IPNC 2023: Developing Standardized Approaches to Evaluate Gonococcal Vaccines and Antimicrobials Workshop

This workshop took place September 26, 2023 at the 22nd International Pathogenic *Neisseria* Conference in Boston, Massachusetts, USA. It was organized by Thomas Rudel (Germany), Alison Criss (USA), Isabel Delany (Italy), Jennifer Edwards (USA), Monica Fabbrini (Italy), Scott Gray-Owen (Canada), Trevor Moraes (Canada), and Kate Seib (Australia). There were 72 participants from academic, government, biotech, and pharmaceutical backgrounds.

The aim of this workshop was to facilitate discussion amongst attendees regarding current issues in gonococcal vaccine and therapeutic development, with the ultimate goal of developing standardized protocols and platforms to evaluate candidate vaccines and therapeutics.

Gonococcal In vivo Challenge Models for Vaccine and AMR Research

Wild type mouse models are cost-effective and are readily available

- Limited to female mice and require antibiotics to suppress the microbiome during infection
- No human-restricted factors, including the major receptors for Opa proteins
- Mice require estradiol treatment to prolong infection; cyclical fluctuation in progesterone levels still occurs, which can impact the expression of host innate effectors
    - progesterone fluctuations may be a strength with respect to hormonal regulation of vaccine-induced immune responses

Humanized mouse models, which currently include 11 human genes relevant to gonococcal infection, allow the establishment of multiple models and inclusion of both sexes (e.g. lower and upper female genital tract models, male urethral model, and disseminated infection models). Models using mice supplemented with human transferrin, or human transferrin and factor H (not yet published), are also used and support ascending gonococcal infection and sustained endometrial and oviduct infection.

- Genetically and immunologically tractable
- Can be manipulated
- More costly
- For humanized transgenic mice, harder to maintain as breeding restricts availability

Human models are restricted to human male urethral challenge

- More labour intensive and costly relative to mouse models
- Restricted access to samples or tissues for analysis
- Limited to analyses of early events during infection

These models are complementary to each other and should be used in conjunction with tractable and relevant cell-based models

- Guided by the research question being asked
- In vivo models are important for identifying correlates of protection and in defining the immune response (e.g. cytokines, resident and recruited immune cells), with access to tissue samples
- Correlates of protection in mouse models have not been defined for mucosal infections by other *Neisseria sp*., and correlates of protection against sepsis do not reflect protection on mucosal tissues, so comparisons cannot be used to predict those required for protection from genital challenge

Questions and Discussion

1. **How do we reliably translate data obtained from animal models to humans?**

a. This question considers protection in humans (e.g. with Bexsero) and on-going mouse models
b. There is a need to establish a minimum standard, which will likely involve combining multiple human (in vitro and ex vivo models) and mouse (immunogenicity, reactogenicity and protection) models – what can help to de-risk studies when making the move into humans?
c. Likely cannot translate data directly, as there are considerable differences in genetic background between human and mice
d. However, it is likely that data beyond simple monitoring of antibody responses will be required to see a correlate of protection, so mouse models may allow an indication of what samples should be collected from clinical studies

2. **Can mice (or other animals) be used to model gonococcal urethritis, rectitis, pharyngitis? For example, can the Nm mouse model be adapted for gonococcal research?**
    - Mice can be used for pre-clinical and pharmacokinetic testing of candidate antibiotics - licensed antibiotics perform similarly in mice as they do in humans.
    - Mice are also a good model to evaluate reactogenicity (safety) and whether efficacy is dose-dependent
    - Need to consider different mechanisms of infection/invasion that potentially exist between mice and humans that could impact drug accessibility to bacteria (e.g. cellular permeability, variable intra- and extracellular Ng niches)
        - The CEACAM-humanized mouse model of pharyngeal infection may be useful

a. Immune cell involvement and activation will differ depending on the site of infection, and for vaccine research, the adjuvant used
    - Protection against sepsis does not indicate mucosal protection, and protection at one mucosal site does not necessarily predict protection at other sites, so must be tested independently
    - The type of adjuvant may determine what sites are protected, so the same immunogen may be more or less effective as a vaccine depending upon the adjuvant used

Complex Human Tissue Models for Vaccine Research

These are cell-based models to more accurately represent the interactions between the bacterium and human cells and are desired by pharmaceutical companies for vaccine research. Moreover, the FDA Modernization Act 2.0 supports the use of ex vivo human models for preclinical drug development.

- Can be used for toxicology studies, which restricts these models to only Phase 1 and 2
- Enable mechanism of action (MOA) analyses for promising antimicrobials
- Can be used to screen and prioritize vaccine antigen candidates

Primary human organ or tissue cultures (e.g. trans-well, organoids, tissue explants), which can be obtained from biopsies, allow one to evaluate different aspects of bacterial-host interactions, especially those that involve long-term infections. Options that are presently available and relevant to gonorrhea include cornea, urethra, endo-/ectocervix, endometrium, fallopian tube, and nasopharynx.

