Randomized double-blind controlled trial of bovine lactoferrin for prevention of diarrhea in children. J Pediatr 2013; 162:349-56; PMID:22939927; http://dx.doi.org/10.1016/j.jpeds.2012.07.043
[26] Nagata S, Asahara T, Ohta T, Yamada T, Kondo S, Bian L, Wang C, Yamashiro Y, Nomoto K. Effect of the continuous intake of

probiotic-fermented milk containing Lactobacillus casei strain Shirota on fever in a mass outbreak of norovirus gastroenteritis and the faecal microflora in a health service facility for the aged. The British journal of nutrition 2011; 106:549-56; PMID:21521545; http://dx.doi.org/10.1017/S000711451100064X

[27] Thorne LG, Goodfellow IG. Norovirus gene expression and replication. J Gen Virol 2014; 95:278-91; PMID:24243731; http://dx.doi.org/10.1099/vir.0.059634-0

[28] Lindesmith LC, Debbink K, Swanstrom J, Vinje J, Costantini V, Baric RS, Donaldson EF. Monoclonal antibody-based antigenic mapping of norovirus GII.4-2002. J Virol 2012; 86:873-83; PMID:22090098; http://dx.doi.org/10.1128/JVI.06200-11

[29] Debbink K, Donaldson EF, Lindesmith LC, Baric RS. Genetic mapping of a highly variable norovirus GII.4 blockade epitope: potential role in escape from human herd immunity. J Virol 2012; 86:1214-26; PMID:22090110; http://dx.doi.org/10.1128/JVI.06189-11

[30] Hoa Tran TN, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. J Clin Virol 2013; 56:185-93; PMID:23218993

[31] Katayama K, Shirato-Horikoshi H, Kojima S, Kageyama T, Oka T, Hoshino F, Fukushi S, Shinohara M, Uchida K, Suzuki Y, et al. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. Virology 2002; 299:225-39; PMID:12202225; http://dx.doi.org/10.1006/viro.2002.1568

[32] Siebenga JJ, Vennema H, Renckens B, de Bruin E, van der Veer B, Siezen RJ, Koopmans M. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J Virol 2007; 81:9932-41; PMID:17609280; http://dx.doi.org/10.1128/JVI.00674-07

[33] Allen DJ, Gray JJ, Gallimore CI, Xerry J, Iturriza-Gomara M. Analysis of amino acid variation in the P2 domain of the GII.4 norovirus VP1 protein reveals putative variant-specific epitopes. PLoS One 2008; 3:e1485; http://dx.doi.org/10.1371/journal.pone.0001485

[34] Allen DJ, Noad R, Samuel D, Gray JJ, Roy P, Iturriza-Gomara M. Characterisation of a GII.4 norovirus variant-specific surface-exposed site involved in antibody binding. Virol J 2009; 6:150; PMID:19781066; http://dx.doi.org/10.1186/1743-422X-6-150

[35] Eden JS, Tanaka MM, Boni MF, Rawlinson WD, White PA. Recombination within the pandemic norovirus GII.4 lineage. J Virol 2013; 87:6270-82; PMID:23536665; http://dx.doi.org/10.1128/JVI.03464-12

[36] Lindesmith LC, Costantini V, Swanstrom J, Debbink K, Donaldson EF, Vinje J, Baric RS. Emergence of a norovirus GII.4 strain correlates with changes in evolving blockade epitopes. J Virol 2013; 87:2803-13; PMID:23269783; http://dx.doi.org/10.1128/JVI.03106-12

[37] Giammanco GM, De Grazia S, Terio V, Lanave G, Catella C, Bonura F, Saporito L, Medici MC, Tummolo F, Calderaro A, et al. Analysis of early strains of the norovirus pandemic variant GII.4 Sydney 2012 identifies mutations in adaptive sites of the capsid protein. Virology 2014; 450–451:355-8; PMID:24503099; http://dx.doi.org/10.1016/j.virol.2013.12.007

[38] Lindesmith LC, Donaldson EF, Lobue AD, Cannon JL, Zheng DP, Vinje J, Baric RS. Mechanisms of GII.4 norovirus persistence in human populations. PLoS Med 2008; 5:e31; PMID:18271619; http://dx.doi.org/10.1371/journal.pmed.0050031

[39] Ruvoen-Clouet N, Belliot G, Le Pendu J. Noroviruses and histo-blood groups: the impact of common host genetic polymorphisms on virus transmission and evolution. Rev Med Virol 2013; 23:355-66; PMID:23959967; http://dx.doi.org/10.1002/rmv.1757

