Summary and Recommendations from the National Institute of Allergy and Infectious Diseases (NIAID) Workshop “Gonorrhea Vaccines: the Way Forward”

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There is an urgent need for the development of an antigonococcal vaccine due to the increasing drug resistance found in this pathogen. The U.S. Centers for Disease Control (CDC) have identified multidrug-resistant gonococci (GC) as among 3 “urgent” hazard-level threats to the U.S. population. In light of this, on 29 to 30 June 2015, the National Institute for Allergy and Infectious Diseases (NIAID) sponsored a workshop entitled “Gonorrhea Vaccines: the Way Forward.” The goal of the workshop was to gather leaders in the field to discuss several key questions on the current status of gonorrhea vaccine research and the path forward to a licensed gonorrhea vaccine. Representatives from academia, industry, U.S. Government agencies, and a state health department were in attendance. This review summarizes each of the 4 scientific sessions and a series of 4 breakout sessions that occurred during the one and a half days of the workshop. Topics raised as high priority for future development included (i) reinvigoration of basic research to understand gonococcal infection and immunity to allow intervention in processes essential for infection; (ii) clinical infection studies to establish parallels and distinctions between in vitro and animal infection models versus natural human genital and pharyngeal infection and to inform in silico modeling of vaccine impact; and (iii) development of an integrated pipeline for preclinical and early clinical evaluation and direct comparisons of potential vaccine antigens and adjuvants and routes of delivery.

The World Health Organization (WHO) estimates that there were 78 million cases of gonorrhea worldwide in 2012 (1). Despite control efforts, the disease remains entrenched and is even increasing in incidence in several at-risk populations. Disturbingly, the causative agent of gonorrhea, the Gram-negative species Neisseria gonorrhoeae (the gonococcus, or GC), has accumulated antibiotic resistance such that it has achieved multidrug resistance (MDR) status. Recently, the U.S. Centers for Disease Control (CDC) identified MDR GC as among 3 “urgent” hazard-level threats to the U.S. population, and MDR GC is featured prominently in U.S. President Obama’s National Action Plan for Combating Antibiotic-Resistant Bacteria (2, 3). The advent of MDR gonorrhea has spurred renewed interest in the development of new antimicrobial products and studies on combination therapies. However, in the end, these activities may only delay the development of untreatable gonorrhea, given the organism’s genetic plasticity and ability to acquire foreign DNA containing resistance genes. Prevention thus appears to be a solution to the issue, and the most proven, reliable, and cost-effective method of prevention would be the implementation of an effective vaccine.

In 2013, the WHO and National Institutes of Health (NIH) organized a technical consultation to evaluate how to advance sexually transmitted infection (STI) vaccine development focused on five STIs, herpes simplex virus, Chlamydia trachomatis (chlamydia), Neisseria gonorrhoeae (gonorrhea), Treponema pallidum (syphilis), and Trichomonas vaginalis (trichomoniasis) infections. The consultation was reported in a special issue of the journal Vaccine (4) which identified gaps in knowledge and concluded with a proposed global roadmap for STI vaccine development outlining critical steps in vaccine development and implementa-

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ward to a licensed gonorrhea vaccine. Representatives from academia, industry, U.S. Government agencies, and a state health department were in attendance. This report summarizes each of the 4 scientific sessions and a series of 4 breakout sessions that occurred during the one and a half days of the workshop.

SESSION 1: GOAL OF A GONOCOCCAL VACCINE
The ultimate goal in the design of any vaccine is to prevent disease and/or infection in a group of vaccinated individuals, with the hope that a comprehensive vaccine program would prevent transmission and eventually eradicate the disease in the general population. Achievement of this goal for a gonorrhea vaccine, however, will require the achievement of several subordinate goals and resolution of other multiple issues, identified and discussed at this workshop. The presentations in this first session focused on 3 main themes: the need for a gonococcal vaccine, the readiness of the scientific field, and the impact of the implementation of an effective gonorrhea vaccine on the adolescent population.

