View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Available online at www.sciencedirect.com
SciVerse ScienceDirect
Procedia in Vaccinology 4 (2011) 19–26
Procedia in Vaccinology
www.elsevier.com/locate/procedia

4th Vaccine and ISV Annual Global Congress

Norovirus P Particle as a Platform for Antigen Presentation

Ming Tan[a,b]*, Ming Xia[a], Pengwei Huang[a], Leyi Wang[a], Weiming Zhong[a], Monica McNeal[a,b], Chao Wei[a], and Xi Jiang[a,b]*

[a] Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA
[b]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA

Abstract

The norovirus P particle, a subviral particle (~20 nanometers in diameter) formed by 24 protrusion (P) domains of the norovirus capsid protein, is easily made, stable, and highly immunogenic and thus an excellent vaccine candidate against noroviruses. Each P domain has three surface loops that have been shown useful for antigen presentation. We have successfully inserted a number of small (5 aa) to large (238 aa) antigens into these loops without affecting P particle formation and production. Increased immune responses were demonstrated by improved antibody titers induced by the P particle presented antigens compared to free antigens. Significantly increased neutralization of virus and/or protection against influenza virus and rotavirus challenges have also been demonstrated in mice after immunization with chimeric P particle vaccines containing flu M2e and rotavirus VP8 antigens, compared to free M2e and VP8 antigens, respectively. The chimeric P particle-induced antibodies also blocked binding of norovirus-like particles (VLPs) to histo-blood group antigen (HBGA) receptors, indicating a potential dual vaccine against norovirus in addition to rotavirus and influenza virus. Taken together, the P particle appears to be an excellent platform for antigen presentation for vaccine development. The multiple surface loops and the large capacity of foreign antigen insertion suggest that this platform may have a wide application in vaccine development against different infectious diseases.

© 2011 Published by Elsevier Ltd. Open access under CC BY-NC-ND license.
Selection and peer-review under responsibility of Prof. Ray Spier

Key words: norovirus; calicivirus; P particle; vaccine platform; antigen presentation; rotavirus vaccine; influenza virus vaccine.

1. Introduction

Biomaterials and bioengineering have become important areas of modern medicine. Because of their versatility and propensity to form arrays, viral and subviral particles are excellent substrates to construct presentation systems. These viral particles can be used as a platform for integration of foreign antigens in designed patterns through genetic engineering, which, in turn, can be developed into vaccines [1-8].

* Corresponding authors. Tel.: +1-513-636-0510, +1-513-636-0119; fax: +1-513-636-7655.
E-mail address: Jason.jiang@cchmc.org; ming.tan@ cchmc.org.

1877-282X © 2011 Published by Elsevier Ltd. Open access under CC BY-NC-ND license.
Selection and peer-review under responsibility of Prof. Ray Spier. doi:10.1016/j.provac.2011.07.004

Examples of successful insertions of antigens into viral particles have been described for several virus particles, including flock house virus (FHV)-like particle (VLP) containing a *Bacillus anthracis* antigen [9], hepatitis B virus (HBV) capsid-like particle (CLP) holding a surface antigen (OspA) of *Borrelia burgdorferi* [10-12], and the cowpea mosaic virus (CPMV) presenting a number of different antigens [13-20], although limitations have also been described in these presentation systems. In this study we assessed the usefulness of the norovirus P particle, a novel subviral particle of norovirus [21-23], as a multifunctional platform for vaccine development against different infectious diseases.

1.1. Norovirus P particle

Noroviruses are a group of non-enveloped RNA viruses within the family *Caliciviridae* causing epidemics of acute gastroenteritis in humans. The viruses are encapsulated by a protein capsid that is constituted by a single major structural protein, the capsid protein VP1. The capsid protein is divided into a shell (S) and a protruding (P) domain that constitute the interior shell and the exterior protrusions of the viral capsid [24]. These two domains, linked by a short hinge, can be structurally and functionally independent. When expressed *in vitro*, the S domain alone forms a smaller particle with smooth surface, the S particle [25, 26], whereas the P domain can form three different complexes, the P dimer [26-28], the P particle [21, 22, 29] and the small P particles [30]. Since all these P complexes are formed by the surface portion of a norovirus capsid and contain all elements required for interaction with carbohydrate receptors, they have been extensively used as models in studies of norovirus-host interaction [21, 22, 26, 29, 31-33].