[40] Lindesmith LC, Donaldson EF, Baric RS. Norovirus GII.4 strain antigenic variation. J Virol 2011; 85:231-42; PMID:20980508; http://dx.doi.org/10.1128/JVI.01364-10

[41] Bull RA, White PA. Mechanisms of GII.4 norovirus evolution. Trends in microbiology 2011; 19:233-40; PMID:21310617; http://dx.doi.org/10.1016/j.tim.2011.01.002

[42] Tan M, Xia M, Chen Y, Bu W, Hegde RS, Meller J, Li X, Jiang X. Conservation of carbohydrate binding interfaces: evidence of human HBGA selection in norovirus evolution. PLoS One 2009; 4:e5058; PMID:19337380; http://dx.doi.org/10.1371/journal.pone.0005058

[43] Bull RA, Eden JS, Rawlinson WD, White PA. Rapid evolution of pandemic noroviruses of the GII.4 lineage. PLoS Pathog 2011; 6:e1000831; http://dx.doi.org/10.1371/journal.ppat.1000831

[44] Iritani N, Kaida A, Kubo H, Abe N, Murakami T, Vennema H, Koopmans M, Takeda N, Ogura H, Seto Y. Epidemic of genotype GII.2 noroviruses during spring 2004 in Osaka City, Japan. J Clin Microbiol 2008; 46:2406-9; PMID:18495859; http://dx.doi.org/10.1128/JCM.01993-07

[45] Bok K, Abente EJ, Realpe-Quintero M, Mitra T, Sosnovtsev SV, Kapikian AZ, Green KY. Evolutionary dynamics of GII.4 noroviruses over a 34-year period. J Virol 2009; 83:11890-901; PMID:19759138; http://dx.doi.org/10.1128/JVI.00864-09

[46] O'Ryan ML, Lucero Y, Prado V, Santolaya ME, Rabello M, Solis Y, Berrios D, O'Ryan-Soriano MA, Cortes H, Mamani N. Symptomatic and asymptomatic rotavirus and norovirus infections during infancy in a Chilean birth cohort. Pediatr Infect Dis J 2009; 28:879-84; PMID:19672213; http://dx.doi.org/10.1097/INF.0b013e3181a4bb60

[47] O'Ryan ML, Pena A, Vergara R, Diaz J, Mamani N, Cortes H, Lucero Y, Vidal R, Osorio G, Santolaya ME, et al. Prospective characterization of norovirus compared with rotavirus acute diarrhea episodes in chilean children. Pediatr Infect Dis J 2010; 29:855-9; PMID:20581736; http://dx.doi.org/10.1097/INF.0b013e3181e8b346

[48] Zhang S, Chen TH, Wang J, Dong C, Pan J, Moe C, Chen W, Yang L, Wang X, Tang H, et al. Symptomatic and asymptomatic infections of rotavirus, norovirus, and adenovirus among hospitalized children in Xi'an, China. J Med Virol 2011; 83:1476-84; PMID:21618552; http://dx.doi.org/10.1002/jmv.22108

[49] Ayukekbong JA, Fobisong C, Tah F, Lindh M, Nkuo-Akenji T, Bergstrom T. Pattern of circulation of norovirus GII strains during natural infection. J Clin Microbiol 2014; 52:4253-9; PMID:25274996; http://dx.doi.org/10.1128/JCM.01896-14

[50] Kabayiza JC, Andersson ME, Welinder-Olsson C, Bergstrom T, Muhirwa G, Lindh M. Comparison of rectal swabs and faeces for real-time PCR detection of enteric agents in Rwandan children with gastroenteritis. BMC Infect Dis 2013; 13:447; PMID:24073740; http://dx.doi.org/10.1186/1471-2334-13-447

[51] Trainor E, Lopman B, Iturriza-Gomara M, Dove W, Ngwira B, Nakagomi O, Nakagomi T, Parashar U, Cunliffe N. Detection and molecular characterisation of noroviruses in hospitalised children in Malawi, 1997–2007. J Med Virol 2013; 85:1299-306; PMID:23918547; http://dx.doi.org/10.1002/jmv.23589

[52] Nurminen K, Blazevic V, Huhti L, Rasanen S, Koho T, Hytonen VP, Vesikari T. Prevalence of norovirus GII.4 antibodies in Finnish children. J Med Virol 2011; 83:525-31; PMID:21264875; http://dx.doi.org/10.1002/jmv.21990

[53] Menon VK, George S, Aladin F, Nawaz S, Sarkar R, Lopman B, Gray JJ, Gomara MI, Kang G. Comparison of age-stratified seroprevalence of antibodies against norovirus GII in India and the United Kingdom. PLoS One 2013; 8:e56239; PMID:23437102; http://dx.doi.org/10.1371/journal.pone.0056239

[54] Mesquita JR, Nascimento MS. Norovirus GII.4 antibodies in the Portuguese population. J Infect Dev Ctries 2014; 8:1201-4; PMID:25212086.

