Norovirus P particle as epitope display platform for vaccine development

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Virus-like particles (VLPs) as a candidate vaccine for NVs

- NVs are an important cause of acute gastroenteritis
- Difficult to study due to lack of a cell culture
- The successful expression of virus-like particles of NVs provides a hope
  - Baculovirus and other expression systems
  - Morphologically and antigenically similar to authentic viruses
  - Scale up production of VLPs is feasible
  - Phase II clinical trials on a divalent VLP vaccine
Structure of Norwalk virus capsid

Science, 1999; J Inf Dis, 2000
VLP and subviral particles of human NVs

180 VP1s (VLP)  
180 S domains (S-particle)  
24 P domains (P particle)  
12 P domains (small P particle)
The P-particles display an enhanced binding affinity to HBGAs

- H  H antigen
- A  A antigen
- B  B antigen
- N  Non-secretor

P mutant (VA387)
P particle revealed an octahedral symmetry by Cryo-EM resolution: 8.6 Å
Discovery of NV P particles

P domain with the hinge (H/P mutant)

P domain w/o the hinge (P mutant)

P domain with an end-linked cysteine (CNGRC-P mutant)
The P particles are easily produced in *E. coli* and yeast.

* P particles are highly stable and tolerate with a wide range of pH and physical conditions.
Binding of NV P particle and VLP to different ABO, Lewis and secretor antigens

* P particles have authentic receptor binding patterns as their parent VLPs
P particle-induced antibody blocked binding of VLP to HBGAs

Both VA387 VLP and P particle induce specific antibody

(through an intranasal route of immunization w/o adjuvant)

VA387 VLP binding to A-type saliva

VA387 VLP binding to B-type saliva

VLP-HBGA binding is blocked by VLP-induced Ab (red line)

VA387 VLP binding to B-type saliva

VLP-HBGA binding is blocked by P particle-induced Ab (red line)

Both VA387 VLP and P particle induce specific antibody

(through an intranasal route of immunization w/o adjuvant)
Advantages of P-particles as a vaccine against NVs

• Self-forming particles, easy production, high yield, easily to purify, low cost
• High affinity of receptor binding/blocking, highly immunogenic
• Structurally stable, tolerate with a wide range of pH, possibly survive in the stomach and intestine
• Induced both cellular and humoral immune responses in animal mouse model
P particle as carrier for foreign antigen presentation

List of small antigens successfully presented by P particles

- His-tag (7 aa)
- T-cell epitope of mouse CMV (9 aa)
- Epi8 epitope of Pseudomonas (14 aa)
- M2e epitope of influenza virus (23 aa)
- Rotavirus VP8 (159 aa)

Genetic insertion
Construction of P particle presenting rotavirus VP8* antigen

* P particles tolerated a large foreign insertion (159 aa)
VP8* is anchored on the tip of the P-2 domain
P-VP8* induced strong immune responses in mice

* Administered intranasally without an adjuvant
The P-VP8* antibodies neutralized rotavirus replication in cell cultures.
Immunization with P-VP8* protected mice from a murine rotavirus infection
High responses against the P-particle backbone
Dual vaccine against RV and NV

- Both NV and RV are endemic in children
- Risk of intussusception of live attenuated vaccine

Age-distribution of NV and RV diarrhea in children under 5 year in China
Additional studies

- The P-VP8* induced strong immune responses in chicken – production of IgY as therapeutic application
- A chimeric P particle presenting the influenza M2e epitope has been constructed
  - Mouse model
  - Chicken and swine model
- All three surface loops are useful for foreign insertion - multi-polyvalent vaccine
- Procedures for optimal expression and purification of the P particle and chimeric particles have been developed – industry partners:
  - PATH Vaccine Development Global Program (Gates Foundation)
  - Takeda/LigoCyte Vaccine
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