Approaching the Apex: Technology Innovations Facilitating the Development of a Gonococcal Vaccine

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Neisseria gonorrhoeae

- 106 million cases/year (WHO 2012)
- sexually active
- gonorrhea - massive neutrophil response
- sequelae include acquired blindness, pelvic inflammatory disease and female infertility
- elicits little immunological memory
- facilitates HIV transmission

Gonococcal infection of the female genital tract

Pelvic inflammatory disease

Uncomplicated infections

Gram stain: urethral exudate

N. gonorrhoeae

Neisseria gonorrhoeae infection of the female genital tract

HIV & Neisseria gonorrhoeae: Clinical and epidemiological synergy

- Positive correlation has been appreciated for over two decades
- Gonorrhea is associated with a 2-5 fold increased rate of male to female HIV transmission (Fleming and Wasserheit, 1999)
- Women with laboratory-diagnosed N. gonorrhoeae infections have a 7-fold increased risk of HIV-1 acquisition (Mlisana et al., 2012)
- Concurrent infection is associated with:
  - Increased HIV-1 viremia (Mlisana et al., 2003; Moss et al., 1995)
  - Decreased CD4+ T lymphocyte counts (Mlisana et al., 2003)
  - Decreased CD8+ T lymphocyte responses (Moss et al., 1995)
- The treatment of symptomatic N. gonorrhoeae infection leads to decreased HIV-1 DNA detected in urogenital tract swabs in HIV+ men and women (Gray-Owen et al., 1997; Moss et al., 1996)
- N. gonorrhoeae directly stimulates HIV replication by shedding HBP, a novel innate immune agonist (Malott et al., 2013; Gaudet et al., 2015)

1. Necessity...
WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health

New WHO report provides the most comprehensive picture of antibiotic resistance to date, with data from 114 countries

Media centre

“Treatment failure to the last resort of treatment for gonorrhea – third generation cephalosporins – has been confirmed in Austria, Australia, Canada, France, Japan, Norway, Slovenia, South Africa, Sweden and the United Kingdom.”

Epistemologic Evidence for the Development of Serovar-specific Immunity after Gonococcal Infection

Abstract

We used a hypothesis that serovar-specific immunity after gonococcal infection is a major cause of the worldwide epidemic of gonorrhea. This hypothesis was tested by comparing the survival of gonococci after infection in patients with and without serovar-specific immunity. Patients were classified as having serovar-specific immunity if they had a history of previous infection with the same serovar. Patients not classified as having serovar-specific immunity were infected with gonococci of a serovar that they had not previously encountered. The hypothesis was tested by comparing the survival of gonococci after infection in patients with and without serovar-specific immunity. Patients were classified as having serovar-specific immunity if they had a history of previous infection with the same serovar. Patients not classified as having serovar-specific immunity were infected with gonococci of a serovar that they had not previously encountered.

2. Potential...

Bactericidal Antibody in Genital Infection Due to Neisseria gonorrhoeae

An assay of bactericidal activity has been developed to study the host response to infection with Neisseria gonorrhoeae. This test for bactericidal activity was performed on the sera of women who were exposed to N. gonorrhoeae but who did not become infected, of women with N. gonorrhoeae infection, and of a control group of unexposed women. Antibody was found in the sera of <30% of normal women with uncomplicated gonococcal infection. Preexposure normal serum, obtained from women with gonococcal infection, demonstrated a specific bactericidal activity in the serum of women who were exposed to N. gonorrhoeae but who did not become infected, of women with N. gonorrhoeae infection, and of a control group of unexposed women. Antibody was found in the sera of <30% of normal women with uncomplicated gonococcal infection.
Vaginal administration of microencapsulated IL-12 administered during primary infection leads to more rapid immune-mediated clearance to secondary infection. This correlates with heightened Th1 response, and generation of gonococcal-specific serum IgG and mucosal IgA and IgG. Microencapsulated anti-IL-10 or anti-TGFβ had a similar effect.

