Immunoprotective effect of plasmid-less Chlamydia strain

A live-attenuated chlamydial vaccine protects against trachoma in nonhuman primates


JEM
Volume 208(11):2217-2223
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In vitro characterization of the C. trachomatis plasmid-deficient trachoma strain A2497P−.

A

Kari L et al. JEM 2011;208:2217-2223

B

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The C. trachomatis plasmid-cured trachoma strain A2497P− is highly attenuated for the monkey eye.

Kari L et al. JEM 2011;208:2217-2223
Repeated infections of nonhuman primates with the plasmid-deficient A2497P− strain do not cause ocular pathology.

Kari L et al. JEM 2011;208:2217-2223

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Monkeys vaccinated with the attenuated A2497P− strain are protected against a challenge infection with virulent A2497 organisms.

Kari L et al. JEM 2011;208:2217-2223
Immune and genetic correlates of vaccine-mediated protective immunity.

Kari L et al. JEM 2011;208:2217-2223
Implications…

- Could you make a human vaccine using CT that lacks the virulence plasmid?...discuss
  - How could this work?
  - What would we need to do?
  - Would cell debris be a problem?

- Or if you make a sub-unit vaccine, which antigens can you choose?
  - Discuss with respect to what you know now about Chlamydia
Development of Mucosal Vaccine against Trachoma

Concepts and current progress
What are mucosal vaccines?

- Protective mucosal immune responses are most effectively induced by mucosal immunization through oral, nasal, rectal or vaginal routes, but the vast majority of vaccines in use today are administered by injection.

- Disadvantages include,
  - dose given is never certain and large amounts of antigen are needed for mucosal immunisation.
  - Antibodies in mucosal secretions are difficult to capture and quantitate
  - Recovery and functional testing of mucosal T cells is labour intensive and technically challenging
Epithelial cells

- Part of innate immune system and important for recognition
- Epithelial cells are active participants in mucosal defence
- Function as sensors that detect dangerous microbial components through pattern recognition receptors, such as Toll-like receptors (TLRs). They respond by sending cytokine and chemokine signals to underlying mucosal cells, such as dendritic cells (DCs) and macrophages, to trigger innate, nonspecific defences and promote adaptive immune responses.
- TLR 5 recognises flagellin, while TLR 4 recognises E.coli LPS
Enhancing the immune response

- Non-living macromolecules, protein sub-units and non-microbial particles evoke weak or undetectable adaptive immune responses when applied mucosally.

- To be distinguish, they need to be coupled with adjuvants to activate innate signalling pathways in epithelial cells or underlying antigen presenting cells.

- Incorporation of cytokines, IL-12, GM-CSF, or both.

- TLR ligands, CpG nucleotides, flagellin and bacterial porins have shown adjuvant activity when administered with antigens.

- Particulate vaccines (up to 1µm) enter mucosal inductive sites (M-cells) more efficiently and are more actively taken up by mucosal DCs and transported to antigen presenting cells.
Influences for choice of vaccination immunisation route

- Primary mode of entry of CT is across mucosal surfaces
- Any efficient vaccine needs to induce a strong mucosal immune response
- Inducing strong mucosal response may protect at time of exposure, and reduce the number of infectious organisms shed by individual
- IM, IP and SC routes in animal models develop strong systemic immunity, but often only weak mucosal immunity
- Strong mucosal immunity achieved by inoculation at local and distant mucosal sites, via common mucosal immune system
- Most common routes include sublingual, intranasal and oral routes
- Choice of ROI will influence level of protection achieved with CT vaccine
Knowledge for global sight solutions

Laura Bassi Centre of Expertise

OCUVAC
Bacterial Ghosts Vaccines - the concept

- To make a vaccine against Trachoma
Research Programme

- How?
  - Bacterial Ghost System

- Why?
  - Conjunctival vaccination strategy

- When?
  - Will it work?
Vaccine Carrier: Bacterial Ghost System
Bacterial Membrane
Production of ‚armed‘ Bacterial Ghosts

• Initially, suitable antigens identified by literature search

• Confirmation of suitability by testing antigens against Trachoma patient serum from field studies
Bacterial Ghosts with Chlamydia Antigens

Surface antigens; promote attachment, adhesion
Advantages of BGs

- Pose no safety or horizontal gene transfer threat
- Topical application to the eye/nose (needle free)
- Cost-effective
- Fast BG production through fermentation
- Straightforward technology can be transferred to developing countries
- Storage at room temperature for several years
• Conjunctival vaccination strategy
Why mucosal vaccines?

- Needle-free administration possible
- Compatible with BG technology platform
- Less difficult to register with FDA
- Promotes protective immunity at site of infection
Stimulating Innate Immunity – adhesion & uptake
Mucosal Immunity – Nasal Route

http://www.nature.com/nm/journal/v11/n4s/pdf/nm1213.pdf
In mice, monkeys and humans, nasal administration of vaccines via intranasal route has induced specific mucosal IgA antibody responses in salivary glands, upper and lower respiratory tracts and the small and large intestine. The nasal route can also induce CTLs in distant mucosal tissues including the female genital tract. Nasal immunization studies in humans and mice produced greater systemic antibody responses than other mucosal routes.