Magnus Unemo (Örebro University) and Edward Hook (University of Alabama) presented evidence to support the idea that there is an immediate need to develop a gonococcal vaccine. Dr. Unemo discussed the history and current antibiotic resistance of the gonococcus. After 70 years of changing treatment regimens, the gonococcus has accumulated resistance such that the extended-spectrum cephalosporins (ESCs) are the last class of antibiotics recommended for monotherapy of the disease in most settings. However, reports of increased ESC MICs, ESC treatment failures, and, most worryingly, isolation of strains with high-level ceftriaxone resistance indicate that global ESC resistance (possibly leading to untreatable gonorrhea) is only a matter of time. To address this problem in the short term, dual antimicrobial therapy (mainly ceftriaxone plus azithromycin) has been introduced in many countries and efforts should be made to repurpose older antibiotics (e.g., gentamicin and fosfomycin) and investigate novel dual therapeutic regimens. Long-term solutions include the development of new antimicrobials or new derivatives/analogues of previous antimicrobials and the development of a vaccine that theoretically would be efficacious regardless of antimicrobial resistance.

Dr. Hook presented an appraisal of gonorrhea control efforts in the United States. The control of gonorrhea depends on a combination of syndromic diagnosis (leading to treatment), follow-up of sexual contacts, and screening. Syndromic diagnosis predominantly identifies cases of gonorrhea in males, as they are more often symptomatic. In contrast, infection is frequently asymptomatic in women means that control of their disease relies heavily on follow-up of sexual contacts and, particularly, screening; current guidelines suggest annual screening of sexually active women ≤25 years of age and risk factor-based screening for older women. Screening is currently performed in the high-risk population of men who have sex with men (MSM), but there are no other current guidelines for males. Dr. Hook posited that future flat or rising rates of gonorrhea are likely to occur with the current effort focusing on the treatment of symptomatic men and screening of women. Additional control efforts, such as routine screening in young men and the development of a gonorrhea vaccine, should be considered if we are to make a significant impact on gonorrhea rates.

P. Frederick Sparling (University of North Carolina) discussed the status of the field of gonococcal vaccinology and the potential to develop an effective vaccine. In general, gonococcal vaccine development is still in a discovery phase, in which the basis of immunity and the identification of immune correlates or surrogate markers of protection still need to be deciphered. Very little evidence of effective immunity to reinfection after uncomplicated genital infection has been observed; infection induces a weak and transient mucosal immune response, and the infecting organism possesses several mechanisms of immune suppression or diversion (6). Despite these limitations, there are some hopeful data to support the successful development of a gonorrhea vaccine. These include the induction of modest protection by a gonococcal pilin vaccine (7) and the recent introduction of an effective protein-based meningococcal group B vaccine that can be used as a prototype of noncapsular vaccines that prevent neisserial infection and/or colonization. Efforts should be focused now on selection of antigen/adjuvant combinations that prevent homologous infection in the mouse or other models to produce candidates for phase I clinical testing. In particular, Dr. Sparling suggested that the human male model of gonorrhea infection could be expanded and employed as a way to select vaccine candidates in modified phase I/II safety and efficacy studies that would include ancillary studies of the vaccine-induced immune response.

The final presentation of the session was given by Katherine Hsu (Massachusetts Department of Public Health/Boston University School of Medicine/Boston Medical Center), who contended that the logical goal of a gonococcal vaccine is the elimination of persistent mucosal infection within priority populations of the United States. Priority populations include nonwhite youths and young adults in urban areas in the South and West and high-risk adults such as MSMs, HIV-positive individuals, and women at high risk. However, implementation of a vaccine in specific target populations would be difficult and unprecedented. Universal implementation in the general adolescent population is more straightforward and would cover the priority populations. Dr. Hsu suggested that much can be learned from the introduction of the human papillomavirus (HPV) vaccines, in particular with respect to measures used to increase vaccine acceptability to parents and young adults who may have to choose vaccination.

In the discussion that followed, the participants agreed that all the groundwork for the development of a gonococcal vaccine has been laid by interested stakeholders. Credible data showing the real possibility of untreatable gonorrhea and the current deficiency in control efforts strongly support the need for vaccine development now. The gonorrhea research community has provided both research and clinical data, including the identification of a series of vaccine candidates and animal and human models of infection, to guide development of a vaccine. In addition, surveillance activities have adequately kept up with changing gonorrhea epidemiology and the collection of recent isolates and their genomic information should be made available to aid in future vaccine design. Finally, HPV vaccines should be used as a model for gonococcal vaccine development and implementation, paying particular attention to the methods used in messaging and increasing vaccine acceptance in the adolescent population.