1.2. The hypothesis: the P particle may be an excellent platform for antigen presentation

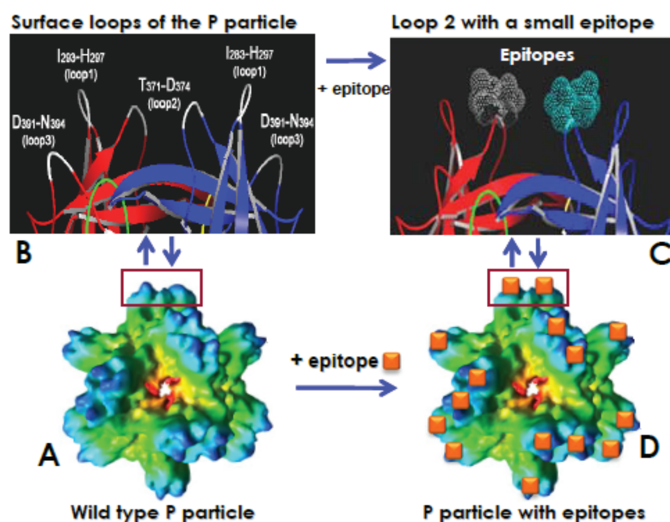

Fig 1. The principle of antigen presentation by a norovirus P particle. (A), the structure of the wild type P particle; (B), crystal structure of a protrusion of the P particle with indications of 3 surface loops of each P domain that may be excellent sites for antigen presentation; (C) and (D), when an epitope is inserted into loop 2 (C), 24 copies of the epitope will be presented on the surface of the chimeric P particle (D).

The P particles (~840 kDa, ~20 nm in diameter) are easily produced in *E. coli* and yeast, are stable and highly immunogenic [34], and thus are an excellent vaccine candidate for human noroviruses [21, 35]. Structural analysis of the P particle by electron cryo-microscopy (cryo-EM) revealed an octahedral

symmetry (Fig 1A) consisting of 24 copies of P domains that organize into 12 P dimers [34, 36]. Further study of the P dimer by crystallography indicated three surface loops on the distal end of each P domain [28], corresponding to the outermost surface of the P particle (Fig 1A and B). These loops now have been shown to be excellent sites for foreign antigen insertion and presentation [23]. Since each P particle contains 24 P monomers and each P monomer has three surface loops, insertion of a single antigen into these loops will produce 24-72 copies of the antigen on the surface of a P particle. By this mean the immunogenicity of the inserted antigens may be significantly increased. Therefore, the P particle may serve as an effective vaccne platform for antigen presentation (Fig 1).

2. Evidence to support the hypothesis

To assess the usefulness of the P particle as a vaccine platform, a number of experiments have been performed to evaluate the capability and capacity of foreign antigen insertions, the production and stability of the chimeric P particle, the immune response and protection of the chimeric P particles when used as a vaccine, as well as the immunogenicity of the P particle backbone. The results that support the hypothesis are described below.

2.1. Small peptide epitopes can be well presented by the P particles

Our experiments started with insertions of seven small peptide epitopes. These included two cysteine-containing peptides, a CNGRC (ligand of aminopeptidase N or CD13) [37] and a CDCRGDCFC (ligand of intergrins $\alpha_v\beta_3$) [38], a 7xhistidine tract (His tag, 7 aa), the T cell epitope of murine cytomegalovirus (YPHFMPTNL, 9 aa), the Epi8 epitope of Pseudomonas (NATAEGRAINRRVE, 14 aa) [39], the CD4+ T cell epitope of murine rotavirus VP6 (RLSFQLMRPPNMTP, 14aa) [40], and the M2 extracellular epitope (M2e) of influenza virus (SLLTEVETPIRNEWGCRCNDSSD, 23 aa) (Table 1). These small epitopes were inserted into loop 2 of the P domain (Fig 1) and the chimeric P proteins were expressed using the GST Gene Fusion System (GE Healthcare) [35]. The results showed that insertion of all these short peptide into loop 2 individually did not affect the production of the chimeric P proteins in *E. coli*. The P particle formation was demonstrated by a defined major peak at ~840 kDa in a gel filtration analysis [35]. The exposure of the inserted epitopes was detected by binding of the chimeric P particle to the Talon resin (His tag) or to antigen-specific antibodies by EIAs.