- Current models usually involve one tissue but, to better study infection, would need multiple models connected in a systematic way (e.g. lower to upper genital tract), one that can include multiple cell types and include immune cell effectors (e.g. T cells)
- Current models can (should?) be modified to mimic physiology with respect to levels of relevant iron sources, oxygen, and for female tissues, reproductive hormones
- Current models are not vascularized and do not contain an intact immune system
    - Models presently in development are addressing these issues

- Models are dependent on a reliable supply of biopsy samples
- Not a replacement for animal models but can be a good complement

Questions and Discussion

1. **Should, and can, complex primary human models of other infection sites be developed?**
   a. Presently, pharma companies are employing organoids for pharmacology and toxicology studies (need to replace animals) for studies prior to vaccine trials.
   b. The FDA recommends that the clinical development of new anti-Ng agents include patients with urethritis, cervicitis, rectitis, and pharyngitis, the latter three of which can presently only be achieved through ex vivo models.
   c. Question: How many tissue models do we need as a field – one for every site of infection? For complex, multicellular models, what immune cell types should be included? What is the main outcome(s) we need to evaluate?
2. **What improvements can be made to these models to more closely simulate the in vivo human environment? Are there limitations to model viability or development that may prevent better simulation of in vivo conditions?**
   a. Need a good understanding of what the system is measuring
   b. How do we model these systems to be comprehensive, for instance including various ages and ethnic / racial backgrounds?
   c. How close are these cell-based models to the native model? Systems are very complex and can be difficult to tease apart, considering that these cell-based models may be at different developmental stages (age-related effects), which can be accounted for through standardization.
   d. We need to understand how the bacterium can persist in different models – are the bacterium growing or staying static?

Assays to Define Correlates of Protection

Need to better understand the immune response desired for a vaccine trial; this has been the “black box” of gonorrhea research for decades

It is a game changer that now there are multiple vaccine trials happening around the world to evaluate how the meningococcal vaccine 4CMenB / Bexsero cross-protects against gonorrhea, because we can evaluate human immune responses in real-world settings

Presently, GSK is evaluating 3 assays to study the immune response

- Antibody binding using Luminex
  - Looks at binding of IgG collected in humans following 2 doses of Bexsero against a panel of strains
  - In individual samples, can only see small shifts between pre- and post-immunization samples as there is the confounding factor of a pre-immunization titre (high baseline)
  - Already selecting for healthy individuals but still seeing a variable response that also seems to be strain-specific
- Functional activity (bactericidal activity)
  - From these immunized individuals, have noticed a lack of correlation between two different strains being tested in SBAs
  - What is a meaningful assay in this context?
  - Define pre-existing immunity?

  - How do we choose a representative gonococcal strain (e.g. MS11)?
  - Should sialylated gonococci and serum-resistant and serum-sensitive strains always be tested?
- Opsonophagocytosis, an assay which involves the phagocyte and a source of complement
  - Questions on strain, different sources of complement (human, rabbit, lyophilized?), how to grow cells/bacterium, way to report results (e.g. CFU, titre)
- Cell-mediated immunity (still being established and optimized)
  - How should we restimulate T cells in PBMCs?
  - Also seeing a strain-dependent baseline, similar to antibody binding Luminex assay
  - Need for standardization – e.g. positive and negative controls, serum source, definition of titres; many variations in protocols, standards and strains, how do we make meaningful comparisons?

Questions and Discussion

1. **What, as a field, should we consider as vaccine-induced protection? What would regulatory agencies (e.g., the US FDA) consider as protection? Is a vaccine that provides any of the below better than not having a vaccine at all??**
   a. prevention of acquisition of the bacteria
   b. accelerated clearance without antibiotic therapy
   c. prevention of symptomatic disease
   d. prevention of ascended or diseminated infection,
   e. prevention of onward transmission

2. **What are the meaningful assays to set up?**
   a. Cell-mediated immunity assays need development
3. **Do we have reason to believe that the same correlate will define protection/killing for all infection sites? How do we search for correlates of protection? How can we understand if protection is antibody/cell-immunity mediated?**
   a. Likely will have multiple correlates to infer protection – is there a surrogate to protection?
   b. For vaccine studies, there is an assumption that immunization will cause relevant immune cells to go to relevant sites of infection, although this can be harder to discern with asymptomatic individuals
   c. Each vaccine candidate may have its own basis/MOA of protection. For instance, the anti-LOS IgG 2C7 requires complement for clearance in the mouse genital tract.
      - There may be differences in efficacy based on known differences between murine and human complement - MOA may differ between mice and humans
      - Whereas each vaccine candidate may need to establish their own surrogate of protection, IgG titers are usually a recommended first step (classic step)
      - Some marketed vaccines do not have defined correlates of protection (acellular pertussis vaccine), but some way is needed to evaluate efficacy

Standardization of Strains and Assays

Based on the above discussion, the following questions for discussion were raised:

1. **What assays are needed?**
   a. Are there better, or additional, ways to test the bactericidal activity of antimicrobials or vaccine antigens?

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2. **Who decides or how do we decide what is the "best protocol" for standardization?**
    a. There are different populations being assessed: those who are infected again and don't have an immune response vs. vaccine-induced immune response
    b. Need multiple correlates to dissect the factors that determine protection vs. no protection
    c. This should be a community effort; the *Neisseria gonorrhoeae* Research Society (NgoRS; ngosociety.org) has offered to host protocols on its website and webinars for further discussion.
3. **How do we maintain a community-based stockpile of resources, strains, reagents, etc?**
    a. Can the NgoRS be leveraged to help in making resources available.
4. **Do we need an international reference standard for our assays (to benchmark)?**
    a. Positive controls that each lab can incorporate for comparison would be useful. This was done for meningococcal vaccine development in the UK.
5. **Should we have a strain library?**
    a. Incorporate sialylation as well as multi-drug resistant isolates (which usually have a high proportion of certain compensatory mutations)
    b. Ideally these strains would be maintained at low passage number, but this can differ amongst different labs. To validate a single strain amongst different labs, is whole genome sequencing and comparison against a reference strain required, and is it sufficient? How would a "reference strain" be determined?
    c. Who or what organization should maintain and distribute these strains?