[55] Son H, Jeong HS, Cho M, Lee J, Lee H, Yoon K, Jeong AY, Jung S, Kim K, Cheon DS. Seroepidemiology of predominant norovirus strains circulating in Korea by using recombinant virus-like particle antigens. Foodborne Pathog Dis 2013; 10:461-6; PMID:23627928; http://dx.doi.org/10.1089/fpd.2012.1300

[56] Liu X, Liu P, Wang J, Moe C, Hu S, Cheng L, Gu W, Wang X. Seroepidemiology of Norovirus GII.3 and GII.4 Infections in Children with Diarrhea in Xi'an, China. Foodborne Pathog Dis 2015; 12(6):500-5.

[57] Carmona-Vicente N, Fernandez-Jimenez M, Ribes JM, Tellez-Castillo CJ, Khodayar-Pardo P, Rodriguez-Diaz J, Buesa J. Norovirus infections and seroprevalence of genotype GII.4-specific antibodies in a Spanish population. J Med Virol 2015; 87:675-82; PMID:25655810; http://dx.doi.org/10.1002/jmv.24112

[58] Blazevic V, Malm M, Salminen M, Oikarinen S, Hyoty H, Veijola R, Vesikari T. Multiple consecutive norovirus infections in the first

2 years of life. Eur J Pediatr 2015; 174(12):1679-83; PMID:26152345.
[59] Blazevic V, Malm M, Vesikari T. Induction of homologous and cross-reactive GII.4-specific blocking antibodies in children after GII.4 New Orleans norovirus infection. J Med Virol 2015; 87:1656-61; PMID:25946711; http://dx.doi.org/10.1002/jmv.24237
[60] 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; PMID:24970849; http://dx.doi.org/10.1093/infdis/jiu361
[61] Hutson AM, Atmar RL, Graham DY, Estes MK. Norwalk virus infection and disease is associated with ABO histo-blood group type. J Infect Dis 2002; 185:1335-7; PMID:12001052; http://dx.doi.org/10.1086/339883
[62] Siebenga JJ, Beersma MF, Vennema H, van Biezen P, Hartwig NJ, Koopmans M. High prevalence of prolonged norovirus shedding and illness among hospitalized patients: a model for in vivo molecular evolution. J Infect Dis 2008; 198:994-1001; PMID:18774885; http://dx.doi.org/10.1086/591627
[63] Murata T, Katsushima N, Mizuta K, Muraki Y, Hongo S, Matsuzaki Y. Prolonged norovirus shedding in infants. Pediatr Infect Dis J 2007; 26:46-9; PMID:17195705; http://dx.doi.org/10.1097/01.inf.0000247102.04997.e0
[64] Tu ET, Bull RA, Kim MJ, McIver CJ, Heron L, Rawlinson WD, White PA. Norovirus excretion in an aged-care setting. J Clin Microbiol 2008; 46:2119-21; PMID:18417655; http://dx.doi.org/10.1128/JCM.02198-07
[65] Ayukekbong J, Lindh M, Nenonen N, Tah F, Nkuo-Akenji T, Bergstrom T. Enteric viruses in healthy children in Cameroon: viral load and genotyping of norovirus strains. J Med Virol 2011; 83:2135-42; PMID:22012721; http://dx.doi.org/10.1002/jmv.22243
[66] Milbrath MO, Spicknall IH, Zelner JL, Moe CL, Eisenberg JN. Heterogeneity in norovirus shedding duration affects community risk. Epidemiol Infect 2013; 141:1572-84; PMID:23507473; http://dx.doi.org/10.1017/S0950268813000496
