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Biological and immunological characterization of norovirus major capsid proteins from three different genotypes

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ABSTRACT

Noroviruses (NoVs) are the leading cause of non-bacterial acute gastroenteritis worldwide. Due to a lack of cell culture system and animal model, our understanding of NoVs has been lagging behind. In this study, NoV major capsid proteins (VP1) from three different genotypes (GI.2, GII.3 and GII.4) were expressed by using recombinant baculovirus expression system and which led to successful assembly of virus-like particles (VLPs). The receptor binding patterns of three kinds of VLPs were characterized by using synthetic and salivary HBGA-VLP binding assay. Cross-reactivity and cross-blocking activity of rabbit hyperimmune sera against these VLPs were determined by ELISA/Western blot analysis and saliva-VLP binding blockade assay, respectively. Expression of the major capsid proteins from three genotypes all led to smaller VLPs in dominance when sf9 cells were cultured in suspension, which was in consistence with our previous report. These smaller VLPs were used for in vitro synthetic and salivary HBGA-VLP binding and binding blockade assays. VLPs from GII.3 strain exhibited no binding to all synthetic HBGAs and saliva samples tested while VLPs from GI.2 and GII.4 strain showed similar binding pattern and bound to all salivary HBGAs tested. Rabbit anti-GII.3 VLPs hyperimmune serum didn't block the binding of GI.2 and GII.4 VLPs to salivary HBGAs while rabbit anti-GI.2 VLP hyperimmune serum blocked the binding of GII.4 VLPs to salivary HBGAs but not vice versa. Our results provide further evidence indirectly in support of presence of other factors involved in receptor binding other than HBGAs for NoVs, and demonstrate poor cross-blocking activities of antibodies against VLPs within or across genogroups.

Noroviruses (NoVs), members of the Caliciviridae family, are the leading cause of non-bacterial acute gastroenteritis worldwide [1–3]. They are currently divided at least into six genogroups (GI-GVI), with GI and GII primarily infect humans and which can be further divided into 9 and 22 genotypes, respectively [4]. NoVs are single-stranded positive sense RNA viruses and its genome encodes three open reading frames. ORF1 encodes a non-structural polyprotein that is co- or posttranslationally cleaved into 6 proteins, including RNA dependent RNA polymerase (RdRp). ORF2 and ORF3 encode major (VP1) and minor (VP2) capsid protein, respectively. Expression of VP1 using recombinant baculovirus expression system leads to formation of virus-like particles with morphology and antigenicity similar to native virions [5]. The VP1 can be structurally divided into two domains, shell (S) domain and protruding (P) domain. The S domain, which assembles into smooth-surfaced particles when expressed using recombinant baculovirus expression system, forms the core of the VLPs [6]. The P domain can be further divided into two subdomains, P1 and P2 domains [7]. The P2 domain is the most surface-exposed region and has been shown to be involved in antibody binding and VLP attachment to histo-blood group antigen (HBGA) ligands [8–10].

HBGAs are complex carbohydrates that are present on surfaces of red blood cells and mucosal epithelial cells, or as free antigens in biological fluids such as saliva and milk [11]. The linkage of HBGA recognition with NoVs infection was first demonstrated by human volunteer challenge study as secretor negative volunteers were not infected by Norwalk virus even at high doses [12]. Secretors are defined as having a functional FUT2 gene which controls secretion of HBGAs at the gut surface. FUT-2 is inactivated in non-secretors.

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Further studies using saliva- and oligosaccharide-VLP binding assay have indicated presence of at least seven binding patterns [11]. The fact that certain NoV strains are capable of binding to many different HBGA antigens indicates HBGA binding might be a required step for virus binding and entry. The GII.4 strains, causing more than 80% of reported cases, have broad HBGA recognition which may be linked with their strength of transmission [13]. It should be noted that certain strains of NoVs exhibited no binding to any human HBGAs or secretor positive saliva samples tested [11]. Further evidence in support of the complexity of NoV infection is that Snow Mountain virus (SMV), a genogroup 2 NoV, was independent of blood group secretor status for infection [14]. Using VLP-cell interaction based assay, it has been shown that binding of NoV VLPs to intestinal epithelial cells is independent of HBGAs for binding and internalization [15].

Serological classification of NoVs has been hindered due to a lack of cell culture system and animal models [16,17]. In vivo assembled VLPs from expressed VP1 in eukaryotic cells as immunogens have greatly expanded our understanding of antigenicity and immunogenicity of diverse genotypes of NoVs. Studies have shown robust antibody cross-reaction within genogroups, but mild or absence of antibody cross-reaction between genogroups [18–20]. Cross-blocking antibodies have been observed within and between genogroups with latter showing poorer blocking efficiency [20–22].

In this study, NoV VP1 from three different genotypes (GI.2, GII.3 and GII.4) was expressed by using recombinant baculovirus expression system. Synthetic or salivary HBGA-VLP binding assay was performed to characterize the binding patterns of the three VLPs. Hyperimmune sera against the three kinds of VLPs were subsequently produced in rabbit and used to characterize the cross-blocking antibodies against salivary HBGA-VLP binding.

