New prospects for vaccination: from polio to dengue and flu

From ancient Chinese variolation to Jenner and cowpox

Paul Young
Australian Infectious Diseases Research Centre
School of Chemistry and Molecular Biosciences
“Modern” vaccines

ACTIVE

INACTIVATED - under conditions that retain immunogenic properties
ATTENUATED - virulent organism weakened by growth in unnatural host

PASSIVE

Instilling the products of the immune response (antibodies or immune cells) into the recipient
- only useful for short term protection, e.g., rabies immune globulin

Prime objective of a vaccine stimulated immune response is to protect against disease - not necessarily prevent infection
Vaccines have an excellent track record

- Smallpox
- Yellow fever
- Polio
- Measles
“Active” vaccines

ADJUVANTS – stimulate immune response

DELIVERY – route can define quality and type of immune response
“Active” vaccines

ADJUVANTS – stimulate immune response

DELIVERY – route can define quality and type of immune response
Successful subunit vaccines

**Influenza virus** – virus grown in eggs, purified and disrupted into immunogenic component

**Hepatitis B virus (HBV)** – HBsAg produced recombinantly in yeast

**Human papillomavirus (HPV)**
- Gardasil (Merck): types 6, 11, 16, 18 produced in *S. cerevisiae*
- Cervarix (GlaxoSmithKline): types 45, 31 produced in insect cells + AS04 (aluminium hydroxide and 3-O-desacyl-4′-monophosphoryl lipid A)
Dengue virus Envelope (E) protein

- Biological properties
  - 3 structural domains
    - Domain 1 (red)
    - Domain 2 (yellow)
    - Domain 3 (blue)
  - Target of neutralizing antibody response
  - Vaccine candidate
  - Contains viral fusion peptide and receptor binding motif
Subunit vaccine

• 80% E protein (sE) produced in S2 cells:
  • Den 1: 258848
  • Den 2: PR159
  • Den 3: CH53489
  • Den 4: H241

• Key target for neutralising antibody
• Balance immune response by varying antigen concentration
• Subunit vaccine suffer from poor immunogenicity
The Nanopatch

- High density microprojection arrays
  - >20,000 microprojections/cm$^2$

http://www.abc.net.au/catalyst/stories/3576755.htm
Correlation between cell death and immunogenicity

Dynamic application of the Nanopatch generates localized transient stresses invoking cell death around each projection.
Dose sparing

- Nanopatch (to skin)
- Needle injection (to muscle)

Adapted from Fernando et al. (2010) PLoS ONE
Aims

• Evaluate the antibody responses to tetravalent dengue sE vaccination:
  – Investigating different delivery methods
• Demonstrate the protective efficacy of Nanopatch delivered sE in a lethal challenge model.
  – Monitoring viral load, NS1 levels and survival
Nanopatch delivery of sE
SV129 Dose ranging experiment

- **Aim**: To determine the best route of immunization to elicit potent anti-sE IgG
- **SV129 mice**: parental background to AG129 dengue model

<table>
<thead>
<tr>
<th>Nanopatch</th>
<th>SC</th>
<th>IM</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 1µg sE</td>
<td>6) 10µg sE</td>
<td>14) 10µg sE</td>
<td>21) 10µg sE</td>
</tr>
<tr>
<td>2) 1µg sE + QA</td>
<td>7) 10µg sE + QA</td>
<td>15) 10µg sE + QA</td>
<td>22) 10µg sE + QA</td>
</tr>
<tr>
<td>3) 0.1µg sE</td>
<td>8) 1µg sE</td>
<td>16) 1µg sE</td>
<td>23) 1µg sE</td>
</tr>
<tr>
<td>4) 0.1µg sE + QA</td>
<td>9) 1µg sE + QA</td>
<td>17) 1µg sE + QA</td>
<td>24) 1µg sE + QA</td>
</tr>
<tr>
<td>5) MC</td>
<td>10) 0.1µg sE</td>
<td>18) 0.1µg sE</td>
<td>25) 0.1µg sE</td>
</tr>
<tr>
<td></td>
<td>11) 0.1µg sE + QA</td>
<td>19) 0.1µg sE + QA</td>
<td>26) 0.1µg sE + QA</td>
</tr>
<tr>
<td></td>
<td>12) PBS</td>
<td>20) PBS</td>
<td>27) PBS</td>
</tr>
</tbody>
</table>

