Filamentous Phage as a Platform for Development of Contraceptive Vaccines for Animals

Phage vectors

Phage particle can be genetically modified to carry antigenic peptides

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Phage vectors
Phage vectors

- Wild type gene 8
- Recombinant gene 8
- Wild type protein
- Recombinant protein + WT protein + antigenic peptide

Why use landscape phage

**Effective immunogen**
- Structure of Ag surface display
- Highly organized, repetitive, high density
- Particulate antigen, size and shape attractive for APCs
- Stimulate strong T helper cell responses
- Low cost to produce
- 1L of ON culture yields ~10 mg phage, 50-100 doses stable to transport, store and deliver
- Years if refrigerated
- Six months at room temperature
- Weeks at 63°C
- Hours at 76°C
- Easily administered via parenteral routes, no adverse reactions
- Tested in mice, dogs, pigs
- Viable pathway to regulatory approval
- Can be used in inactivated form
- Retains immunogenicity (Samoylova et al., Virological Methods, 2012)

Phage viability

Phage absorbance profiles

Antibody responses in mice

Sequential bleedings, weeks after primary immunization
Phage-GnRH constructs can be generated via:

1. Cloning of GnRH inserts in phage vector
2. Selection of GnRH-like clones from phage display libraries

Peptide of interest: GnRH

Phage-GnRH construct

GnRH-like peptide
4000 copies per phage particle

Design of phage-GnRH constructs

- Known receptor binding epitopes
- Studies on a.a. critical for contraception
- Arl/A/AAAAA
  - Testosterone - immunocastration
  - In mice (Hu et al., 2007)
- Short peptide GLRPG
  - Fertility trials
  - In dogs (Hu et al., 2004)
  - XXXXXX
  - In cats

Cloning of phage-GnRH constructs

- Design insert sequences
- Synthesize oligonucleotides
- Prepare dsDNA inserts; prepare phage vector DNA
- Cut insert & phage DNAs with restriction enzymes
- Ligate insert in phage DNA
- Electroporate obtained constructs in E. coli
- Sequence phage-harbor E. coli colonies
- Propagate correct phage clone
**Phage-GnRH constructs obtained via cloning: example**

<table>
<thead>
<tr>
<th>Insert sequence</th>
<th>Titer, cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRGGGS</td>
<td>4x10¹⁵</td>
</tr>
<tr>
<td>ENWVYGL</td>
<td>low</td>
</tr>
<tr>
<td>GGGYNVGGGS</td>
<td>1.4x10¹²</td>
</tr>
<tr>
<td>GGGPQGGGS</td>
<td>1.2x10¹¹</td>
</tr>
<tr>
<td>ENVGRPGGS</td>
<td>low</td>
</tr>
<tr>
<td>ENVGGGVRPG</td>
<td>low</td>
</tr>
</tbody>
</table>

Constructs obtained = 61
Constructs that propagate relatively well = 10
Constructs that propagate well and show good binding to GnRH Ab - several Stable?

**Phage display library is multibillion mixture of phage clones**

Phage display library is a multibillion mixture of phage clones.

**How phage display works...**

**Selection from phage library**

- Add phage library
- Incubate
- Wash
- Recover bound phage
- Propagate
- Add to GnRH Ab for next round
- Purify
- Selection

Ab = anti-GnRH antibodies
3-4 rounds required
**Monitoring of selection steps**

<table>
<thead>
<tr>
<th>Round 1</th>
<th>Round 2</th>
<th>Round 3</th>
<th>Round 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propagate/purify phage pool</td>
<td>Comparative ELISA with anti-GnRH Abs</td>
<td>Identify round with highest ELISA response</td>
<td>Propagate phage from random individual colonies</td>
</tr>
<tr>
<td>ELISA of individual phage clones</td>
<td>Sequence clones with highest ELISA responses</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**8-mer library selection: example**

Round-to-round enrichment in GnRH Ab-binding clones (ELISA)

**Phage libraries selection using different Abs**

- 8-mer on Ab 16216
- 9-mer on Ab 16216
- 8-mer on cat Ab Protein A purified
- 8-mer on cat Ab GnRH pep purified

<table>
<thead>
<tr>
<th>8-mer on Ab 16216</th>
<th>9-mer on Ab 16216</th>
<th>8-mer on cat Ab Protein A purified</th>
<th>8-mer on cat Ab GnRH pep purified</th>
</tr>
</thead>
<tbody>
<tr>
<td>XGLRPXXX</td>
<td>ALRPGLDES</td>
<td>DQQGNNXXX</td>
<td>DGLRPQAP</td>
</tr>
<tr>
<td>GPTPXXX</td>
<td>XIXPGXXX</td>
<td>GANRXXX</td>
<td>EHFTGNG</td>
</tr>
<tr>
<td>XIXPXXX</td>
<td>AIXXXXXX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTPVXXX</td>
<td>GTPIXXX</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Candidate clones for in vivo studies: ELISA signals**

**Green**: GnRH epitope C terminus
**Red**: GnRH epitope N terminus
**Others colors**: GnRH mimics or non-GnRH epitopes
**Black**: any amino acid residue

**Phage clones**

- 1: DGLRPQAP
- 2: EHFTGNG
- 3: EKLAVSQG
- 4: ALRPGLDES
- 5: DPTFPTWTS
- 6: GLLPQGS

**Phages clones**

- Ab 16216
- Cat 1 serum
- Cat 2 serum
**Phage-peptide conjugates**

Phage: wild type
Peptide: EHWSYGLRPG[Ahx]-COOH
- [Ahx] 6-aminohexanoic acid derivative of lysine

Conjugation: EDC 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
- activates carboxyl groups for coupling to primary amines
- yields amide bonds

**ELISA signals**

- Pep 1: EHWSYGLRPG[Ahx]-COOH
- Pep 2: EHWSYKLPG[Ahx]-COOH
- Pep 3: reverse GPRLGYSWHE[Ahx]-COOH
- Pep 4: EHWSYGLPG-amide

**Antibody responses in mice**

- Pre-immunization
- Post-immunization

**ELISA responses to cat & dog sera**

- cat sera
- dog sera
Another target – ZP on oocytes

- ZP: zona pellucida
- Protective membrane surrounding oocyte
- 3 or 4 glycoproteins depending on the species
- Play critical role in sperm-oocyte binding and induction of acrosome reaction

ZP-binding epitopes on sperm and on phage

Incubation: phage-ZP binding
Fertilization: sperm-ZP binding

Hypothesis: phage epitopes mimic sperm epitopes

Potential contraceptive mechanisms

Inject ZP-binding phage
Anti-sperm antibodies

Mechanisms of action:
- Inhibition of sperm capacitation, acrosome reaction
- Decreasing sperm motility
- Interference with sperm-ZP interaction/penetration/fusion

Antibody responses in dogs

Sperm cells reacted with serum from immunized dog

- Anti-sperm antibodies
- High, long-lasting Ab responses
- Phage is safe (7 dogs, dose 500µg)
Fertility trials in mice

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Pups/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>13.50</td>
</tr>
<tr>
<td>Phage vector</td>
<td>13.25</td>
</tr>
<tr>
<td>Phage clone 3.1</td>
<td>11.33</td>
</tr>
<tr>
<td>Phage clone 2.26</td>
<td>9.89</td>
</tr>
<tr>
<td>Phage clone 3.7</td>
<td>7.89</td>
</tr>
</tbody>
</table>

p<0.001
p=0.029

Thank you!!!

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