Vaccine Development for *Neisseria gonorrhoeae*

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Presentation Overview

- Background of *Neisseria gonorrhoeae* and the Rapid Emergence of Antimicrobial Resistance (AMR)

- Short- and Long-term Interventions
  - Surveillance and Treatment
  - Vaccination

- Summary and Next Steps
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Genetically flexible Gram-negative diplococci

- Require increased iron and CO₂ for growth
- Human-specific
- Exquisitely well-adapted to withstand host inflammatory response

Genetically flexible

- High-level antigenic and phase variation of surface molecules
- Easily evolves antibiotic resistance
  - Conjugative plasmids carry penicillin and tetracycline resistance
  - Picks up naked DNA from other *Neisseria* sp.
The Steady Pace of Gc Antibiotic Resistance

Today: No single class of antibiotics that is reliably effective for empirical treatment of gonorrhea.

Current recommendation: Dual therapy with high dose ceftriaxone (Cro) and azithromycin (Az)
Global Response: Surveillance Networks

WHO Global GASP: Global sentinel surveillance system
- Initiated in 1990s; revamped in 2009
- Headquarters: Geneva
- Collaborating nations in Africa, Asia, the Americas, Europe, Western Pacific

CDC: GISP, Gonococcal Isolate Surveillance Program
- Established in 1986
- Represents < 2% of all reported cases; samples from men only
- Has detected entry of strains from Asia to Hawaii and the US west coast

GEIS: Global Emerging Infectious Disease Surveillance Program
- CONUS sites: Six US military bases in the continental U.S.
  - Men and women; cervical, urine, urethral
- OCONUS sites: Ghana, Kenya, Georgia, Thailand, Peru
  - Men and women
Current status

- Increasing Cro and cefixime (Cfx) MICs in many countries and several Cro\textsuperscript{R} strains have now been isolated
- Increasing Az MICs; recent outbreaks of high-level Az\textsuperscript{R} strains

Five Az\textsuperscript{R} isolates with elevated Cro MICs were recently isolated in Hawaii (also resistant to Pen, Tet and Cip) (Katz 2017)
Due to diminishing treatment options Gc has been designated

“a high priority antibiotic resistant pathogen” (WHO, 2017)

“a microorganism with an urgent threat level” (CDC, 2013)
Gonorrhea: The Disease

- 106 million infections each year
- Uncomplicated lower urogenital tract infections (urethritis, cervicitis)
- Pharyngeal and rectal infections also very common
- 18-20% of cervical infections ascend to pelvic inflammatory disease (PID) and associated sequelae
- Maternal gonorrhea leads to neonatal conjunctivitis, adverse pregnancy outcomes
- Untreated urethritis in men results in epididymitis, infertility
- Disseminated gonococcal infection (DGI) occurs in 0.5-3% of infected individuals
  - Septic arthritis (leading cause in young adults)
  - Dermatitis, petechial rash
Gonorrhea Disproportionately Affects Women and Neonates

- Fertility and reproductive health
- Pelvic Inflammatory Disease
- Infertility
- Ectopic pregnancy
- Chronic pelvic pain
- Pre-mature rupture of membranes
- Low birth-weight, failure to thrive
- 3rd trimester miscarriage
- Ophthalmia neonatorum
- Infertility
Gonorrhea Is a Strong Co-Factor for Increased HIV Spread

- HIV-infected individuals with a gonorrhea have higher level of HIV transcripts in body fluids

- Individuals with gonorrhea are more susceptible to HIV infection due to increased presence of susceptible immune cells

- STI intervention/treatment reduces incidence of HIV within communities

**Durban South Africa – prospective study to identify predictors of HIV acquisition**

Women with Gc (most inflammatory of the STDs) had a 7-fold higher risk of HIV acquisition

**Mwanza (Tanzania) STD intervention trial**

40% reduction in HIV incidence in communities that received enhanced STD care

(Galvin and Cohen Nat Rev Microbiol, 2004; and Cohen MS J Infect Dis, 2012)
Burden of Sequelae and Costs

- **2300 deaths from gonorrhea per year** (Lancet 2016)
- **Disability-adjusted life years (DALYs)**
  - 282,000 and 440,000 lost DALYs / year due to Gc-associated morbidity (Murray 2010)
- **Treatment Costs**

  **Uncomplicated urogenital infection; low and middle income countries (LMIC)**
  - $86.49/case, > $9 billion USD annually (Terris-Prestholt 2006)