- **Dose 1**: 0 day
- **Dose 2**: 28 day
- **Dose 3**: 56 day
NP immunization with Quil A produces significantly more anti-sE IgG than all other delivery methods.
AG129 dengue challenge study

- Small animal model for dengue virus infection
- Interferon α, β and γ receptor knockouts

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination</th>
<th>Mouse number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NP 1µg sE + QA</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>NP 1µg sE</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>ID 1µg sE + QA</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>ID 1µg sE</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Virus control</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Naïve</td>
<td>5</td>
</tr>
</tbody>
</table>

Dose 1 → Dose 2 → Dose 3 → challenge
Results: Anti-sE IgG response

**DENV-1 anti-sE IgG titres**

- NP 1μg sE + Quil A
- ID 1μg sE + Quil A
- ID 1μg sE
- Virus
- Naive

**DENV-2 anti-sE IgG titres**

- NP 1μg sE + Quil A
- ID 1μg sE + Quil A
- ID 1μg sE
- Virus
- Naive

**DENV-3 anti-sE IgG titres**

- NP 1μg sE + Quil A
- ID 1μg sE + Quil A
- ID 1μg sE
- Virus
- Naive

**DENV-4 anti-sE IgG titres**

- NP 1μg sE + Quil A
- ID 1μg sE + Quil A
- ID 1μg sE
- Virus
- Naive
Survival to lethal challenge
• Nanopatch can deliver dengue virus sE antigen
• Saponin adjuvant QuilA enhances antibody titres
• Enhanced immune response to the four dengue serotypes
• Elicits balanced neutralising antibody response
• Anti-sE responses in AG129 mice were protective to lethal challenge
SEM - uncoated Nanopatch

fluorescent microspheres deposited into the ear

Cryo SEM of Nanopatch projections in place within the viable epidermal and dermal layers of ear skin.

microsphere depth in skin
Dose-sparing and efficient one dose response

**a** 21 days post prime

**b** 21 days post boost 1 (day 42)

**c** 21 days post boost 2 (day 63)

**d** Seroconversion: IPV2

- NP, 1 DU
- IM, 1 DU
- NP, 1/5 DU
- IM, 1/5 DU
- IM, 8 DU
- IM, Negative
Nanopatch: Features and Benefits

Key Features:
- Immune enhancer
- Dose sparing
- Adjuvant sparing
- Thermostability
- Versatility
- Scalable manufacturing

Key Benefits:
- No needles
- Less discomfort than needles
- Improved immunogenicity
- No cold-chain
Viral fusion proteins

Process of membrane fusion is driven by conformational change in structure of the virion surface fusion protein.

Pre-fusion → Post-fusion

Potent neutralizing Abs target the pre-fusion form.
**Envelope Viruses encoding Class I and III Fusion Proteins**

<table>
<thead>
<tr>
<th>Virus family</th>
<th>Fusion Protein</th>
<th>Fusion subunit</th>
<th>Class</th>
<th>Fusion pH</th>
<th>Fusion Peptide Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthomyxviridae</td>
<td>HA</td>
<td>HA1-S-S-HA2</td>
<td>I</td>
<td>Low</td>
<td>N-terminal</td>
</tr>
<tr>
<td>Retroviridae</td>
<td>Env</td>
<td>SU-S-TM, SU/TM</td>
<td>I</td>
<td>Neutral (Low)</td>
<td>N-terminal (most) Internal (ASLV)</td>
</tr>
<tr>
<td>Paramyxoviridae</td>
<td>F, HN</td>
<td>F2-S-S-F1</td>
<td>I</td>
<td>Neutral</td>
<td>N-terminal</td>
</tr>
<tr>
<td>Coronaviridae</td>
<td>S</td>
<td>S1/S2</td>
<td>I</td>
<td>Neutral (Low)</td>
<td>Internal</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>GP</td>
<td>GP1-S-S-GP2</td>
<td>I</td>
<td>Low</td>
<td>Internal</td>
</tr>
<tr>
<td>Arenaviridae</td>
<td>GP, SSP</td>
<td>GP1/GP2/SSP</td>
<td>I</td>
<td>Low</td>
<td>N-terminal</td>
</tr>
<tr>
<td>Rhabdoviridae</td>
<td>G</td>
<td>G</td>
<td>III</td>
<td>Low</td>
<td>Internal (bipartite)</td>
</tr>
<tr>
<td>Herpesviridae</td>
<td>gB, gD, gH/L</td>
<td>gB(h), gH/gL</td>
<td>III</td>
<td>Neutral (Low)</td>
<td>Internal (bipartite)</td>
</tr>
</tbody>
</table>