2.2. Large protein antigens can also be presented by the P particle platform

To examine the capacity of the loops for foreign antigen insertion, we chose the core proteins (159 aa) of rotavirus spike protein VP8 of two serotypes, Wa (G1P8) and DS1 (G2P4), and the green fluorescence protein (GFP, 238 aa) to determine if the P particle can handle larger antigens (Table 1). These three proteins were inserted into loop 2 of the P domain through a cloning cassette containing two restriction enzyme sites (SpeI/ClaI) [35]. The chimeric P proteins were generated using the same approach described above. In all cases, the chimeric P proteins were expressed well and formed chimeric P particles. The particle formation of the P-VP8 chimeric protein has also been confirmed by cryo electron microscopy. The presentation of the VP8 on the surface of the particle was further demonstrated by fitting of the crystal structure of the VP8 into the density map of the extended protrusion of the chimeric P particle [35]. Successful insertion of the GFP in the chimeric P particle was shown by the size of the chimeric protein on a SDS PAGE, the defined particle peak in gel filtration and the emission of green fluorescence of the chimeric P particle. This data indicated that the P particle platform has a capacity of antigen insertion of at least 238 amino acids.

2.3. All three loops of the P particle are capable of presenting foreign antigens

Table 1. A list of small to large antigens that have been inserted into the surface loops of the norovirus P particles and their immune responses in mice.

Inserted antigens	size (aa)	Inserted location	Particle formation	Yield in soluble protein (mg/liter culture)	Increased antibody response [1]	Increased neutralizing titer [1]	Increased protection [1]
CNGRC (ligand of cd13)	5	Loop 2	yes	~7	/	/	/
CDCRGDCFC (ligand of intergrins)	9	Loop 2	yes	~7	/	/	/
7xHistidine	7	Loop 1	yes	~5	yes	/	/
7xHistidine	7	Loop 2	yes	~5	yes	/	/
7xHistidine	7	Loop 3	yes	~5	yes	/	/
T cell epitope of murine CMV	9	Loop 2	yes	~3	/	/	/
Epi8 epitope of Pseudomonas	14	Loop 2	yes	~3	/	/	/
T cell epitope of murine RV VP6	14	Loop 1	yes	~3	/	/	/
T cell epitope of murine RV VP6	14	Loop 2	yes	~3	/	/	/
M2e epitope of influenza virus	23	Loop 2	yes	~5	yes	/	yes
Human RV VP8 (Wa, G1P8)	159	Loop 1	yes	<0.1	/	/	/
Human RV VP8 (Wa, G1P8)	159	Loop 2	yes	~15	yes	yes	/
Human RV VP8 (Wa, G1P8)	159	Loop 3	yes	~5	/	/	/
Human RV VP8 (DS1, G2P4)	159	Loop 2	yes	~10	yes	yes	/
Murine RV VP8 (EDIM)	159	Loop 2	yes	~2	yes	/	yes
Green fluorescence protein (GFP)	238	Loop 1	yes	~1	/	/	/
Green fluorescence protein (GFP)	238	Loop 2	yes	~1	/	/	/
Green fluorescence protein (GFP)	238	Loop 3	yes	~1	/	/	/

[1] For detailed data of these measurements please refer to reference [35]; / indicates that the measurement has not yet been performed.

We then examined whether the other two surface loops (loops 1 and 3) are also capable of presenting foreign antigens. Two small epitopes (His tag and the T cell epitope of murine rotavirus VP6) and two large protein antigens (VP8 of human rotavirus and GFP) were tested (Table 1). Most of these insertions did not affect the production of the chimeric P proteins. Gel filtration followed by Talon resin binding and antigen detection EIA confirmed the P particle formation and the exposure of the His tag and the other inserted antigens. The only failure that occurred was the insertion of VP8 into loop 1. This insertion resulted in a low yield (<0.1 mg/liter bacterial culture) of the chimeric protein, possibly because of an instability of the resulting chimeric P protein (Table 1). These data indicated that all of the three loops are useful in presenting an antigen, although loop 1 may be more selective for certain antigens.