  **Sequelae management**
  - (Compiled data from UK, Netherlands, Sweden, Australia, Ireland, Canada and Denmark) (Ong 2017)

Treatment failures due to antimicrobial resistance (AMR)
- Estimated 3 million treatment failures due to AMR/year world-wide
- Conservative cost of $500 million USD per year (Tapsall 2005)
Infertility and Adverse Pregnancy Outcomes

Infertility

Low and middle income countries (LMIC)
- Infertility rates 15-30% in some countries, IVF usually unaffordable
- Economic loss of reduced future work-force
- Can have great social and economic costs to women

http://www.who.int/bulletin/volumes/88/12/10-011210

Adverse Pregnancy Outcomes

Ectopic pregnancy (EP)
- Cause of 1/20 maternal deaths in Papua New Guinea (Sanga 2010)
- EP in 0.41% to 1.5% of annual deliveries in Guinea (Thonneau 2002)

Pre-mature rupture of membranes (PROM)
- 19.7% of referrals for OB complications in Addis Ababa (Mirkuzie 2016)
- Huge long-term medical and financial burdens for affected children, families and the health care system (Butali 2016, Nigeria)
Impact on Neonatal Ocular Health

Ophthalmia neonatorum (ON)

- Most preventable cause of neonatal blindness
- 1-5% of newborns globally are at risk of Gc ON
  - 12% of neonates, and 23% of neonates have Chlamydia or Gc ON in parts of the world
- Transmission rate from an infected mother to newborn: 30-45%

Diagnosis and treatment is poor in many countries

Malawi, large tertiary care hospital  (Ranjit 2014)
- 231 cases of ON over a three year period; variable treatment, tetracycline + saline wash most common

Southern Tanzania study  (Hanson 2013)
- Prevention of ON offered by only 5% of dispensaries, 38% of health centers and 50% of hospitals consistently

Ophthalmology clinic, Angkor Hospital for Children  (Khauv 2014)
- Found Gc and, or Ct the cause of 10 cases of ON. Maternal screening for STI and tetracycline eye ointment for newborns are not routine practice in Cambodia.
How do we realize the goal of eradicating gonorrhea as a global public health problem?
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Diagnosis and treatment of patients and their partners

Nucleic acid amplification tests (NAAT)
- If positive, treat patient with ceftriaxone + azithromycin
- Offer condoms, refer for risk-reduction counseling
- Re-test for Gc three months later
- Treat sex partners from within the last two months
- Test all patients with Gc for HIV; if negative re-test 3-6 months later

If persistent or recurrent symptoms occur
CULTURE and SENSITIVITY-- Report to surveillance system

Empirical/syndromic treatment (with or w/o Gram stain)
- Not definitive
- May miss coinfection with Chlamydia, M. genitalium, other STI pathogens
- Risks inappropriate treatment

Many labs are not equipped to perform culture and sensitivities for expected treatment failures
Short-term Interventions (2): Identify Back-up Therapies, Develop New Anti-infectives

Gentamycin (Gm)
- Recommended back-up for CroR strains
- Gm plus Az or gemifloxacin plus Az (single dose): cleared 100% of urogenital, rectal and pharyngeal infections in a clinical trial (Kirkaldy 2014)

New antibiotics in the pipeline
- EXT0914 (Entasis): Phase II trial
- GSK compound: Phase II trial

Development of new anti-infectives for gonorrhea
- Large increase in funding and activity in this area the last 5-7 years (government, academic, corporate)
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Long-term Intervention: A Gonorrhea Vaccine

35-40 years ago…

Chimpanzee Model
- Killed whole cell vaccine: increased resistance to infection

Published clinical trials
- Whole cell killed vaccine, Inuit volunteers: no protection
- Purified pilin vaccine, U.S. soldiers: no protection

(Reviewed in Zhu, Front Microbiol. 2011)

Efforts waned in 1980s…
- Antigenically variable surface molecules (pilin, LOS, Opa proteins)
- No evidence of protective immunity
- Lack of an animal model other than chimpanzees
- Challenges of immunizing the genital tract / scant information on genital tract immunology
Recently reported association between immunization with a Group B meningococcal outer membrane-based vaccine and a reduced risk of gonorrhea

Biological Feasibility a Gonorrhea Vaccine

- Several stably expressed conserved or semi-conserved antigens have been identified
- Female mouse model for systematic testing of antigens and immunology studies
- Male urethral challenge model for human studies
- Identification of immunosuppressive and protective pathways in human cells or mice

