Trachoma

Global Scourge causing Blindness
How would your life change if you became blind?

- Imagine you live in a poor rural community....
What is Trachoma?

- Historical context of trachoma
- Clinical diagnosis of trachoma
- Treatment for trachoma
- SAFE strategy
- *Chlamydia trachomatis*
- Other Chlamydiae
- Developing a mucosal vaccine against trachoma
What is Trachoma?

Bacterial infection of the conjunctivae caused by the intracellular bacteria, *Chlamydia trachomatis*
Clinical Signs of Trachoma
History of Trachoma

Epilation forceps known from 2600BC
Early History of Trachoma

- Texts from Ancient China (27th Century BC) references suggest Emperor Huang Ti Nei had surgery for trichiasis
- 420-581 AD Trachoma lesions described as ‘pepper-seed-like’ or ‘millet-like lesions. Pannus and trichiasis were well known
- Treatments included copper compounds, rubbing eyelids with octopus bone and garlic.
1553-1550 BC Ebbers Papyrus

First known medical text, contains over 700 prescriptions for treating diseases; 1 in 10 were to treat eye disease

Topical eye treatments included:
• Onions, myrrh & gazelle excrement applied with the feather of a vulture
• “the blood of lizards or bats was to be applied to the eyelid after epilation”
• Mineral components i.e. lead sulphate, lead acetate, antimony and soot, still used in Kohl eye preparations
Hippocrates 460-380BC

- Described trachoma as ‘ophthalmia’ and ‘lippitudo’ ‘trichosis’ for inturned lashes
- Recognised fig-like appearance of everted upper lid
- Described 4 eye operations; 3 of these were for trachoma**
  - Excision of fleshy granular tissue
  - Cauterisation with heated iron and copper bloom treatment
  - Suturing with hairs through the eyelid to evert the lid (the sutures would eventually slough out)

**These operations were still recommended by physicians in the 20thC
Bible reference:
When St. Tobias used fish bile to cure his father of blindness, he was probably treating trachoma.

Tobias cures his father's blindness – Bernardo Strozzi 1581 – 1644
Scourge of trachoma was endemic in Palestine during the Crusades. Crusaders and pilgrims to the Holy Land may have returned infected with trachoma which they then introduced Italy and elsewhere into Europe in the 13th C.

St Francis of Assisi (1182-1226) visited Palestine on at least two occasions between 1218-1221. By 1223 he had severe trachoma and trichiasis. He was blind when he died in 1226.
Under Napoleon, French troops were devastated by infections. In one battalion, 125 out of 350 men had ophthalmia, …many cases were self limiting, but others persisted for months. An expedition to upper Egypt had to be aborted in 1798, when 1400 developed ophthalmia out of a force of 3000 men. “there were more blind men than healthy”.
Infectious and contagious nature of ophthalmia was noted, and described by Vetch:
In one battalion; 606 out of 700 soldiers developed ophthalmia, 90 subsequently developed unilateral or bilateral blindness.

British Army enforced strict hygiene measures, including isolation of ophthalmia cases, importance of cleanliness, faces washed under running water, no sharing of towels, pillowcases were introduced.
MacCallan Classification of Trachoma

(first published in 1908, world standard for over 60 years)

- **Trachoma I:** soon after infection has occurred
- **Trachoma IIa:** follicles predominate
- **Trachoma IIb:** papillary hypertrophy coexists with follicles
- **Trachoma III:** cicatrization is beginning
- **Trachoma IV:** cicatrization is complete
Was Trachoma infectious?

• First suggestions that trachoma was infectious were made by Plato (427-347). Aristotle (384-322 BC) went further and concluded that it could be caught by looking at someone who was infected.

• Debate raged until the 1800s.; ‘Contagionists’ vs. ‘Compressionists’

• A major epidemic broke out among Dutch and Belgian Armies in 1815, but a further severe epidemic occurred in Belgium in 1834. 4000 Belgian soldiers were total blind and another 10,000 partially blinded. This led to the First International Congress in Ophthalmology in Brussels in 1857.
Trachoma screening – Ellis Island
Mapping Trachoma

- First efforts to develop a global picture of trachoma was the work of F. Wibaut in Amsterdam. Following a two year extensive survey of all existing Ophthalmic societies; he summarised current knowledge and was subsequently presented in ICO, Amsterdam 1929.