**Class I and III envelope viruses of human and veterinary importance**

<table>
<thead>
<tr>
<th>Virus family</th>
<th>Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthomyxviridae</td>
<td>Influenza A, B, C</td>
</tr>
<tr>
<td>Retroviridae</td>
<td>HTLV-1, 2, 3, HIV-1, HIV-2</td>
</tr>
<tr>
<td>Paramyxoviridae</td>
<td>Measles (MeV), Rinderpest virus (RPV), Canine distemper virus (CDV)</td>
</tr>
<tr>
<td></td>
<td>Respiratory syncytial virus (RSV) – human and bovine</td>
</tr>
<tr>
<td></td>
<td>Human Meta-pneumovirus (HMPV)</td>
</tr>
<tr>
<td></td>
<td>Human Parainfluenza virus (HPIV)</td>
</tr>
<tr>
<td></td>
<td>Mumps virus (MuV)</td>
</tr>
<tr>
<td></td>
<td>Hendra virus (HeV), Nipha viruses (NiV)</td>
</tr>
<tr>
<td></td>
<td>Newcastle Disease virus (NDV)</td>
</tr>
<tr>
<td>Coronaviridae</td>
<td>Human Coronavirus (HCoV 229E)</td>
</tr>
<tr>
<td></td>
<td>Human Coronavirus (HCoV OC43, HCoV HKU1, HCoV EMC)</td>
</tr>
<tr>
<td></td>
<td>Human Torovirus (HToV)</td>
</tr>
<tr>
<td></td>
<td>SAS-CoV, MERS-CoV</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>Zaire Ebola virus (ZEBOV), Reston (REBOV), Sudan (SEBOV)</td>
</tr>
<tr>
<td></td>
<td>Marburg (MARV)</td>
</tr>
<tr>
<td>Arenaviridae</td>
<td>Lassa virus (LASV), Lymphocytic choriomeningitis virus (LCMV), Junin virus (JUNV)</td>
</tr>
<tr>
<td>Rhabdoviridae</td>
<td>Rabies (RABV), Australian Bat Lyssavirus (ABLV), Bovine ephemeral fever virus (BEFV), Vesicular stomatitis virus (VSV)</td>
</tr>
<tr>
<td>Herpesviridae</td>
<td>HHV-1 (HSV-1), HHV-2 (HSV-2), HHV-3 (VZV), HHV-4 (Epstein-Barr virus - EBV), HHV-5 (CMV)</td>
</tr>
</tbody>
</table>
Evidence for pre-fusion stabilization for a range of viral fusion proteins

A  Ebola GP reactivity with Kz52

B  Influenza HA reactivity with FI6V3

C  RSV F sucrose gradient separation

D  Measles F reactivity with human sera
Influenza HA Clamp

**Stabilised HA Vaccine**

- **αHead Abs**
  - Strain Specific
  - Highly Neutralizing

- **αStem Abs**
  - Cross-reactive
  - Broadly protective

**Comparison to Seasonal Vaccine**

- **Head**: 80 fold Improvement
- **Stem**: 27 fold Improvement
- **Clamp**: Conformationally Stabilised
The Future?

• Synthetic peptides

• DNA vaccines
  • (DNA launched virus vaccines)

• Improved adjuvants, liposomes, ISCOMS etc

• Edible vaccines – transgenic plants - algae

• New delivery methods – eg “Nanopatches”
Acknowledgements

PRY Lab
- Keith Chappell
- Daniel Watterson
- David Muller
- Vernon Seow
- Naphak Modhiran
- Sue Liebscher
- Ashleigh Shannon
- Imogen Bermingham
- Jaelle Brealey
- Stacey Cheung
- Andrew Young
- Chris McMillan

Mark Kendall, UQ
Vaxxas
WHO

Stradbroke Island retreat Jan 2015