2.4. The P particle presented small and large antigens induced enhanced immune responses

The chimeric P particles containing small peptides, His tag or M2e, induced significantly higher antibody responses in mice against the inserted peptides than that induced by the free peptides alone and by the P dimer presented His tag after an intranasal immunization without adjuvant [35]. Similarly, the chimeric P particles containing rotavirus VP8s resulted in significantly higher antibody responses in mice than that induced by the free VP8s for both types [8] (Wa) and [4] (DS1). The antisera induced by the P particles-VP8 chimera also revealed significantly higher neutralizing titers against rotavirus than the antisera induced by the free VP8 [35]. These data indicated that the P particles are a useful platform for immune enhancement of antigens.

2.5. The chimeric P particles protected mice from virus infection

Two chimeric P particles containing either the influenza virus M2e or the murine rotavirus VP8 were studied. The P particle-M2e chimera was delivered intranasally without an adjuvant or subcutaneously with Montanide ISA 720 adjuvant (Sepic, France). Mice were fully protected (100% survived) against the lethal challenge of an mouse adapted human influenza virus PR8 (H1N1), while very low survival rates (<10%) were found in control mice immunized with free M2e peptide, wild type P particle, or phosphate buffer saline (PBS), respectively.

The chimeric P particle containing murine rotavirus (EDIM) VP8 was also delivered intranasally to mice. Mice were then challenged with EDIM and the quantity of viral shedding in the stool was determined by ELISA. When compared to control mice that were immunized by PBS, wild type P particle and free EDIM VP8, respectively, the group that received the chimeric P particle containing EDIM VP8 shed the lowest amount of rotavirus [35]. These data further demonstrated that the P particles can be used as a platform for vaccine development and both the P particle-M2e and -VP8 chimeras are promising vaccine candidates against influenza virus and rotavirus.

2.6. The chimeric P particles induced antibody against norovirus that block binding of norovirus to viral receptors

In addition to producing antibodies against influenza virus and rotavirus, the chimeric P particle-M2e and -VP8 also induced high antibody titers against norovirus in mice. The mouse sera were able to block the binding of norovirus VLPs to histo-blood group antigens (HBGAs), the host receptors of human noroviruses [41-43], in both saliva- and synthetic oligosaccharide-based binding assays [21, 35, 44, 45]. These results suggest that the chimeric P particles can be used as dual vaccines against norovirus in addition to rotavirus and influenza virus, respectively.

3. Conclusions

In this study we have provided solid evidence on the usefulness of the norovirus P particle as a novel platform for antigen presentation for immune enhancement. Significantly increased immune responses and protection were observed for both small and large antigens that are presented by the P particles. The two chimeric P particles created in this study, the P particle-M2e and the P particle-VP8, are promising vaccine candidates against influenza virus, rotavirus and norovirus and further development of these chimeric P particles into a useful vaccine is warranted. Further studies to explore diverse applications of the platform for other antigens by taking advantage of all three surface loops and the large capacity of the loops for foreign antigen insertion also are necessary.

Acknowledgements

The research described in this article was supported by the National Institute of Health, the National Institute of Allergy and Infectious diseases (R01 AI37093, R01 AI055649 and R01 AI089634), and the Department of Defense (PR033018) to X.J. This study was also supported by an Institutional Clinical and Translational Science Award, NIH/NCRR Grant Number 1UL1RR026314-01 to M. T. In addition, the Infectious Disease Grant to M. T. sponsored by Nipert Foundation contributed to this research.

References

[1] Dragnea B, Chen C, Kwak ES, Stein B, Kao CC. Gold nanoparticles as spectroscopic enhancers for in vitro studies on single viruses. *J Am Chem Soc* 2003;**125**:6374-5.

[2] Douglas T, Young M. Viruses: making friends with old foes. *Science* 2006;**312**:873-5.

[3] Douglas T. Materials science. A bright bio-inspired future. *Science* 2003;**299**:1192-3.

[4] Peabody DS. A Viral Platform for Chemical Modification and Multivalent Display. *J Nanobiotechnology* 2003;**1**:5.

[5] Chen C, Daniel MC, Quinkert ZT, De M, Stein B, Bowman VD, et al. Nanoparticle-templated assembly of viral protein cages. *Nano Lett* 2006;**6**:611-5.

[6] Chen C, Kwak ES, Stein B, Kao CC, Dragnea B. Packaging of gold particles in viral capsids. *J Nanosci Nanotechnol* 2005;**5**:2029-33.