- No races were immune
- Women affected more severely than men
- Trachoma was a disease of the poor
- Secondary infections are important
- Incubation period was 4-10 days
- Transmission took place in the family
- Children aged less than 7 yr had highest prevalence
- Younger children were infected by older brothers and sisters
F. Wibaut (1929) “Mappa Mundi trachomae”
Where is Trachoma in 2005?

Country trachoma status:
- Green: No active trachoma
- Red: Data confirmed endemic active trachoma
- Yellow: No data identified, believed endemic active trachoma

Epidemiology of Trachoma

- Family based disease
- Women affected more than men
- Mother has trachoma, children at higher risk
- Facial cleanliness (+/- nasal discharge) and eye-seeking fly density
- Access to water
- Repeated infections lead to worsening infections and disease progression
Clinical diagnosis of Trachoma

Normal tarsal conjunctiva

(TF) Trachomatous inflammation, follicular

(TI) Trachomatous inflammation, intense

(TS) Trachomatous scarring

(TT) Trachomatous trichiasis (eyelashes inturned)

(CO) Corneal opacity

The WHO simplified grading scheme for assessment of trachoma. Reproduced from Thylefors, 1987
What is in the Universal Transport Medium?

Used for collection of cells from patients subsequent isolation and growth of viruses, Chlamydia and mycoplasma

- Flocked swab
- Beads for mixing
- UT Medium

Contains:
Hanks Balanced salts, bovine serum albumin, gelatin, sucrose, L-glutamic acid, HEPES buffer, Vancomycin, Amphotericin B, Colistin, Phenol Red

\[ \text{pH 7.3 +/- 0.2 @ 25°C} \]

- Cryoprotective – samples can be frozen (-80°C or liquid nitrogen)
- Antibiotics reduce growth of other bacteria and fungi
- Medium not toxic to cell monolayers
Laboratory testing for Chlamydia

- DFA cytology
- Giemsa
- EIA kits for LPS and MOMP
- Dipstick EIA
- (Presence of secondary or co-infections)

- Cell culture in HeLa229 and McCoy cells remains current method for isolation of strains

- NAAT testing: Amplicor (Roche), ProbeTEC (BD), APTIMA Combo2 (GenPROBE)

  (OmpA PCR – sequencing may be used for strain differentiation)

- are the current standard laboratory tests for detection of *C. trachomatis* from clinical samples
Eye diseases; but is it trachoma?
Patient with malignant melanoma and a secondary bacterial infection
# Differential diagnosis

Other ocular infections that may be distinguished from trachoma

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Distinguishing features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachoma</td>
<td>Should be suspected in endemic trachoma area, confirmed by lab</td>
</tr>
<tr>
<td>Inclusion conjunctivitis</td>
<td>In adults in non-trachoma endemic areas, related to genital <em>C. trachomatis</em> strains</td>
</tr>
<tr>
<td>Viral conjunctivitis</td>
<td>Common cause of follicles, acute history and mucopurulent discharge</td>
</tr>
<tr>
<td>Bacterial conjunctivitis</td>
<td>Bacterial infection, e.g. Moraxella, Haemophilus, aegyptus, cocci, Neisseria etc.</td>
</tr>
<tr>
<td></td>
<td>2° infections common in trachoma sufferers</td>
</tr>
<tr>
<td>Hypersensitivity conjunctivitis</td>
<td>Chronic exposure to drugs or cosmetics, clinical Hx important</td>
</tr>
<tr>
<td>Vernal conjunctivitis</td>
<td>Allergic disorder, often associated with atrophy</td>
</tr>
<tr>
<td>Parinarud ocularglandular syndrome</td>
<td>Rare condition may cause follicles, assoc. with cat scratch fever, TB, syphilis, LGV and glandular fever</td>
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</tbody>
</table>
Ocuvac Partners in Endemic Countries,