[67] Teunis PF, Sukhrie FH, Vennema H, Bogerman J, Beersma MF, Koopmans MP. Shedding of norovirus in symptomatic and asymptomatic infections. Epidemiol Infect 2015; 143:1710-17.
[68] Kirby AE, Shi J, Montes J, Lichtenstein M, Moe CL. Disease course and viral shedding in experimental Norwalk virus and Snow Mountain virus infection. J Med Virol 2014; 86:2055-64; PMID:24531909; http://dx.doi.org/10.1002/jmv.23905
[69] Al-Mashhadani MN, Nakagomi O, Dove W, Ahmed H, Nakagomi T, Hart CA, Cunliffe NA. Norovirus gastroenteritis among children in Iraqi Kurdistan. J Med Virol 2008; 80:506-9; PMID:18205231; http://dx.doi.org/10.1002/jmv.21099
[70] Sukhrie FH, Teunis P, Vennema H, Copra C, Thijs Beersma MF, Bogerman J, Koopmans M. Nosocomial transmission of norovirus is mainly caused by symptomatic cases. Clin Infect Dis 2012; 54:931-7; PMID:22291099; http://dx.doi.org/10.1093/cid/cir971
[71] Jones MK, Watanabe M, Zhu S, Graves CL, Keyes LR, Grau KR, Gonzalez-Hernandez MB, Iovine NM, Wobus CE, Vinje J, et al. Enteric bacteria promote human and mouse norovirus infection of B cells. Science 2014; 346:755-9; PMID:25378626; http://dx.doi.org/10.1126/science.1257147
[72] Richardson C, Bargatze RF, Goodwin R, Mendelman PM. Norovirus virus-like particle vaccines for the prevention of acute gastroenteritis. Expert review of vaccines 2013; 12:155-67; PMID:23414407; http://dx.doi.org/10.1586/erv.12.145
[73] Jiang X, Wang M, Graham DY, Estes MK. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol 1992; 66:6527-32; PMID:1328679
[74] Baric RS, Yount B, Lindesmith L, Harrington PR, Greene SR, Tseng FC, Davis N, Johnston RE, Klapper DG, Moe CL. Expression and self-assembly of norwalk virus capsid protein from venezuelan equine encephalitis virus replicons. J Virol 2002; 76:3023-30; PMID:11861868; http://dx.doi.org/10.1128/JVI.76.6.3023-3030.2002
[75] Reeck A, Kavanagh O, Estes MK, Opekun AR, Gilger MA, Graham DY, Atmar RL. Serological correlate of protection against norovirus-induced gastroenteritis. J Infect Dis 2010; 202:1212-8; PMID:20815703; http://dx.doi.org/10.1086/656364
[76] LoBue AD, Lindesmith L, Yount B, Harrington PR, Thompson JM, Johnston RE, Moe CL, Baric RS. Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains. Vaccine 2006; 24:5220-34; PMID:16650512; http://dx.doi.org/10.1016/j.vaccine.2006.03.080
[77] Lindesmith LC, Donaldson E, Leon J, Moe CL, Frelinger JA, Johnston RE, Weber DJ, Baric RS. Heterotypic humoral and cellular immune responses following Norwalk virus infection. J Virol 2010; 84:1800-15; PMID:20007270; http://dx.doi.org/10.1128/JVI.02179-09
[78] Harrington PR, Lindesmith L, Yount B, Moe CL, Baric RS. Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. J Virol 2002; 76:12335-43; PMID:12414974; http://dx.doi.org/10.1128/JVI.76.23.12335-12343.2002
