Delivery Dystems in ETEC vaccination: Complexity, Failure, Success

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Worldwide deaths annually from mucosal infections

- Acute respiratory infections (4 million)
- Diarrheal diseases (1.8 million)
- Tuberculosis (1.5 million)
- HIV (2.9 million)
- Measles (600,000)
- Hepatitis B (103,000*)
- Whooping cough (294,000)
- Roundworm and hookworm (12,000)

Figure 11-2 Immunobiology, 7ed. (© Garland Science 2008)
Vaccines

**Replicating (alive)**
- Avirulent strains
  - E.g. no toxines
- Attenuated strains
- Recombinant (living subunits)
  - recombinant vectors

**Non-replicating (dead)**
*To be targeted?*
- Particles
  - Microparticles
  - Nanoparticles
- Soluble proteins
  - anatoxins
  - envelope proteins
- Produced proteins (dead subunits)
# Human oral vaccines

**Polio**
- Live attenuated vaccine (OPV)  
  Oral  Many Producents

**Cholera**
- Cholera toxin B subunit (CTB) + inactivated *V. cholerae* O1  
  Oral  Dukoral (Crucell)
- Inactivated *V. cholerae*, no CTB  
  Oral  Shanchol (Santa Biotechnics)
- CVD 103.HgR live recombinant *V. cholerae* O1 strain lacking CTA  
  Oral  Orochol (Crucell)

**Typhoid**
- Ty21a live attenuated vaccine  
  Oral  Vivotif (Crucell)

**Rotavirus**
- Live attenuated monovalent human rotavirus strain  
  Oral  RotaRix (GlaxoSmithKline)
- Multivalent human-animal reassortant Strain  
  Oral  RotaTeq (Merck)
Protection small intestine

**Virulence**
- Too attenuated => no danger
- Too virulent => disease

**ROUTE**
- Parenteral ↔ Mucosal
- Oral preferred route
- Not mucosa

**Can immunomodulation change this?**

**VACCINE**
- Alive ↔ Dead

**Translation from mice?**

**AGE**
- Passive ↔ Active

**Immune status**
- Colostral/placental passive Immunity
- Lactogenic Immunity

**Passive immunity**

**Active immunity**

**Intestinal Mucosa**
FOLLICLE-ASSOCIATED EPITHELIALM

Enteropathogens (enterotoxigenic *E. coli*)
Particulated antigens

Immunity

T helper cells

MUCOSA

Non-replicating soluble antigens

Epithelium

IgA production

Dentritic cells

Immature antigen-presenting cells

Draining lymph node

Oral tolerance

Mucosa-associated lymphoid tissue

Danger

Epithelium
Oral vaccination remains difficult

Follicle-associated epithelium

M-cells
Particulated antigen

Pathogen
Live vaccine

Enterocytes
Few soluble antigens
Virulence factors

Enterocytes

M-cells
Particulated antigen

Mature DC

Gut-associated lymphoid tissue

Immunity

Mature DC

Lamina propria
Mesenteric Lnd
Oral vaccination remains difficult.

Follicle-associated epithelium

- M-cells
  - Particulated antigen
  - e.g. Killed Vibrio cholerae

Enterocytes

- Soluble antigens
  - Virulence factors
  - e.g. CT, LT, F4

Selection of antigens

- Antigens remain immunogenic (production process)
- Reach FAE (target)
- Transcytose by M cells (target?)
- Danger signals (adjuvant?)
- Uptake by DCs
- Maturation of DCs

Pathogen

- Live vaccine

• Antigens retain immunogenic (protection)
• Reach enterocytes (target?)
• Transcytose by enterocytes (target?)
• Danger signals (adjuvant?)
• Uptake by DCs
• Maturation of DCs

Immunity
Pigs

- Diarrhoea = 11% of all post-weaning mortality
- ± 10 million piglets die annually worldwide
- 50% is caused by enterotoxigenic E. coli

**Enterotoxigenic Escherichia coli (ETEC) in piglets**

- **Fimbriae** (F4, F5, F6, F41, F18)
- **Colonisation**
- **Enterotoxins** (LT, STa, STb)

**Neonatal diarrhoea**

**Postweaning diarrhoea**
1. **Oral Immunisation** with purified F4 (and dissolved in PBS)

2. **Infection challenge with** F4+ETEC

3. **Faecal excretion of F4+ ETEC**
The mucosal response is protective

- **IgA**
  - PBS: Flat line
  - F4-immunized: Steep increase to high levels

- **IgG**
  - PBS: Flat line
  - F4-immunized: Steep increase to high levels

- **IgM**
  - PBS: Flat line
  - F4-immunized: Steep increase to high levels

**ETEC excretion after infection**

- **Days post infection**
  - 2: 0
  - 3: 15
  - 4: 3
  - 5: 3
  - 6: 0

**F4 + ETEC**

- **Oral F4**
  - No boost after infection
  - No faecal excretion!

- **F4**
  - Mucosal response is protective

=> **Oral F4 induces protective mucosal response!**

*Van den Broeck et al., 1999. Infect Imm*
Binding and uptake of F4 fimbriae

Ligated loops injected with F4

**FLUOS-labeled F4**

Snoeck et al., 2008. Vet Imm Immunopath.

F4 fimbriae bind to **Aminopeptidase N (APN)**

Blotting of brush border proteins and staining with F4
Uptake of APN-specific antibodies by enterocytes in vivo (intestinal loops)

IgA response in pigs against rabbit IgG

Immunization with 1mg anti-APN rabbit IgG ± 50 µg CT of 26 days old piglets seronegative for anti-rabbit IgG

Rasschaert et al., 2012. Mucosal Immunology
Conclusions

- Targeting soluble antigens towards a receptor on enterocytes can lead to a protective intestinal mucosal immune response if:
  - Antigen is stable
  - Binding to the receptor results in transcytosis
  - There is activation of DCs
- Targeting aminopeptidase N is a promising strategy to induce an intestinal IgA response against soluble antigens.
Protective immunity at weaning:

- Encapsulation to protect F4 antigen
- Digestion
- Denaturation
- Inhibition by milk glycoproteins and sugars

**Gantrez nanoparticles**

poly(methylvinylether-co-maleic anhydride)

\[ \approx 100 \text{ nm} \]

Forms covalent, ionic and H-bounds with the F4

F4 only released by hydrolysis of the polymer

Gantrez nanoparticles (gNP) oral in weaned pigs

**F4-specific Ab response**

- **Serum IgA**
  - F4
  - g(F4) NP
  - F4+gNP
  - gNP

- **Serum IgG**
  - F4
  - g(F4) NP
  - F4+gNP
  - gNP

**Ab response:** gNP < F4 < (F4)NP < F4+NP

**Excretion:** F4+ NP < F4 < gNP < (F4)NP

**best effect with empty NP**

⇒ **Adjuvant effect mainly caused by polymer properties** ↔ **encapsulation!!**

Targeting to enterocytes

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<th>Positive</th>
<th>Weak positive</th>
<th>Negative</th>
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<tr>
<td>SNA-I, -II, -III, -IV</td>
<td>Jacalin</td>
<td>GNA</td>
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<tr>
<td>SRA</td>
<td>STL</td>
<td>NPA</td>
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WGA: Wheat germ agglutinin binds N-acetyl-glucosamine (EGFR)
Protection against infection with F4+E. coli

Faecal excretion of F4+ ETEC

Addition of gNP: excretion reduced by at least 3 days!!!; WGA no effect
Conclusions

- Particles are not very successful in vaccine trials in large animals.
- There is no optimal targeting
- Live attenuated vaccines are still the most successful oral vaccines.
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