SESSION 2: HUMAN DISEASE MODELING TO EVALUATE THE IMPACT OF VACCINATION
Developing a vaccine from research concept to licensed product is a long and expensive process, costing on the order of hundreds of millions of dollars. Commercial vaccine producers are therefore
unlikely to commit themselves to the development of a vaccine unless there is some certainty that the vaccine would have a market and would deliver a reasonable return on their investment. Consequently, impact, cost-effectiveness, and affordability modeling plays a major part in influencing both the development and implementation of vaccines. For example, modeling the impact of immunization with human papillomavirus (HPV) vaccine has demonstrated the cost-effectiveness of vaccination for the prevention of STI caused by this virus (8, 9). A further example of the importance of cost-effectiveness modeling in vaccine development programs is provided by the recently licensed vaccines against meningococcal disease, which is caused by Neisseria meningitidis, a bacterium closely related to the gonococcus (10, 11). Guided by presentations from Kate Seib (Griffith University) and Kwame Owusu-Edusei (U.S. CDC), the workshop participants discussed modeling the potential impact of gonococcal vaccines.

The models presented fall into two categories: those that assume that the vaccine simply prevents disease in an individual and those that also take into account the potential of a vaccine to disrupt transmission of the disease. Whichever type of model is applied, values have to be estimated for the many parameters upon which the model is based and may be varied to assess the sensitivity of the model to changes in each parameter. The parameters can be grouped into the following categories: vaccine efficacy; the target population; disease-related factors; and vaccine coverage. If cost-effectiveness is to be modeled, the costs of the disease itself, of the vaccine, and of its delivery to the target population are also to be considered. The workshop participants recognized that in the case of gonococcal disease, the amount of data available to underpin models currently ranges from reasonable to nonexistent, depending on the parameter.

In the absence of a gonococcal vaccine, clinical trial data, or an immunological correlate of protection, various theoretical efficacy values can be used to model whether a gonococcal vaccine needs to induce sterilizing protection or simply a modest reduction in transmission to be effective. Similarly, varying theoretical vaccine coverage values and whether both sexes are immunized can be used to identify potential immunization strategies. In contrast to the vaccine-related parameters, the target population, its sexual behavior, and many of the disease-related parameters are better understood and information is available to help estimate values. Improved models of infection are still required to provide the quality of data necessary for more-accurate model simulations. Furthermore, specific information needs to be extracted from pathological and epidemiological studies of gonococcal disease to address limitations of the current models. Similarities with Chlamydia trachomatis disease, such as clinical outcomes (e.g., pelvic inflammatory disease [PID], infertility, and ectopic pregnancy), natural history, the potential for reinfection, and existing screening recommendations, can also be informative in modeling the prospective impact of gonococcal vaccination.

The models presented to the workshop suggested that a hypothetical gonococcal vaccine with partial efficacy could have a significant impact on disease prevalence, as long as coverage is high and protection lasts through the period of highest risk of disease among young people (36). There are currently insufficient data on the cost of disease, of its sequelae, or of prospective implementation strategies to provide a realistic appraisal of the potential cost-effectiveness of GC vaccination. However, in the case of the gonococcus, it is important that such models take notice of the potential impact of a vaccine on both the disease burden and the ongoing emergence of antimicrobial resistance.

**SESSION 3: HUMAN STUDIES**

A major impediment in gonococcal vaccine development is the lack of correlation between infection and subsequent protection. This stands apart from numerous infectious diseases in humans where infection is an immunizing event that protects against future bouts of infection. While numerous well-characterized adaptive immune responses occur in gonococcal infection, no single response and, indeed, no combinations of responses have been shown to protect subsequently against human infection and disease.