[79] Czako R, Atmar RL, Opekun AR, Gilger MA, Graham DY, Estes MK. Serum hemagglutination inhibition activity correlates with protection from gastroenteritis in persons infected with Norwalk virus. Clin Vaccine Immunol : CVI 2012; 19:284-7; PMID:22190401; http://dx.doi.org/10.1128/CVI.05592-11
[80] El-Kamary SS, Pasetti MF, Mendelman PM, Frey SE, Bernstein DI, Treanor JJ, Ferreira J, Chen WH, Sublett R, Richardson C, et al. Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. J Infect Dis 2010; 202:1649-58; PMID:20979455; http://dx.doi.org/10.1086/657087
[81] Atmar RL, Bernstein DI, Lyon GM, Treanor JJ, Al-Ibrahim MS, Graham DY, Vinje J, Jiang X, Gregoricus N, Frenck RW, et al. Serological Correlates of Protection against a GII.4 Norovirus. Clin Vaccine Immunol : CVI 2015; 22:923-9; PMID:26041041; http://dx.doi.org/10.1128/CVI.00196-15
[82] Lindesmith LC, Ferris MT, Mullan CW, Ferreira J, Debbink K, Swanstrom J, Richardson C, Goodwin RR, Baehner F, Mendelman PM, et al. Broad blockade antibody responses in human volunteers after immunization with a multivalent norovirus VLP candidate vaccine: immunological analyses from a phase I clinical trial. PLoS Med 2015; 12:e1001807; PMID:25803642; http://dx.doi.org/10.1371/journal.pmed.1001807
[83] Ramani S, Neill FH, Opekun AR, Gilger MA, Graham DY, Estes MK, Atmar RL. Mucosal and Cellular Immune Responses to Norwalk Virus. J Infect Dis 2015; 212:397-405; PMID:25635121; http://dx.doi.org/10.1093/infdis/jiv053
[84] Newman KL, Moe CL, Kirby AE, Flanders WD, Parkos CA, Leon JS. Human norovirus infection and the acute serum cytokine response. Clin Exp Immunol 2015; 182:195-203; PMID:26178578; http://dx.doi.org/10.1111/cei.12681
[85] Lindesmith LC, Beltramello M, Swanstrom J, Jones TA, Corti D, Lanzavecchia A, Baric RS. Serum Immunoglobulin A Cross-Strain Blockade of Human Noroviruses. Open forum infectious diseases 2015; 2:ofv084; PMID:26180833; http://dx.doi.org/10.1093/ofid/ofv084
[86] Simmons K, Gambhir M, Leon J, Lopman B. Duration of immunity to norovirus gastroenteritis. Emerg Infect Dis 2013; 19:1260-7; PMID:23876612; http://dx.doi.org/10.3201/eid1908.130472
[87] Ball JM, Hardy ME, Atmar RL, Conner ME, Estes MK. Oral immunization with recombinant Norwalk virus-like particles induces a systemic and mucosal immune response in mice. J Virol 1998; 72:1345-53; PMID:9445035
[88] Periwal SB, Kourie KR, Ramachandaran N, Blakeney SJ, DeBruin S, Zhu D, Zamb TJ, Smith L, Udem S, Eldridge JH, et al. A modified cholera holotoxin CT-E29H enhances systemic and mucosal immune responses to recombinant Norwalk virus-virus like particle vaccine. Vaccine 2003; 21:376-85; PMID:12531635; http://dx.doi.org/10.1016/S0264-410X(02)00618-7
[89] Bok K, Parra GI, Mitra T, Abente E, Shaver CK, Boon D, Engle R, Yu C, Kapikian AZ, Sosnovtsev SV, et al. Chimpanzees as an animal model for human norovirus infection and vaccine development.