- Jimma University. Ethiopia
- Mission Trust, Shillong Meghalaya, India
- With our international partners: Vietnam, Nepal, Cameroon & Mali

Mutual Benefits

- Ethiopia is a country that has high level of incidence of trachoma
- Establishment of a research laboratory for trachoma diagnostics and therapy in Africa
Jimma University, Ethiopia

37,000 students and biggest university in Ethiopia

Education is important to the people and FREE
Jimma, Ophthalmology Clinic
At the AKH, this patient load is shared with 59 ophthalmologists.

In Jimma, there are 9 ophthalmologists.

Very busy doctors:

200-300 patients per day, same patient load as Eye Clinic at AHK, Vienna
Trichiasis operation – Jimma, Ethiopia

- Modern treatment for trichiasis (inturning of the eyelashes) is surgery to turn the eyelid back so that the eyelashes no longer rub the corneal surface
- Operation not dissimilar to ones described by Hippocrates
SAFE strategy

- S = surgery
- A = antibiotics
- F = facial cleanliness
- E = environmental improvement

WHO guidelines....
VISION 2020 TRACHOMA


- http://www.youtube.com/watch?feature=player_embedded&v=ra301elj9cw
Biology of Chlamydia
Development cycle “inclusion conjunctivitis agent”

- Thygeson (1943) published the complex development cycle of *C. trachomatis*
- Earlier (1930) proved the causal connection of baby-mother-NGU transmission
Chlamydia trachomatis first cultured

T’ang, Chang, Huang, Wang (Beijing, 1957)….. Yolk sac in fertilized eggs at 35°C, presence of Pen-Strep antibiotics, repeated blind passage.
Cause of Trachoma

- The obligate intracellular gram-negative bacteria, *Chlamydia trachomatis*
- Identified in 1907, Halberstaedter and Prowazek (Vienna)
- Life cycle described by Lindner, 1910-13 and demonstrated RBs and EBs
Elementary & Reticulate Bodies

1 hour → Differentiation → 8 hours → Replication

12-30 hours → Redifferentiation

40 hours

48 hours

Reticulate Body (RB) - Elementary Body (EB)
Phylum of Chlamydia
Host Range of Chlamydia

- **Chlamydia trachomatis**: Humans; ocular & genital strains
- **Chlamydia pneumoniae**: Broad range incl. Humans; pneumonia,
- **Chlamydia caviae**: Guinea pigs; commensal, ocular, genital
- **Chlamydia muridarum**: Mice; genital
- **Chlamydia pecorum**: Mammals, incl. Koalas; ocular, genital
- **Chlamydia suis**: Pigs; respiratory infection
- **Chlamydia felis**: Cats; pneumonia
- **Chlamydia abortus**: Cattle, sheep; genital, abortion
- **Chlamydia psittici**: Birds; commensal, respiratory
- **Pischichlamydia salmonis**: Atlantic salmon; gill disease
- **Protochlamydia amoebophila**: Amoebae symbiont
- **Parachlamydia acathamoebae**: Amoebae pathogen
- **Rhabdochlamydia crassificens**: Cockroach; intestine
Animal Models of Chlamydial ocular infection

Guinea Pigs
- Ocular infection with *C. caviae* mimics ocular pathology seen in human infections with *C. trachomatis*
- Established animal model
- Guinea pigs have M-cells
- Limited reagents for testing of acquired immune response
- Expensive

Mice
- Disease in eyes with *C. muridarum* difficult (usu. IN or genital infections), ocular *C. trachomatis* infections will be tested later this year
- Broad range of reagents for elucidating acquired and innate immune response after infection and rechallenge
- Less expensive, large numbers of animals easily handled

Non-human primates
- Not widely available at this time
Pathogenesis of Chlamydial infections in humans

*C. trachomatis* is spread by contact, with the EB binding only to receptor-bearing, nonciliated epithelial cells of mucous membranes.