Additional nonimmune factors undoubtedly contribute in an adjunct fashion to susceptibility; a robust vaccine(s) should also strive to overcome these nonimmune barriers to prevent infection under diverse circumstances. The biologic considerations that were discussed and found important to consider in achieving vaccine efficacy included (i) racial and gender differences, and the impact of genetic differences and hormones; (ii) efficacy at different clinical (tissue) sites of infection; (iii) the importance of ancillary microbial flora (e.g., microbiome); and (d) genotypic and phenotypic strain variations. The antimicrobial resistance of gonococci, while not specifically discussed, may contribute its own biologic phenotype(s) and is a major driver of vaccine development.

Peter Rice (University of Massachusetts) began by noting that three gonococcal vaccine trials (whole cell, pilus, and Por), conducted since 1970, were unsuccessful in protecting vaccinees from either natural (whole-cell trial [12] and pilus trial [7]) or experimental (Por trial [13]) infection. Limited in vivo studies of the pilus vaccine and in vitro studies of the Por vaccines had shown efficacy. However, in the case of the pilus vaccination trials, the vaccine was pilus variant specific but the organisms were from the community and, therefore, antigenically distinct wild types. In the case of the Por vaccine, the homologous strain used to prepare the vaccine was used for challenge. The vaccine was contaminated with other outer membrane components; at least one that was present (Rmp antigen) is known to subvert the killing function of the Por-specific immune response (14). In the Por vaccine study, and in several additional contemporaneously performed natural history studies in women, modeling of the immune status of subjects exposed to N. gonorrhoeae has suggested that immunity against several outer membrane components together may predict protection from infection (15, 16) or, alternatively, may enhance the likelihood of infection (17), depending on the relative contributions of specific immune responses. Therefore, a successful vaccine may have to be multivariate, targeting conserved epitopes while avoiding subversive immunogens. If univariate, the vaccine must force a protective immune response that exceeds what is seen in natural infection; that is, “nurture must trump nature.” The most successful of such single antigens used as vaccines have been bacterial capsular polysaccharides. While these are not present in N. gonorrhoeae, conserved saccharides are present in gonococcal lipooligosaccharides (LOs) (18) and should be exploited further as vaccine candidates (19).

Peter Leone (University of North Carolina) provided a perspective on conducting clinical vaccine trials and confronting challenges. Enrolling specific populations where gonorrhea is prevalent may be difficult, particularly when there may be a stigma...
attached and a distrust of the research enterprise in certain communities. Furthermore, enrollment of such populations may be more cumbersome because they are viewed as more vulnerable by institutional review boards (IRBs) and therefore require greater safeguards. Adolescents may be particularly hard to reach because of IRB consent requirements, which differ across jurisdictions. It will also be important to include women, minority populations, MSM, and HIV-positive persons in gonococcal vaccine trials because they are more likely to be exposed to gonococcal infection and may sustain greater consequences of gonococcal diseases. The involvement of these groups will also be necessary to test vaccine efficacy against gonococcal infections that involve a greater number and variety of tissue sites of infection. Increasingly, patients infected with *N. gonorrhoeae* have STIs managed outside the sexually transmitted disease (STD) clinic. More extensive and more efficient recruitment at STD clinics will be necessary, and, where possible, recruitment should also be performed at alternative locations that can be supported by STD clinic research staff and that permit follow-up of subjects at the local STD clinic. Staff members at vaccine trial sites should be experienced in performing clinical research and should have academic connections that can assist in providing key laboratory measurements.

Martin Maiden (Oxford University) pointed out that, in large part, pathogenic *Neisseria* species (*N. gonorrhoeae* and *N. meningitidis*) share most of their genomic sequences and share sequences with each other to a greater extent than with nonpathogenic *Neisseria* species. As shown by whole-genome sequencing methodology, the pathogenic *Neisseria core* genome is made up of 1,800 genes (of a total of 2,000); ~17% are specific to *N. gonorrhoeae*. With the possible exception of the gonococcal genetic island (GGI; 50 kb) which is present in some strains of *N. gonorrhoeae*, genomic uniformity is the rule, although some loci and therefore the antigens that they encode are highly variable. Consequently, sequencing many gonococcal strains and combining the results with bioinformatics data will be useful to separate highly variable from conserved genes across the species and may represent a useful step in identifying potential vaccine candidates, particularly candidates that are unique to *N. gonorrhoeae*.