Proc Natl Acad Sci U S A 2011; 108:325-30; PMID:21173246; http://dx.doi.org/10.1073/pnas.1014577107
[90] Cohen J. NIH Curtails Chimpanzee Research in Wake of IOM Report Science 2011; 334:1484-5.
[91] Fang H, Tan M, Xia M, Wang L, Jiang X. Norovirus P particle efficiently elicits innate, humoral and cellular immunity. PLoS One 2013; 8:e63269; PMID:23638188; http://dx.doi.org/10.1371/journal.pone.0063269
[92] Cheetham S, Souza M, Meulia T, Grimes S, Han MG, Saif LJ. Pathogenesis of a genogroup II human norovirus in gnotobiotic pigs. J Virol 2006; 80:10372-81; PMID:17041218; http://dx.doi.org/10.1128/JVI.00809-06
[93] Souza M, Azevedo MS, Jung K, Cheetham S, Saif LJ. Pathogenesis and immune responses in gnotobiotic calves after infection with the genogroup II.4-HS66 strain of human norovirus. J Virol 2008; 82:1777-86; PMID:18045944; http://dx.doi.org/10.1128/JVI.01347-07
[94] Souza M, Costantini V, Azevedo MS, Saif LJ. A human norovirus-like particle vaccine adjuvanted with ISCOM or mLT induces cytokine and antibody responses and protection to the homologous GII.4 human norovirus in a gnotobiotic pig disease model. Vaccine 2007; 25:8448-59; PMID:18022293; http://dx.doi.org/10.1016/j.vaccine.2007.09.040
[95] Kocher J, Bui T, Giri-Rachman E, Wen K, Li G, Yang X, Liu F, Tan M, Xia M, Zhong W, et al. Intranasal P particle vaccine provided partial cross-variant protection against human GII.4 norovirus diarrhea in gnotobiotic pigs. J Virol 2014; 88:9728-43; PMID:24920797; http://dx.doi.org/10.1128/JVI.01249-14
[96] Lindesmith L, Moe C, Lependu J, Frelinger JA, Treanor J, Baric RS. Cellular and humoral immunity following Snow Mountain virus challenge. J Virol 2005; 79:2900-9; PMID:15709009; http://dx.doi.org/10.1128/JVI.79.5.2900-2909.2005
[97] Tamminen K, Huhti L, Koho T, Lappalainen S, Hytonen VP, Vesikari T, Blazevic V. A comparison of immunogenicity of norovirus GII.4 virus-like particles and P-particles. Immunology 2012; 135:89-99; PMID:22044070; http://dx.doi.org/10.1111/j.1365-2567.2011.03516.x
[98] Wobus CE, Thackray LB, Virgin HWt. Murine norovirus: a model system to study norovirus biology and pathogenesis. J Virol 2006; 80:5104-12; PMID:16698991; http://dx.doi.org/10.1128/JVI.02346-05
[99] Chachu KA, LoBue AD, Strong DW, Baric RS, Virgin HW. Immune mechanisms responsible for vaccination against and clearance of mucosal and lymphatic norovirus infection. PLoS Pathog 2008; 4:e1000236; PMID:19079577; http://dx.doi.org/10.1371/journal.ppat.1000236
[100] Zhu S, Regev D, Watanabe M, Hickman D, Moussatche N, Jesus DM, Kahan SM, Napthine S, Brierley I, Hunter RN, 3rd, et al. Identification of immune and viral correlates of norovirus protective immunity through comparative study of intra-cluster norovirus strains. PLoS Pathog 2013; 9:e1003592; PMID:24039576; http://dx.doi.org/10.1371/journal.ppat.1003592
[101] Elftman MD, Gonzalez-Hernandez MB, Kamada N, Perkins C, Henderson KS, Nunez G, Wobus CE. Multiple effects of dendritic cell depletion on murine norovirus infection. J Gen Virol 2013; 94:1761-8; PMID:23636823; http://dx.doi.org/10.1099/vir.0.052134-0
[102] Troeger H, Loddenkemper C, Schneider T, Schreier E, Epple HJ, Zeitz M, Fromm M, Schulzke JD. Structural and functional changes of the duodenum in human norovirus infection. Gut 2009; 58:1070-7; PMID:19036950; http://dx.doi.org/10.1136/gut.2008.160150
[103] LoBue AD, Lindesmith LC, Baric RS. Identification of cross-reactive norovirus CD4+ T cell epitopes. J Virol 2010; 84:8530-8; PMID:20573810; http://dx.doi.org/10.1128/JVI.00727-10
[104] Tacket CO, Sztein MB, Losonsky GA, Wasserman SS, Estes MK. Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers. Clin Immunol 2003; 108:241-7; PMID:14499247; http://dx.doi.org/10.1016/S1521-6616(03)00120-7
[105] LoBue AD, Thompson JM, Lindesmith L, Johnston RE, Baric RS. Alphavirus-adjuvanted norovirus-like particle vaccines: heterologous, humoral, and mucosal immune responses protect against murine norovirus challenge. J Virol 2009; 83:3212-27; PMID:19176631; http://dx.doi.org/10.1128/JVI.01650-08