- Urethra (including the epithelial cells of the prostatic part of the urethra — so called "pars prostatica urethrae")
- Cervix
- Fallopian tubes
- Anorectal tract
- Respiratory tract
- Conjunctiva
- Synovium (joints)
Detailed life cycle of Chlamydia
Choosing Immuno-protective vaccine candidates

- **MOMP**: abundant protein, serovar specific, tissue tropism
- **CPAF**: serine-like protease, inclusion integrity, survival
- **PmpD**: outer membrane protein, autotransporter, adhesive, entry to cell in early stages; as soluble form in late stage develop. cycle, genus specific, homologues in all chlamydial spp.
- **Other members of Pmp family** (other members, A-I) in CT but up to 21 members in Cpn. Early stage attachment and adhesion of EB to epithelial cells
- **ArtJ**: ABC transporter, arginine binding protein, outer membrane, adhesion, attachment
- **OmcB**: cysteine-rich outer membrane polypeptide, binds heparan-sulphate like GAGs, functions as adhesin, genus specific, homologues in all chlamydial spp.
Requirements for

"Potential vaccine candidates must not only provide protection, but must also demonstrate vaccine-induced immunity does not increase immune pathology”...Cochrane et al, 2010

Complete sterilizing immunity is not required to effectively eliminate CT infections in 20 yrs if given prior to sexual debut (both sexes)

However a separate vaccine for males and females may be required

Th1 response, esp. IFN-gamma, is useful in females but may lead to infertility in males; Th2 (neutralizing IgA) and minimizing Th1 response may be needed for a male specific vaccine
CT Vaccine design

- Should induce sterilizing immunity
- Reduction of peak load/duration of acute infection (reduce transmission)
- Prevention of ascending infection in URT or...
- Must target multiple serovars of CT
- Induce protective mucosal immunity at site of infection and disease
- Ensure that vaccine induced responses confer long lasting protection without enhancing immune pathology
Antibiotic Therapy

- Current antibiotic therapy is effective in clearing diagnosed CT infections, however asymptomatic patients may unwittingly spread infection.

- Screening at risk populations and treating with early antibiotics resulted in short term decrease followed by return to increased infection (blunting effect on protective immunity).

- Delivery....
Whole-organism Vaccines

- Veterinary examples: live attenuated or fixed EBs used to prevent ovine abortion in sheep, feline chlamydial pneumonia in cats
- Protection is incomplete, short-lived and adverse secondary sequelae not of concern
- Human examples: Inactivated organisms used in attempt to prevent trachoma
- Inactivated organisms with alum or mineral oil IM were given multiple occasions to children. Short term protection only. Transient decline in rate of disease compared to placebo controls.
- Exacerbated disease was seen in many vaccine recipients following subsequent infection.
Details on Whole cell vaccine studies

- WCV were attempted as early as 1907, the same year Chlamydia were identified.

- Nicolle and Tunis group used subconjunctival or intravenous injections of filtered material from conjunctival scrapings of patients with trachoma.

- Studies included human and monkeys and vaccines gave variable and inconclusive results. They noted some resistance to rechallenge suggesting immunity in some cases, but in others, the response to rechallenge led to worse disease.
Early vaccine studies

- Following isolation of CT in 1957, now possible to measure infectious dose, MOI, infectious load after challenge and develop antibody assays…renewed interest in vaccine for next 15 years

- 1960’s 4 groups, all with initial work in subhuman primates

- London gp. inoculated live LGV in monkeys– partial resistance at 2-6 months. IV inoculation led to dissemination of LGV to LN and spleen. Human trials in Gambia were inconclusive. Follow up with live or formalin killed bivalent vaccines in Iran. IM with 2/52 booster. Mild transient reduction of infection at 1 year, no effect at 2 years.