Marcia Hobb (University of North Carolina), who has advanced the idea of the use of experimental gonococcal infection in men, highlighted three initiatives using this approach: (i) observational studies of the natural history of experimental gonococcal infection with “wild-type” strains; (ii) pathogenesis studies performed with isogenic mutants; and (iii) vaccine and treatment studies (the latter yet to be performed). Experimental infection of male volunteers is safe and reproduces the clinical features of naturally acquired gonococcal urethritis. This model has helped define the natural history of experimental infection with two well-characterized strains (reviewed in reference 20), FA1090 (21) and MS11mkC (22). Studies performed with isogenic mutants have improved understanding of the requirements for specific LOS structures, pili, opacity proteins (reviewed in reference 23), and IgA1 protease (24) and the ability of infecting organisms to obtain iron from human transferrin and lactoferrin (25). In addition, host factors such as cytokine development (26) and the role of LOS antibodies (Abs) in resistance to reinfection (27) have also been characterized. However, in order to amplify this approach, expanded clinical capacity will be needed to broadly apply vaccine testing in human experimental infection.

Considerable discussion was devoted to the use of experimen-
saccharide epitope given by intraperitoneal injection with monophosphoryl lipid A (19). However, not all antigens confer protection. Several unpublished immunization/challenge studies have been conducted with other antigens through collaborations with the Jersey laboratory that did not show protection. Each of these antigens induced high-level serum and vaginal IgG and bactericidal antibodies. Most were given with cholera toxin, which is a known Th2-inducing adjuvant. Perhaps importantly, two of the three published antigens that showed protection, rPorB-VRP and MAP1, elicited Th1 responses. The immune bias of the third antigen, OMV, was not measured.

Michael Russell (State University of New York/Buffalo) discussed studies in mice that have allowed dissection of the immunological pathways induced by *N. gonorrhoeae*, including the discovery that induction of Th1 responses correlates with protection in this model. A Th17 response which is not protective drives the neutrophil response to infection in mice; this finding is consistent with the detection of Th17 responses in naturally infected humans. In mice, lower-genital-tract infection induces a poor adaptive response, and *N. gonorrhoeae* suppresses the development of Th1 and Th2-driven adaptive immune responses by mechanisms dependent on transforming growth factor β (TGF-β) and interleukin-10 (IL-10) and the induction of type 1 regulatory T cells. This immunosuppression can be reversed by blocking expression of TGF-β and/or IL-10 or by topical administration of microencapsulated IL-12, which clears infection and induces a protective memory response that is characterized by high antibody titers (reviewed in reference 6). Both B cells and gamma interferon (IFN-γ) contribute to the generation of protection. These results suggest that Th1-driving cytokines could be used to induce protective responses. Intravaginal immunization with gonococcal OMV plus microencapsulated IL-12 induces similar protective immunity, including the generation of gonococcus-specific antibodies and IFN-γ. Protection is effective against challenge with the homologous strain (FA1090) and against heterologous strains (MS11 and a clinical isolate) (31).

Scott Gray-Owen (University of Toronto) discussed new transgenic (Tg) mouse models that not only better mimic the human host but also are valuable for assessing the importance of selected host-restricted factors for infection. Higher numbers of gonococci associate with the tissues of human carcinoembryonic antigen-related cell adhesion molecule (hCEACAM)-expressing mice than with those of non-Tg mice due to the Opa protein-dependent binding to mucosally expressed hCEACAM1 and hCEACAM5, while the opsonin-independent binding and engulfment of gonococci by neutrophils are mediated by hCEACAM3. These interactions reflect what is presumed to occur in humans, since expression of individual hCEACAMs in these things. The potential hierarchies of assays that could be utilized for assessing both correlates and mechanisms of immunity were discussed above, but understanding the hierarchy of these correlates is important, including the methods by which these correlates can be determined and measured in improved animal models (as discussed above), how these correlates can be used to help obtain FDA approval, and how adjuvants can help obtain these correlates. These are all critical issues that this workshop was intended to begin to address.

