Bacterial expression of a VLP Sub-unit for rapid and cheap influenza vaccination

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Engineering VLP and Capsomere Vaccines

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Introduction

- Vaccination enormously successful
  - Smallpox eradicated, polio close
- More to do
  - 15 M people still die annually, half children <5 yrs
  - Emergent and re-emergent disease
- Technological gap in approaches
  - Pasteur’s “Isolate-Inactivate-Inject” dominates
  - Opportunity to engineer better systems
Modular Vaccine Design

BASE with sites for insertion of optional modules

MODULE

MODULAR DESIGN

Front guard

Viral antigen

http://www.indiamart.com/ajantaautoacc/front-guard.html#ajanta-grill-guard-for-tavera
Murine Polyomavirus

Capsomere

VP1 Protein

Virus-like Particle (VLP or Capsid)
Antigen Insertion Sites on VLP Surface
Bioprocess Engineering

**pGEX-4T-1-VP1**
- **6112 bp**
- GST
- Thrombin Cleavage Site + GG
- **BamHI (931)**
- **VP1**
- **XhoI (2098)**
- Ampicillin Resistance Gene

Best available expression in literature: 1 mg/L.OD

After factorial optimisation, Host selection and redesign: 15-20 mg/L.OD

*J. Biotechnol. (2008), 134(1-2): 64-71*

Confirmed 2-4 g/L in fed-batch *E. coli* fermentation.

*J. Biotechnol. (2010), 150(2): 224-231*
VP1 self-assembly *in vitro*
Process Flowsheet
The UQ Microbial Vaccine Platform (MVP)

- **Speed**
  - same process for different viruses
  - time from DNA to purified antigen < 1 week
  - processing can be automated

- **Scale**
  - Makes protein using industrial biotechnology tools
  - 100M doses per kL of bacterial culture in 24 h

- **Safety**
  - we make protein, not virus
  - we can sterile filter before virus assembly
The UQ Microbial Vaccine Platform (MVP)

Water
Glucose
Salts
Bacteria

2 g/l Soluble Antigen
Within 24 h

Purification and Assembly

>100,000 doses per litre
1000L pilot = 100M doses
In 24 h

“Dose Excess” regime.
Everyone can cultivate bacteria.
Pre-existing immunity against the platform?
Pre-existing immunity

Response against

J8i
(Group A Streptoccocus)

Endpoint titer
(Anti-antigen specific IgG)

Day 21
Day 77

wt VLP

ns = not significant

wt primed
PBS primed
wt primed
PBS primed
Application of the Platform: Influenza
Influenza

Global Challenge
When the virus changes, existing vaccine does not work.
H1N1 (2009): April 27th
H1N1 (2009): May 27th

Pandemic (H1N1) 2009
Countries, territories and areas with lab confirmed cases and number of deaths as reported to WHO

Cumulative deaths
- 1 - 10
- 11 - 50
- 51 - 100
- 101 and more
Country/territory/area with confirmed cases

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be a full agreement.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

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Map produced: 08 October 2009 09:56 GMT
H1N1 (2009): September 27th

Pandemic (H1N1) 2009
Countries, territories and areas with lab confirmed cases and number of deaths as reported to WHO

Cumulative deaths
- 1 - 10
- 11 - 50
- 51 - 100
- 101 and more

Chinese Taipei has reported sixteen deaths associated with pandemic (H1N1) 2009.

Map produced: 30 September 2009 07:54 GMT

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization
People die while they wait for the new vaccine.
Rapid response for emergent virus

Laboratory Timeline (Days):

0 2 4 5 7

Influenza A virus

Murine polyomavirus VLP

Microbial culture

Modular capsomere

Modular VLP

Vaccine available for in vivo testing
Initial Target Epitopes

- Completely Non-Universal
  - HA1 – receptor binding regions
  - Other HA1 epitopes

- Somewhat Universal
  - M2e of matrix protein 2
  - HA stalk regions
Haemagglutinin Helix 190

- Receptor binding site.
- Biology 101 – blocking the receptor binding site will block viral entry.
- Glycosylation?
- Structure?
Modularize into VLP format

Target epitope

Viral protein

VP1

Capsomere presenting target epitope

VLP presenting target epitope

x5

x72
Structural analysis of helix 190 peptide

MD simulation
- Gromacs
- In PBS solution
- 20 ns

Helix 190 in native HA

Peptide B1

Peptide B2
RMSD

Structural deviation of designed helix 190 peptide from native
Module Structure Matters

Peptide

Glycosylated HA

ns = not significant
Glycosylation Matters Less

![Graph showing the comparison of A450 nm vs Equivalent total HA (μg/well) for B2 VLPs, B1 VLPs, and controls. The graph indicates that A450 nm increases with increasing HA concentration for all three groups, but the increase is more pronounced for B2 VLPs compared to B1 VLPs and controls.]
Initial Target Epitopes

- **Completely Non-Universal**
  - HA1 – receptor binding regions
  - Other HA1 epitopes
  - Biology 101

- **Somewhat Universal**
  - *M2e of matrix protein 2*
  - HA stalk regions
Matrix Protein M2e

- Immunogenic
- Broad cross protection
- Complementary mechanism

Modularize into capsomere format

Engineering VP1 for antigen modules

- Assembly incompetent
- Improved stability
- Trypsin digestion site
- Antigen insertion:
  - two surface loops
  - N-terminus
  - C-terminus
Modular capsomere
Screening of modularized capsomere

- Expression level
- Solubility level
- Downstream bioprocessing yield

1011 and 2022
Capsomere format improves immunogenicity

Endpoint titre (Anti-M2e specific IgG)

- 1st immunization
- boost 1
- boost 2

PBS

Alhydrogel

Alhydrogel

$p = 0.0005$
Modular capsomere 1011 vs 2022

- Excellent IgG titres
- Little sensitivity to more modules
- Adjuvant necessary for capsomere format
- Excellent epitope tolerance
Conclusions

- VLP and capsomere platform developed
  - Remarkable productivity, protein not virus based
- Excellent developability and manufacturability
- Excellent end point titres
  - Moving to protection studies
- Multitude of insertions successfully handled
  - Flexibility afforded by VLP and Capsomere formats
Influenza in a Connected World

Control

Share

Identify
Acknowledgements

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