[7] Radloff C, Vaia RA, Brunton J, Bouwer GT, Ward VK. Metal nanoshell assembly on a virus bioscaffold. *Nano Lett* 2005;**5**:1187-91.

[8] Ochoa WF, Chatterji A, Lin T, Johnson JE. Generation and structural analysis of reactive empty particles derived from an icosahedral virus. *Chem Biol* 2006;**13**:771-8.

[9] Manayani DJ, Thomas D, Dryden KA, Reddy V, Siladi ME, Marlett JM, et al. A viral nanoparticle with dual function as an anthrax antitoxin and vaccine. *PLoS Pathog* 2007;**3**:e142.

[10] Kratz PA, Bottcher B, Nassal M. Native display of complete foreign protein domains on the surface of hepatitis B virus capsids. *Proc Natl Acad Sci U S A* 1999;**96**:1915-20.

[11] Nassal M, Skamel C, Kratz PA, Wallich R, Stehle T, Simon MM. A fusion product of the complete Borrelia burgdorferi outer surface protein A (OspA) and the hepatitis B virus capsid protein is highly immunogenic and induces protective immunity similar to that seen with an effective lipidated OspA vaccine formula. *Eur J Immunol* 2005;**35**:655-65.

[12] Siebenga JJ, Vennema H, Duizer E, Koopmans MP. Gastroenteritis caused by norovirus GGII.4, The Netherlands, 1994-2005. *Emerg Infect Dis* 2007;**13**:144-6.

[13] Lin TaJ, J. Structures of Picorna-like plant viruses: implications and applications. *Advances in virus research* 2003;**62**:167-239.

[14] Johnson J, Lin T, Lomonossoff G. Presentation of heterologous peptides on plant viruses: genetics, structure, and function. *Annu Rev Phytopathol* 1997;**35**:67-86.

[15] Lomonossoff GP, Johnson JE. Use of macromolecular assemblies as expression systems for peptides and synthetic vaccines. *Curr Opin Struct Biol* 1996;**6**:176-82.

[16] Porta C, Spall VE, Lin T, Johnson JE, Lomonossoff GP. The development of cowpea mosaic virus as a potential source of novel vaccines. *Intervirology* 1996;**39**:79-84.

[17] Porta C, Spall VE, Loveland J, Johnson JE, Barker PJ, Lomonossoff GP. Development of cowpea mosaic virus as a high-yielding system for the presentation of foreign peptides. *Virology* 1994;**202**:949-55.

[18] Chatterji A, Burns LL, Taylor SS, Lomonossoff GP, Johnson JE, Lin T, et al. Cowpea mosaic virus: from the presentation of antigenic peptides to the display of active biomaterials. *Intervirology* 2002;**45**:362-70.

[19] Chatterji A, Ochoa W, Shamieh L, Salakian SP, Wong SM, Clinton G, et al. Chemical conjugation of heterologous proteins on the surface of Cowpea mosaic virus. *Bioconjug Chem* 2004;**15**:807-13.

[20] Taylor KM, Lin T, Porta C, Mosser AG, Giesing HA, Lomonossoff GP, et al. Influence of three-dimensional structure on the immunogenicity of a peptide expressed on the surface of a plant virus. *J Mol Recognit* 2000;**13**:71-82.

[21] Tan M, Fang P, Chachiyo T, Xia M, Huang P, Fang Z, et al. Noroviral P particle: Structure, function and applications in virus-host interaction. *Virology* 2008;**382**:115-23.

[22] Tan M, Jiang X. The p domain of norovirus capsid protein forms a subviral particle that binds to histo-blood group antigen receptors. *Journal of Virology* 2005;**79**:14017-30.

[23] Tan M, Huang P, Xia M, Fang PA, Zhong W, McNeal M, et al. Norovirus P particle, a novel platform for vaccine development and antibody production. *J Virol* 2011;**85**:753-64.

[24] Prasad BV, Hardy ME, Dokland T, Bella J, Rossmann MG, Estes MK. X-ray crystallographic structure of the Norwalk virus capsid. *Science* 1999;**286**:287-90.

[25] Bertolotti-Ciarlet A, White LJ, Chen R, Prasad BV, Estes MK. Structural requirements for the assembly of Norwalk virus-like particles. *J Virol* 2002;**76**:4044-55.