1. Materials and methods

1.1. NoV strains

The NoV strains used in this study were isolated from patients presenting with acute non-bacterial gastroenteritis as reported previously [23]. The Jingzhou401, Jingzhou402 and Jingzhou403 isolates were GI.2, GII.3 and GII.4 sydney-2012-like strain, respectively (GenBank accession No. KF306212, KF306213 and KF306214).

1.2. Recombinant VLPs

The ORF2 complementary DNA of each strain was PCR amplified and inserted into pVL1393 transfer vector. The recombinant baculovirus harboring target coding sequence was generated by homologous recombination as described previously [24]. Sf9 cells were cultured in suspension in vented flasks throughout this study. The VLPs were purified from culture medium of sf9 cells infected with recombinant baculovirus at a multiplicity of infection (MOI) of 1. The purification procedure was the same as described previously with minor modifications [24]. In brief, harvested cell medium was clarified at 3000 g to remove cell debris followed by ultracentrifugation at 141,000× g for 3 h at 8 °C in a SW 28 rotor to pellet VLPs. Pellets were resuspended in PBS (pH 7.2–7.4) prefiltered with 0.22 μm membrane. VLPs in PBS were mixed with equal volume of CsCl (1.6 g/ml) and centrifuged at 288,000× g for 24 h at 8 °C in a Beckman SW 41 Ti rotor. Visible bands were collected and analyzed by western blotting using rabbit anti-GII.3 VP1 (GST-fusion protein) specific hyperimmune serum and presence of VLPs was confirmed by electron microscopy after negative staining.

1.3. Production of rabbit anti-VLPs polyclonal antibodies

The concentration of CsCl purified VLPs was determined by BCA method. Japan big ear rabbits were subcutaneously immunized four times at 2 week intervals with approximately 50 μg of CsCl purified VLPs mixed 1:1 with Freund's complete adjuvant (first immunization) or Freund's incomplete adjuvant (subsequent immunizations) for a total of four doses. Rabbits were sacrificed and antisera were collected 1 week after the last immunization and VLP-specific IgG titers were determined by indirect Enzyme linked immunosorbent assay (ELISA).

1.4. Western blot analysis

Western blot was used to determine cross-reactivity of rabbit anti-VLPs polyclonal antibodies against each VLPs. Purified VLPs (400 ng) were boiled in sample buffer for 10 min and loaded onto 12% SDS-PAGE gel. Separated proteins were transferred to nitrocellulose membrane followed by blocking with PBS (pH 7.2) containing 0.5% Tween-20 (PBS-T) and 1% BSA at 37 °C for 1 h. The capsid proteins were detected by rabbit anti-VLPs (GI.2, GII.3 and GII.4) hyperimmune sera diluted in PBS-T containing 1% BSA at 1:2000 separately. Unbound rabbit anti-VLPs antibodies were removed by washing membrane three times with 5-min intervals in PBS-T. HRP-conjugated goat anti-rabbit IgG antibodies diluted in PBS-T were added. The membrane was washed for another three times using PBS-T and developed with DAB by following the manufacturer's instructions.

1.5. ELISA

Cross-reactivity of rabbit anti-VLPs antibodies against each VLPs was also determined by ELISA. CsCl purified VLPs diluted in carbonate-bicarbonate buffer (pH 9.6) at 2 μg/ml were coated onto 96-well plates (100 μl/well) by incubating at 4 °C overnight. The plates were then blocked with PBS-T containing 1% BSA (150 μl/well) by incubating at 37 °C for 1 h. Rabbit anti-VLP hyperimmune sera diluted in PBS-T containing 1% BSA at 4-fold serial dilutions starting from 1:500 were added to above wells followed by an incubation time of 1 h at 37 °C. The plate was washed five times with PBS-T and HRP-conjugated goat anti-rabbit IgG polyclonal antibody was added followed by an incubation period of 30 min at 37 °C. After washing for another five times with PBS-T, the final product was detected by addition of peroxidase substrate TMB and urea peroxide followed by an incubation period of 10–30 min. The reaction was stopped by addition of 100 μl/well, 2 M sulfuric acid. The optical density was read with a Multiscan plate reader at 450 nm. The end-point titers were calculated as the reciprocal of the highest serum dilutions that gave an absorbance value greater than 0.1.

1.6. Saliva-VLP binding assay

For saliva-VLP binding assay, secretor positive, blood type A, B, AB and O saliva samples were used. In brief, saliva samples were boiled for 10 min to inactivate native antibodies. Saliva samples were then diluted in carbonate-bicarbonate buffer (pH 9.6) at 1:2000 and coated onto 96-well microplates by incubating at 37 °C overnight. The plates were then washed and blocked with PBS-T containing 1% BSA by incubating at 37 °C for 1 h. Purified VLPs (0.2 μg/ml) diluted in PBS-T containing 1% BSA were added to above wells in triplicate (100 μl/well) and incubated at 37 °C for 1 h. Wells (in triplicate) added with PBS-T containing 1% BSA only were selected as negative control. After washing for five times with PBS-T, rabbit anti-genotype specific VLP hyperimmune sera diluted in