[106] Ma Y, Duan Y, Wei Y, Liang X, Niewiesk S, Oglesbee M, Li J. Heat shock protein 70 enhances mucosal immunity against human norovirus when coexpressed from a vesicular stomatitis virus vector. J Virol 2014; 88:5122-37; PMID:24574391; http://dx.doi.org/10.1128/JVI.00019-14
[107] Guo L, Wang J, Zhou H, Si H, Wang M, Song J, Han B, Shu Y, Ren L, Qu J, et al. Intranasal administration of a recombinant adenovirus expressing the norovirus capsid protein stimulates specific humoral, mucosal, and cellular immune responses in mice. Vaccine 2008; 26:460-8; PMID:18160189; http://dx.doi.org/10.1016/j.vaccine.2007.11.039
[108] Guo L, Zhou H, Wang M, Song J, Han B, Shu Y, Ren L, Si H, Qu J, Zhao Z, et al. A recombinant adenovirus prime-virus-like particle boost regimen elicits effective and specific immunities against norovirus in mice. Vaccine 2009; 27:5233-8; PMID:19589399; http://dx.doi.org/10.1016/j.vaccine.2009.06.065
[109] Tan M, Huang P, Xia M, Fang PA, Zhong W, McNeal M, Wei C, Jiang W, Jiang X. Norovirus P particle, a novel platform for vaccine development and antibody production. J Virol 2011; 85:753-64; PMID:21068235; http://dx.doi.org/10.1128/JVI.01835-10
[110] Xia M, Tan M, Wei C, Zhong W, Wang L, McNeal M, Jiang X. A candidate dual vaccine against influenza and noroviruses. Vaccine 2011; 29:7670-7; PMID:21839795; http://dx.doi.org/10.1016/j.vaccine.2011.07.139
[111] Blazevic V, Lappalainen S, Nurminen K, Huhti L, Vesikari T. Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. Vaccine 2011; 29:8126-33; PMID:21854823; http://dx.doi.org/10.1016/j.vaccine.2011.08.026
[112] Tamminen K, Lappalainen S, Huhti L, Vesikari T, Blazevic V. Trivalent combination vaccine induces broad heterologous immune responses to norovirus and rotavirus in mice. PLoS One 2013; 8:e70409; PMID:23922988; http://dx.doi.org/10.1371/journal.pone.0070409
[113] Parra GI, Bok K, Taylor R, Haynes JR, Sosnovtsev SV, Richardson C, Green KY. Immunogenicity and specificity of norovirus Consensus GII.4 virus-like particles in monovalent and bivalent vaccine formulations. Vaccine 2012; 30:3580-6; PMID:22469864; http://dx.doi.org/10.1016/j.vaccine.2012.03.050
[114] Rocha-Pereira J, Jochmans D, Debing Y, Verbeken E, Nascimento MS, Neyts J. The viral polymerase inhibitor 2′-C-methylcytidine inhibits Norwalk virus replication and protects against norovirus-induced diarrhea and mortality in a mouse model. J Virol 2013; 87:11798-805; PMID:23986582; http://dx.doi.org/10.1128/JVI.02064-13
[115] Farkas T. Rhesus enteric calicivirus surrogate model for human norovirus gastroenteritis. J Gen Virol 2015; 96:1504-14; PMID:25502652; http://dx.doi.org/10.1099/jgv.0.000020
[116] Farkas T, Sestak K, Wei C, Jiang X. Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. J Virol 2008; 82:5408-16; PMID:18385231; http://dx.doi.org/10.1128/JVI.00070-08
[117] Farkas T, Dufour J, Jiang X, Sestak K. Detection of norovirus-, sapovirus- and rhesus enteric calicivirus-specific antibodies in captive juvenile macaques. J Gen Virol 2010; 91:734-8; PMID:19889933; http://dx.doi.org/10.1099/vir.0.015263-0
[118] Farkas T, Cross RW, Hargitt E, 3rd, Lerche NW, Morrow AL, Sestak K. Genetic diversity and histo-blood group antigen interactions of rhesus enteric caliciviruses. J Virol 2010; 84:8617-25; PMID:20554772; http://dx.doi.org/10.1128/JVI.00630-10
[119] Farkas T, Wong Ping Lun C. Prevalence of recovirus-neutralizing antibodies in human serum samples. J Clin Microbiol 2014; 52:3088-90; PMID:24899037; http://dx.doi.org/10.1128/JCM.01187-14
[120] Farkas T, Lun CW, Fey B. Relationship between genotypes and serotypes of genogroup 1 recoviruses: a model for human norovirus antigenic diversity. J Gen Virol 2014; 95:1469-78; PMID:24700099; http://dx.doi.org/10.1099/vir.0.064675-0
[121] Newman KL, Leon JS. Norovirus immunology: Of mice and mechanisms. Eur J Immunol 2015; 45:2742-57; PMID:26256101; http://dx.doi.org/10.1002/eji.201545512
[122] Taube S, Kolawole AO, Hohne M, Wilkinson JE, Handley SA, Perry JW, Thackray LB, Akkina R, Wobus CE. A mouse model for human