- Short-lived, moderately beneficial – not sufficient practical value

- Boston group …working in Saudi Arabia

- Bivalent vaccine in children <3yo. Less clinical disease at 3/12, effect disappeared at 18/12. Children who received higher dose aqueous vaccine were 3 times more likely to develop disease than control group.
Early vaccine studies

- Seattle group used Taiwan monkeys as model
- Formalin-killed EB were used gave short-lived partial protection
- Lower doses ($5 \times 10^7$ organisms) did not protect or only partially and monkeys that were infected developed more severe disease, pannus and scaring.
- Hypersensitivity and increased disease symptoms on rechallenge lasted longer than the partial protection
- Trials in children, (alum) vaccinating the younger siblings of school children with trachoma. 1 year reduced incidence, no protection at 6 yrs, infections in vaccine recipients were more severe and were more frequent.
- 100 children (oil IFA) showed reactions to booster injection. More immunized children developed trachoma at 2.5 yo than placebo children
- 450 3-5 yo children without trachoma ($10^9$ formalin-killed organisms), booster 3/12 later. 12 months, vaccinated gp had half incidence, at 12 yrs pi, no difference. Importantly, vaccinated children did not have more severe disease
Early vaccine studies

- Italian group…working in Ethiopia showed only successful vaccine which led to commercial production.

- $5 \times 10^8$ EB formalin killed monovalent vaccine (aq, IFA, alum), half dose booster at 6/52. Several thousand children vaccinated in 3 separate studies 1960-2. Vaccine prevented new infection, hastened healing; stage II or III active trachoma moved to inactive disease with scaring.

- 1964; new trial with 5000 vaccinated children & 5000 controls. At 12 months, less vaccinated children had disease and more had resolved. Preventive effect lasted 1 year, therapeutic effect 3 years. More marked effect in children >6yo in areas of mild disease. More effect on Grade II disease, no effect on Grade III disease. Commercial vaccine was produced, but was not used!
Purified Antigen in Monkey model

- None of immunizing regimes or vaccines gave better protection than a single, primary ocular infection.
- IM immunisation led to CMI and serum antibody response.
- Oral immunisation gave good serum antibody but not tear antibody titres (max. immunisation gave low level tear antibodies).
- Ocular immunisation +/- boosting gave high titres of antigen specific tear IgA, IgG, IgM with antigen-specific IgA and IgG B cells and antigen-specific conjunctival T cells. After challenge, ocular boosting gave amnestic antibody response with increased CD4+ cells in follicles and extrafollicular CD8+ cells.
- Presence of tear or serum antibodies did not prevent infection.
- Presence of chlamydial EB or MOMP antigen-specific T-cells or B-cells in conjunctiva did not prevent ocular infection.
- Vaccination could induce an antibody response to Hsp60, but this was neither protective or harmful.
Fig. 1. Mean clinical disease score of: (A) four monkeys that received ocular vaccination with MOMP; (B) four monkeys that received enteric vaccination with MOMP; (C) four monkeys that received combined vaccination with MOMP; (D) five ocular-immune monkeys challenged 18 weeks after primary infection (derived from ref. 1). The shaded area shows the normal response in naive monkeys receiving primary infection. It is the mean score of nine animals, the four reported in this study and the five previously reported.
Immunoprotective effect of plasmid-less Chlamydia strain

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


JEM
Volume 208(11):2217-2223
October 24, 2011
In vitro characterization of the C. trachomatis plasmid-deficient trachoma strain A2497P−.

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

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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 1 µm) 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, ID 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
OBJECTIVE: TO MAKE AN OCULAR VACCINE AGAINST TRACHOMA
Pillars of the Research Programme

- Bacterial Ghost System
- Ocular surface immunology
- Conjunctiva vaccination strategy
- Ethical research leading to mutual benefits
Bacterial Ghosts

- Ghosts are empty Gram negative bacterial shells without cytoplasm and DNA
- They share functional and antigenic determinants of their living counterparts
- BGs can express foreign antigens on membrane to become vaccine delivery platform for these antigens
- Can also contain subunit protein antigens or plasmids for DNA vaccines
Advantages of BGs

- Pose no safety or horizontal gene transfer threat
- Administration topically to the eye/nose (needle free)
- Cost-effective
- Fast BG production through fermentation
- Straightforward technology can be transferred to developing countries
- Storage at ambient temperature for several years
E. coli Nissle 1917 as BG platform