ALSO:
- Advances in pathogenesis, immunology, molecular epidemiology, infection models, genomics, proteomics and glycomics
- Success of HPV vaccine, Group B meningococcal vaccines

◆ Recently reported association between immunization with a Group B meningococcal outer membrane-based vaccine and a reduced risk of gonorrhea
### Candidate Gonorrhea Vaccine Antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Function</th>
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<tbody>
<tr>
<td>2C7 mimetic</td>
<td>Bactericidal LOS epitope</td>
</tr>
<tr>
<td>TbpB, TbpA</td>
<td>Transferrin receptor</td>
</tr>
<tr>
<td>MtrE</td>
<td>OM channel of MtrCDE active efflux pump system</td>
</tr>
<tr>
<td>PorB</td>
<td>Nutrient acquisition, serum resistance, invasion</td>
</tr>
<tr>
<td>AniA</td>
<td>Anaerobic growth, biofilm formation</td>
</tr>
<tr>
<td>MetQ, NGO2139</td>
<td>Methionine transporter</td>
</tr>
<tr>
<td>OpcA</td>
<td>Adhesin, invasin</td>
</tr>
<tr>
<td>PilQ</td>
<td>OM porin for pilin extrusion</td>
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- **Conserved surface proteins identified by proteomics approach** (Zielke 2014)
- **Novel *in vivo*-induced antigens**
- **Outer membrane vesicles** (Plante 2001; Lui et al., in press)
Challenge: No Known Correlates of Protection in Humans

- Repeat infections are common

- Can be reinfected with the same strain, same serotype
  - Outbreak study, Durham, North Carolina
  - Rural community, North Carolina (Fox 1999)
  - Human volunteer studies

- Evidence of immunity to reinfection
  - High risk women, South Africa: Reduced risk of upper reproductive tract reinfection
    - Associated with bactericidal Abs, Opa-specific Abs and an absence of blocking Abs (Plummer 1989, 1991)
  - Inverse correlation with successful transmission and presence of blocking Abs
Exposure/Transmission Rates Suggest Mechanisms of Natural Immunity May Exist

- Serum and mucosal antibodies: specificity, isotype, bactericidal/opsonic activity
- Cellular factors: Th1, Th17, PMNs, macrophages
- Receptor polymorphisms: adherence, invasion receptors: CR3, CEACAMs, ASGPR, innate receptors: TLRs, other PRRs, lectins

- Stage of menstrual cycle

- Soluble factors: complement, antimicrobial peptides
- Vaginal microbiota:

Jerse, Bash and Russell, 2013
Discovery of Immunosuppressive Pathways

- Gc induces a robust inflammatory response (Th17 pathway), but a transient, poor Ab response to infection
- No protective memory response
- Analysis of immunosuppressive pathways has identified points of intervention
  - Th1-inducing adjuvants (ex. microencapsulated IL-12) (Liu 2014)
  - Possible immunotherapies (i.e. anti-PD1 antibodies)
Stimulation of Th1 Responses Is Protective in Mice: Adjuvants May Be Key

- The four candidate gonorrhea vaccines that showed protection in mice all stimulated Th1 responses
  - PorB/VRPs (viral replication particles) (Zhu 2011)
  - 2C7 LOS epitope peptide given with MAP1 adjuvant (Gulati 2014)
  - MtrE given with CpG adjuvant (Derocco, IPNC 2014)
  - OMV given with IL-12 (Liu et al, in press)

- In contrast, all vaccine antigens tested with Th2-inducing adjuvants in mice failed to show protection.
Composition of 4CMenB (Bexsero-GSK)

Outer membrane vesicles (OMV) from non-encapsulated strain MC58
- (Group B, New Zealand epidemic strain)

Three purified proteins
- rNadA
  - Autotransporter, adhesion
- NHBA
  - Neisserial heparin-binding protein; fused to GNA10030
- FHBP
  - Factor H-binding protein; fused to GNA2091

Adjuvant: Alum
Norwegian study (Whelan 2016)

- Examined national gonorrhea rates in subjects > 16 years old (1993-2008)
  - (1 year after MenB window closed)

Results:
- Overall decrease in gonorrhea rates in this time period
- Limited age-specific vaccine effect in men and women 20-24 yrs old but not in other groups
- **Confounder:** Promotion of condom use was already causing declining rates

New Zealand study (Petousis-Harris, 2016 IPNC)


Results:
- 877 diagnoses of gonorrhea (cases), 7132 diagnoses of chlamydia only (controls)
- Gonorrhea cases less likely to have been vaccinated with MenB after adjustment
- Effectiveness of MenB vaccine against gonorrhea estimated to be 33%
What Are the Cross-protective Antigens in Bexsero and *N. gonorrhoeae*?