For many pathogens, optimal protection is likely to require both mucosal and systemic immune effectors and perhaps using a mucosal primary inoculation, followed by a parental booster inoculation.
Mucosal Immunity – Nasal Route

Administration of a mucosal vaccine via IN route in children, may be problematic…
Intranasal route – not ideal

- Appears to be best ROI for eliciting protective immunity in the reproductive tract
- Best known mucosal adjuvants are secreted endotoxins of *Vibrio cholerae* and *E.coli*
- Nasal administration of cholera toxin and CTB-based adjuvants has been shown to accumulate in olfactory nerves, mutated B subunit was transported to the brain in experimental animals
- *E.coli* HLT, (inactivated IN influenza vaccine) has been associated with increase incidence of Bell’s Palsy in humans
- CTA1-DD (chimeric toxin with Protein A) and rVCG (recombinant V.cholera bacterial ghosts) are potentially safer adjuvant alternatives
- Mucosal routes can be used to elicit partial protection against infection and immunopathology in animal models, but have not been evaluated in primate or human trials
Conjunctiva Anatomy

The ocular surface is constantly exposed to microorganisms.

Mechanical, anatomical, and immunological defense mechanisms protect the outer eye.
Why? Conjunctival vaccination strategy

- Same route as *Chlamydia trachomatis* infection
- Topical Eye drops: Needlefree application of BGs
- Activates innate immunity to stimulate mucosal immunity
- No health care professional – imp. 3rd World
In the possum: vaccination via conjunctiva gave strongest immune response

“It was demonstrated for the first time that mucosal immunization with BG expressing possum ZP2C terminal protein by the nasal/conjunctival route elicited an immune response and significantly reduced the fertilisation rate of eggs in vaccinated female brushtail possums by 36%”

*E. coli* expressing Chlamydia antigens
## Some Chlamydial Antigens used to make ‘armed BGs’

<table>
<thead>
<tr>
<th>Protein</th>
<th>Localization</th>
<th>Mechanism</th>
<th>Immunogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>PorB</td>
<td>Surface of Ebs</td>
<td>Porin function</td>
<td>Yes</td>
</tr>
<tr>
<td>OmcB</td>
<td>Periplasm EB &amp; RB</td>
<td>Mediates adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>PmpA</td>
<td>Surface of EBs</td>
<td>Mediates host-cell adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>PmpB</td>
<td>Surface of EBs</td>
<td>Mediates host-cell adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>PmpC</td>
<td>Surface of EBs</td>
<td>Mediates host-cell adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>PmpD</td>
<td>Surface of EBs</td>
<td>Mediates host-cell adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>PmpI</td>
<td>Surface of EBs</td>
<td>Mediates host-cell adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>ArtJ</td>
<td>Periplasm and surface of bacteria</td>
<td>Mediates host-cell adhesion</td>
<td>Yes in <em>C.pn</em> only</td>
</tr>
<tr>
<td>PmpG</td>
<td>Surface of EBs</td>
<td>Mediates host-cell adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>PmpE</td>
<td>Surface of EBs</td>
<td>Mediates host-cell adhesion</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Immuno-relevant Antigens/ Proteomics

FIELD COLLECTION of TEARS; SERUM; CHLAMYDIAL ISOLATES.....
Ocular route

- Trachoma is an infection confined to the ocular mucosal surface

- Search of literature suggests that ocular route of vaccination was not previously investigated as a ROI for mucosal vaccines
Ocular surface as an immunization site

Tetanus vaccine delivered into mouse conjunctiva

Immunization induced TTd (tetanus toxin)-specific local and systemic immune response

Partial protection against tetanus toxin (systemic lethal challenge)
HCJE cells internalized BGs in a time- and dose- dependent manner
Uptake of Atto488-labelled *E. coli* Nissle BGs into Human Conjunctival cells

10 BG/cell (green), 10^1 BGs/cell (blue), 10^2 BGs/cell (red), 10^3 BGs/cell (orange)

30 min: 22.7

120 min: 44.7
Uptake of Atto 488-labelled naked-BGs into Human Conjunctival Cells

100x magnification after incubation with E. coli Nissle (right) and M. haemolytica BGs (left) for 40 min
Human Conjunctival cells incubated 120 min
Atto488-labelled *E.coli* Nissle BGs

**Quenched Trypan Blue**

**Unquenched**
HCjE cells with attached BGs
Uptake of BGs in HuConj. Epithelial Cells by TEM
Small animal studies

BGs into guinea pig eyes

Tolerability, Inflammatory reactions and specific immune responses 2 hours after inoculation

No evidence of differences between control and treated animals
Trachoma vaccine: Summary

What? Produce vaccine against trachoma

How? Immunogenic antigens carried on bacterial ghosts

Why? Stimulate protective mucosal immunity via conjunctiva

When? First results are promising

Goal: Reduce blindness due to trachoma in 3rd world
Trachoma vaccine: why now?

- New insights into the localized immunology of the ocular surface
- Topical (needle-free) application of the vaccine is feasible
- Protective antigens/epitopes = good candidates for soliciting immunity
- An effective delivery system using non-living recombinant bacterial ghosts (BG) is available
- BG-based vaccines promise to be safe and cost-effective
- BGs are stable for several years and require no refrigeration
- The production process of BG based vaccines is transferable
- Training developing countries in the manufacture process of the vaccine would help to promote local development
Knowledge for global sight solutions

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Fred Hollows Foundation.....


- http://www.youtube.com/watch?feature=player_embedded&v=V6Qsjtloj8E
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Differences between human and mouse models of persistence

R.V. Schoborg: Microbes and Infection Volume 13, Issue 7 2011 649 - 662