norovirus. mBio 2013; 4:pii: e00450-13; PMID:23860770; http://dx.doi.org/10.1128/mBio.00450-13

[123] Ball JM, Graham DY, Opekun AR, Gilger MA, Guerrero RA, Estes MK. Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. Gastroenterology 1999; 117:40-8; PMID:10381908; http://dx.doi.org/10.1016/S0016-5085(99)70548-2

[124] Ramirez K, Wahid R, Richardson C, Bargatze RF, El-Kamary SS, Sztein MB, Pasetti MF. Intranasal vaccination with an adjuvanted Norwalk virus-like particle vaccine elicits antigen-specific B memory responses in human adult volunteers. Clin Immunol 2012; 144:98-108; PMID:22710446; http://dx.doi.org/10.1016/j.clim.2012.05.006

[125] Atmar RL, Bernstein DI, Harro CD, Al-Ibrahim MS, Chen WH, Ferreira J, Estes MK, Graham DY, Opekun AR, Richardson C, et al. Norovirus vaccine against experimental human Norwalk Virus illness. N Engl J Med 2011; 365:2178-87; PMID:22150036; http://dx.doi.org/10.1056/NEJMoa1101245

[126] Muller S. Avoiding deceptive imprinting of the immune response to HIV-1 infection in vaccine development. International reviews of immunology 2004; 23:423-36; PMID:15370274; http://dx.doi.org/10.1080/08830180490432802

[127] Frenck R, Bernstein DI, Xia M, Huang P, Zhong W, Parker S, Dickey M, McNeal M, Jiang X. Predicting susceptibility to norovirus GII.4 by use of a challenge model involving humans. J Infect Dis 2012; 206:1386-93; PMID:22927452; http://dx.doi.org/10.1093/infdis/jis514

[128] Treanor JJ, Atmar RL, Frey SE, Gormley R, Chen WH, Ferreira J, Goodwin R, Borkowski A, Clemens R, Mendelman PM. A novel intramuscular bivalent norovirus virus-like particle vaccine candidate–reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults. J Infect Dis 2014; 210:1763-71; PMID:24951828; http://dx.doi.org/10.1093/infdis/jiu337

[129] Bernstein DI, Atmar RL, Lyon GM, Treanor JJ, Chen WH, Jiang X, Vinje J, Gregoricus N, Frenck RW, Jr., Moe CL, et al. Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults. J Infect Dis 2015; 211:870-8; PMID:25210140; http://dx.doi.org/10.1093/infdis/jiu497

[130] Sundararajan A, Sangster MY, Frey S, Atmar RL, Chen WH, Ferreira J, Bargatze R, Mendelman PM, Treanor JJ, Topham DJ. Robust mucosal-homing antibody-secreting B cell responses induced by intramuscular administration of adjuvanted bivalent human norovirus-like particle vaccine. Vaccine 2015; 33:568-76; PMID:25444793; http://dx.doi.org/10.1016/j.vaccine.2014.09.073

[131] Lay MK, Atmar RL, Guix S, Bharadwaj U, He H, Neill FH, Sastry KJ, Yao Q, Estes MK. Norwalk virus does not replicate in human macrophages or dendritic cells derived from the peripheral blood of susceptible humans. Virology 2010; 406:1-11; PMID:20667573; http://dx.doi.org/10.1016/j.virol.2010.07.001

[132] Duizer E, Schwab KJ, Neill FH, Atmar RL, Koopmans MP, Estes MK. Laboratory efforts to cultivate noroviruses. J Gen Virol 2004; 85:79-87; PMID:14718622; http://dx.doi.org/10.1099/vir.0.19478-0

[133] La Gruta NL, Turner SJ. T cell mediated immunity to influenza: mechanisms of viral control. Trends Immunol 2014; 35:396-402; PMID:25043801; http://dx.doi.org/10.1016/j.it.2014.06.004

[134] Soema PC, van Riet E, Kersten G, Amorij JP. Development of cross-protective influenza a vaccines based on cellular responses. Front Immunol 2015; 6:237; PMID:26029218; http://dx.doi.org/10.3389/fimmu.2015.00237

[135] Sridhar S, Begom S, Bermingham A, Hoschler K, Adamson W, Carman W, Bean T, Barclay W, Deeks JJ, Lalvani A. Cellular immune correlates of protection against symptomatic pandemic influenza. Nat Med 2013; 19:1305-12; PMID:24056771; http://dx.doi.org/10.1038/nm.3350

[136] Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, Liebner JC, Lambkin-Williams R, Gilbert A, Oxford J, Nicholas B, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. Nat Med 2012; 18:274-80; PMID:22286307; http://dx.doi.org/10.1038/nm.2612

[137] Clarke E, Desselberger U. Correlates of protection against human rotavirus disease and the factors influencing protection in low-income settings. Mocosal Immunol 2015; 8:1-17; PMID:25465100; http://dx.doi.org/10.1038/mi.2014.114

[138] Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 385:117-71; PMID:25530442; http://dx.doi.org/10.1016/S0140-6736(14)61682-2

[139] Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380:2095-128; PMID:23245604; http://dx.doi.org/10.1016/S0140-6736(12)61728-0