- Many shared protein and carbohydrate antigens in Gc and meningococcal OMVs.
- fHBP, NHBA, and NadA are not expressed by all Gc or vary between strains.

*(Hadad 2012)*

- **NIAID/USU (interagency agreement)**
  - What Gc proteins does antiserum from Bexsero-immunized mice recognize?
  - Are Bexsero-immunized mice protected from Gc infection?

- **Human trials are needed**
  - What Gc surface molecules are recognized by serum from Bexsero-vaccinated humans?
  - Clinical trials? – In-house, urethral challenge model versus phase 3 trial?
Several groups are currently working on Gc OMV vaccines

Some unanswered challenges:
- The correlate of protection for meningitis is bactericidal activity, however, the correlate of protection for gonorrhea is NOT KNOWN
  - Mouse studies: Th1 responses are needed, bactericidal
  - Ab titers are not predictive of protection

- Route and adjuvants may need optimization for inducing mucosal responses
  - Mucosal sites colonized by Gc: urethra, cervix, endometrium, oviducts, pharynx, rectum, conjunctiva
  - Don’t yet know if Bexsero eliminates Mc carriage from the nasopharynx

Carriage of *N. meningitidis* is acceptable; “carriage” of *N. gonorrhoeae* is not!!
Vaccine Targeting Strategy: Core Groups, High Risk Groups versus Adolescents

- Core group transmission: gonorrhea, syphilis, HIV, hepatitis B

- Adolescents between 10-19 make up 18% of the world population

- Gonorrhea rates dramatically increase after sexual debut

Adapted from MMWR 2010
Gonorrhea Vaccine Modeling Studies

(Regnier and Huels, 2014)
- Modeling the effectiveness of Bexsero against gonorrhea
- Assuming 20% efficacy with a 2-dose campaign targeting adolescents in the U.S.:
  - Prevention of 83,167 infections, reduction in direct medical costs: $28.7 million, decrease in HIV infections: 55 per vaccinated birth cohort
  - Income and productivity lost: $40 million (mostly due to avoidance of HIV infection)

(Craig 2015)
- If all 13-year olds were given a non-waning vaccine with 50% efficacy OR a vaccine with 100% efficacy that wanes after 7.5 years:
  - The prevalence of gonorrhea could be reduced by 90% after 20 years
  - For a non-waning vaccine of only 20% efficacy: 40% reduction in prevalence of gonorrhea

Predictions rely on: Coverage being high and protection lasting over the highest risk period (sexual partner change) in young people
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Summary

1. Several new developments suggest a gonorrhea vaccine is possible

2. Research in this area has increased due to increased funding and the alarm of potentially untreatable gonorrhea

   Pubmed: gonorrhea, vaccine, immune responses:
   - 80 citations between 1960 and 2006 (46 years)
   - 80 citations between 2007 and 2017 (last 10 years)

3. A gonorrhea vaccine initiative (GVI) working group has been formed to synergize efforts, coordinate networks between disciplines, and to provide SOPs for various assays and vaccine preparation

   NIAID-sponsored Gonorrhea Vaccine Initiative (April 2015)

Workshop proceedings and recommendations

   Summary and Recommendations from the National Institute of Allergy and Infectious Diseases (NIAID) Workshop “Gonorrhea Vaccines: the Way Forward”
   Wetzler LM, Feavers IM, Gray-Owen SD, Jerse AE, Rice PA, Deal CD.
Summary (continued)

4. Gonorrhea vaccine development is in the stage of antigen discovery and identification of protective responses.

5. There is a need for human studies to:

   ▪ Elucidate the mechanisms by which Gc subverts the host immune response in humans
     ▪ Is it as predicted by studies with human cells?
     ▪ By mouse studies?

   ▪ Identify protective mechanisms in exposed individuals who do not become infected (natural history studies)

   ▪ Prospective and retrospective studies on Bexsero efficacy against gonorrhea

   ▪ Test efficacy of candidate vaccines and identify immune correlates
Next Steps: Opportunities for WHO Engagement

- Improve global disease burden estimates
- Support cost-effectiveness modeling
- In areas with syndromic management, explore the Ct/Gc relative contributions to urethritis and cervicitis
  - Impact modeling of Gc vaccine vs. dual Ct/Gc vaccine
Thank You

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