[26] Tan M, Hegde RS, Jiang X. The P domain of norovirus capsid protein forms dimer and binds to histo-blood group antigen receptors. *J Virol* 2004;**78**:6233-42.

[27] Bu W, Mamedova A, Tan M, Xia M, Jiang X, Hegde RS. Structural basis for the receptor binding specificity of Norwalk virus. *J Virol* 2008;**82**:5340-7.

[28] Cao S, Lou Z, Tan M, Chen Y, Liu Y, Zhang Z, et al. Structural basis for the recognition of blood group trisaccharides by norovirus. *J Virol* 2007;**81**:5949-57.

[29] Tan M, Meller J, Jiang X. C-terminal arginine cluster is essential for receptor binding of norovirus capsid protein. *Journal of Virology* 2006;**80**:7322-31.

[30] Tan M, Fang PA, Xia M, Chachiyo T, Jiang W, Jiang X. Terminal modifications of norovirus P domain resulted in a new type of subviral particles, the small P particles. *Virology* 2011;**410**:345-52.

[31] Tan M, Jin M, Xie H, Duan Z, Jiang X, Fang Z. Outbreak studies of a GII-3 and a GII-4 norovirus revealed an association between HBGA phenotypes and viral infection. *J Med Virol* 2008;**80**:1296-301.

[32] Tan M, Xia M, Cao S, Huang P, Farkas T, Meller J, et al. Elucidation of strain-specific interaction of a GII-4 norovirus with HBGA receptors by site-directed mutagenesis study. *Virology* 2008;**379**:324-34.

[33] Tan M, Xia M, Chen Y, Bu W, Hegde RS, Meller J, et al. Conservation of carbohydrate binding interfaces: evidence of human HBGA selection in norovirus evolution. *PLoS One* 2009;**4**:e5058.

[34] Jiang X, Tan M, inventors; Norovirus P particles as a carrier for presentation of foreign antigens and other molecules. US Patent application number: 61185564. 2009.

[35] Tan M, Huang P, Xia M, Fang P, Zhong W, Wei C, et al. Norovirus P particle, a multifunctional vaccine platform. *J Virol* 2011;**85**:753-64.

[36] Tan M, Jiang X. The p domain of norovirus capsid protein forms a subviral particle that binds to histo-blood group antigen receptors. *J Virol* 2005;**79**:14017-30.

[37] Curnis F, Arrigoni G, Sacchi A, Fischetti L, Arap W, Pasqualini R, et al. Differential binding of drugs containing the NGR motif to CD13 isoforms in tumor vessels, epithelia, and myeloid cells. *Cancer Res* 2002;**62**:867-74.

[38] Maeshima Y, Colorado PC, Kalluri R. Two RGD-independent alpha vbeta 3 integrin binding sites on tumstatin regulate distinct anti-tumor properties. *J Biol Chem* 2000;**275**:23745-50.

[39] Worgall S, Krause A, Qiu J, Joh J, Hackett NR, Crystal RG. Protective immunity to pseudomonas aeruginosa induced with a capsid-modified adenovirus expressing P. aeruginosa OprF. *J Virol* 2007;**81**:13801-8.

[40] Choi AH, Basu M, McNeal MM, Flint J, VanCott JL, Clements JD, et al. Functional mapping of protective domains and epitopes in the rotavirus VP6 protein. *J Virol* 2000;**74**:11574-80.

[41] Tan M, Jiang X. Norovirus and its histo-blood group antigen receptors: an answer to a historical puzzle. *Trends Microbiol* 2005;**13**:285-93.

[42] Tan M, Jiang X. Norovirus-host interaction: implications for disease control and prevention. *Expert Rev Mol Med* 2007;**9**:1-22.

[43] Tan M, Jiang X. Norovirus gastroenteritis, carbohydrate receptors, and animal models. *PLoS Pathog* 2010;**6**:e1000983.

[44] Tan M, Zhong W, Song D, Thornton S, Jiang X. E. coli-expressed recombinant norovirus capsid proteins maintain authentic antigenicity and receptor binding capability. *J Med Virol* 2004;**74**:641-9.

[45] Huang P, Farkas T, Zhong W, Tan M, Thornton S, Morrow AL, et al. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. *J Virol* 2005;**79**:6714-22.