- E. coli Nissle 1917 bears defect in LPS biosynthesis (shorter oligosaccharide antigen chains)
- Originally recovered as a commensal bacteria, since 1917 has been used as a probiotic and registered as a therapeutic ‘Multiflor’
- As a therapy for IBD and CD able to modulate cytokine and inflammatory responses, even if a pathogenic bacterial strain is already established, and restore Th1-Th2 balance
<table>
<thead>
<tr>
<th>Subunit vaccine/Location in BG</th>
<th>Bacterial ghost Carrier</th>
<th>Animal model</th>
<th>Route of immunization/challenge</th>
<th>Immune Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-1-gp41/IMA</td>
<td><em>K. pneumoniae</em></td>
<td>Piglets</td>
<td>SC</td>
<td>Serum IgG</td>
</tr>
<tr>
<td></td>
<td><em>S. typhimurium</em></td>
<td>Mouse</td>
<td>Oral/lethal challenge</td>
<td>Survival time</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>Mouse/Rabbit s</td>
<td>IP, SC</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>HIV-1 RT/IMA</td>
<td><em>E. coli</em></td>
<td>Mouse</td>
<td>IP</td>
<td>Serum IgG; T-cell</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>SC</td>
<td>stimulation index</td>
<td></td>
</tr>
<tr>
<td><strong>Ch. trachomatis</strong> MOMP inner membrane anchored</td>
<td><em>V. cholera</em></td>
<td>Mouse</td>
<td>IM / Intravaginal</td>
<td>IFN-γ; IgA, IgG2a in Genital tract, spleen</td>
</tr>
<tr>
<td><strong>Ch. trachomatis</strong> MOMP/OMP2 inner membrane anchored</td>
<td><em>V. cholera</em></td>
<td>Mouse</td>
<td>IM /intravaginal</td>
<td>IFN-γ; IgA, IgG2a in Genital tract, T-cell proliferation in spleen</td>
</tr>
<tr>
<td><strong>Ch. trachomatis</strong> MOMP/PorB inner membrane anchored</td>
<td><em>V. cholera</em></td>
<td>Mouse</td>
<td>IM /intravaginal</td>
<td>IFN-γ; IgA, IgG2a in vaginal wash outs, T-cell proliferation in spleen</td>
</tr>
<tr>
<td><strong>Trichosurus vulpecula</strong> ZP2/ZP3 periplasmic space</td>
<td><em>E. coli</em></td>
<td>Brushtail possum</td>
<td>Eye/nose/oral</td>
<td>IgA, serum ABs outs, T-cell proliferation blood, mandibular and mesenteric isolated lymphocytes</td>
</tr>
</tbody>
</table>
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%”

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
Mucosal Immunity – Nasal Route

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.
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
Administration of a mucosal vaccine via IN route in children, may be problematic...
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
Conjunctiva Anatomy

The ocular surface is constantly exposed to microorganisms.

A combination of mechanical, anatomical, and immunological defense mechanisms protect the outer eye.
Ocular Surface Immunology

- CALT = “conjunctival-associated lymphoid tissue“
- CALT can detect ocular surface antigens
- CALT can supply other organs and the ocular surface with specific effector cells via the regulated recirculation of lymphoid cells
- Increasing evidence that ocular surface diseases are related to local innate immunity response
Conjunctival vaccination strategy

- Same route as *Chlamydia trachomatis* infection
- Possibility to influence topical innate immunity balance/imbalance
- Conjunctival vaccination proved efficient in one animal model
- Needlefree application of BGs
- No health care professional needed
So far.....

- Compare subcutaneous/im route with conjunctival route vaccination in mice
- Test suitable naked BG carriers for mucosal (CALT) route
- Choosing suitable CT antigens; making ‘armed-BGs’
- Test effect of naked BG and ‘armed’-BGs on innate immunity responses of human conjunctival cells in culture
- Develop model of CT infection in human conjunctival cells and animal models
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

LAURA BASSI CENTRE OF EXPERTISE

OCUVAC