### Gonococcal Antigens under Investigation

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Function</th>
<th>Evidence for protective potential</th>
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</thead>
<tbody>
<tr>
<td>2C7 mimetic</td>
<td>Bactericidal LOS epitope</td>
<td>Protective by active and passive immunization</td>
</tr>
<tr>
<td>TbpB, TbpA</td>
<td>Transferrin receptor</td>
<td>Ab block uptake of iron from Tf</td>
</tr>
<tr>
<td>MtrE</td>
<td>OM channel of MtrCDE active efflux pump system</td>
<td>Protective with VRP; loop-specific peptides induce cross-reactive, bactericidal Abs</td>
</tr>
<tr>
<td>PorB</td>
<td>Nutrient acquisition, serum resistance, invasion</td>
<td>Abs reduce surface sialylation</td>
</tr>
<tr>
<td>AniA</td>
<td>Anaerobic growth, biofilm formation</td>
<td>Protective with VRP; loop-specific peptides induce cross-reactive, bactericidal Abs</td>
</tr>
<tr>
<td>Lst</td>
<td>LOS sialylation; protects against innate effectors</td>
<td>Protective by active immunization</td>
</tr>
<tr>
<td>OmpA</td>
<td>Adhesin, invasin</td>
<td>Bactericidal Abs</td>
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### Characteristics of Infection:
- Vaginal colonization for 10-12 days
- Gc in cervicovaginal lumen and tissue and within the lamina propria
- Influx of PMNs in vaginal smears, tissue
- Proinflammatory response due to TLR4-dependent induction of Th17 responses (Feinen, 2010)
- Poor antibody response; susceptible to reinfection

### Proven useful for:
- Studying gonococcal evasion of innate effectors
- Identifying immunological pathways
- Product testing (antibiotics, vaginal microbicides, vaccines)

- 168 common surface proteins identified by proteomic comparison of gonococcal strains
- Immunogenicity and functional studies on-going
Moving Ahead: 'Humanizing’ the Mouse

- Hysterectomy samples from 23 patients
- Stained with monoclonal antibodies specific to CEACAM1, CEACAM5 or CEACAM6

Local and systemic cytokine induction during uterine infection of CEACAM-humanized mice

Experimental Human Gonorrhea

- Since the late 1980’s, controlled human infection studies conducted in the US under appropriate ethical review with full written informed consent
- Existing program at the University of North Carolina provides a unique opportunity to study pathogen factors and host responses in human infection under controlled conditions.
- Experimental urethral infection of male volunteers is safe (>200 subjects inoculated without serious complications)
- Wild-type GC elicit signs and symptoms of natural infection.

Marcia Hobbs
Current eligibility criteria

- **Inclusion**
  - Healthy male, 18-35 years old
  - Normal genital exam
  - Willing to abstain from sexual activity during study

- **Exclusion**
  - History of sexually transmitted infection
  - Positive serology for HIV, syphilis, HBV, HCV
  - Abnormal complement activity
  - Allergy to penicillin, ceftriaxone, ciprofloxacin or lidocaine

Current experimental protocol

- Written informed consent obtained at screening followed by a T/F test of understanding.
- On day of inoculation, subjects admitted to inpatient unit of clinical research center at UNC Hospitals for 6 day trial; written informed consent obtained again.
- ~240 μL of PBS containing 10^4-10^6 organisms instilled into anterior urethra through sterile #8 French pediatric catheter.
- Subjects examined daily for signs & symptoms of urethritis up to 5 days after inoculation. May leave the unit during the day if asymptomatic.

Treatment and follow-up

- All subjects receive ceftriaxone (250mg IM), either on request due to symptoms, or prior to end of trial, whether or not cultures are positive.
- Follow-up visit within 1 week for targeted clinical exam and test of cure.
- Final 2-week follow-up phone call to assess potential AEs.

3 Phases of experimental human gonorrhea studies

- Past observational studies of the natural history of experimental gonococcal infection with “wild-type” strains (Hobbs et al. 2011 Frontiers in Microbiology)
- Ongoing pathogenesis and host response studies with isogenic mutants
- Future vaccine & treatment studies

Future Priorities

1. **Greater focus on vaccine development**
   - Antigens
   - Surrogate measures for assessing immunity
   - Continued improvement of preclinical models

2. **Ongoing research on basic aspects of pathogenesis and host response**
   - Genome-based analyses
   - Gonococcal lifestyle within mucosal tissues
   - Human experimental and natural history studies

3. **Concerted effort toward the goal of vaccine development and implementation**
   - Leadership to coordinate interactions between disciplines
   - Sustained funding by funding agencies and nonprofit organizations

6. Looking ahead...
The Gonococcal Vaccine Consortium

Tom Hiltke
Carolyn Deal
Anne Jerse (Uniformed Health Services University)
Peter Rice (University of Massachusetts Medical School)
Lee Wetzler (Boston University)
Ian Feavers (NIBSC, UK)
Scott Gray-Owen (University of Toronto)

GVC Participants (International)