Lee Wetzler (Boston University School of Medicine/Boston Medical Center) began by discussing the issue of correlates versus mechanisms of immunity, which are not necessarily the same things. The potential hierarchies of assays that could be utilized to assess both correlates and mechanisms of immunity were then discussed. These included (i) antibody (Ab) binding to the pathogen to prevent adhesion or invasion; (ii) Ab binding to the organism to allow bactericidal complement activation; (iii) Ab binding to allow both complement activation and subsequent opsonophagocytic activity and killing by neutrophils of *Neisseria* (this includes induction of more-functional immunoglobulin isotypes like IgG2 and IgG3 in humans—better at activating complement and being bound to FcR); and (iv) T cell involvement, including induction of Th cells to augment and improve Ab responses such as by inducing isotype switching and somatic hy-
permutation to increase Ab affinity and possible auxiliary roles of Th17 cells that may increase neutrophil influx and CD8 T cells (even though the role of the CD8 T cells is unproven). It was felt that the induction of Ab that recognizes the intact organism may be the correlate or biomarker that can be most easily measured, while mechanisms of immunity may include 1 or more of the 4 types of immune functions mentioned. Moreover, more studies are absolutely necessary to help determine whether these or other correlates can be used as valid predictors of immunity; animal models may prove useful for this purpose, with subsequent validation in humans. Indeed, phase 1 human trials may even be needed to help determine correlates, and the FDA representatives suggested that it may not be necessary to define “correlates” prior to initiation of any human trials.

Discussion led to the issue of whether the mechanisms of protective immunity and/or measurable correlates of immunity might differ in males versus females or among the members of other, more restricted target populations. It was posited that immunizing men to protect them from infection, thereby preventing subsequent transmission, may be an easier task than targeting women, since the human infection model utilizes men only and correlates of protection may therefore be more readily defined in men. However, there was significant concern regarding the approach of immunizing only one gender, with the following points mentioned: (i) not enough is known regarding infection or colonization in women to justify this approach; (ii) if a vaccine were to be approved only for males, how such an approach could be justified to parents who have both boys and girls is unclear, given that only the boys would get the vaccine and be “protected” (a concern likely easy to address with information gleaned from other studies); and (iii) this approach may be counterproductive if vaccination prevents symptoms (disease) but not colonization (infection), which would reduce the chance that male partners would inform female partners of potential infection. A counterpoint was, however, made that targeting men would have the benefit of preventing male-male transmission in high-risk MSMs.

Next, Margaret Bash (U.S. Food and Drug Administration) discussed the role of the FDA in helping define correlates of immunity. She pointed out that the FDA does not require understanding or delineation of the mechanism of immunity and/or correlates of immunity induced by a vaccine prior to its licensure. Moreover, she highlighted that adjuvants themselves are not licensed alone; they are licensed only as part of a vaccine product. She did emphasize that “adequate and well-controlled clinical studies that demonstrate efficacy” are absolutely necessary prior to licensure and that such studies should be a priority for any vaccine development plan. Finally, she mentioned that accelerated approval for a vaccine requires serious epidemiological conditions and unmet medical needs (35) and that the urgency of gonococcal vaccine development due to increased antibiotic resistance likely fulfills these criteria, so accelerated approval for a gonococcal vaccine is certainly possible.

Darrick Carter (Infectious Disease Research Institute [IDRI]) then discussed the role of adjuvants in vaccine development. He began by mentioning that the IDRI has a battery of lipid A (Toll-like receptor 4 [TLR4] ligand)-derived adjuvants that have decreased toxicity but remain immunologically active. He mentioned that adjuvants increase immune responses to antigens of interest not just by increasing the quantity of Ab to a particular epitope but also by increasing the number and breadth of epitopes recognized by the Ab response through enhancement of the B cell response. Carter presented novel adjuvants, formulation studies, and human data on vaccines for many infectious diseases, including influenza, malaria, and tuberculosis, which provided a glimpse into a potential pathway toward a gonococcal vaccine.

BREAKOUT SESSION: ADJUVANTS AND ROUTES OF ADMINISTRATION (CHAIRS: DARRICK CARTER [INFECTIOUS DISEASE RESEARCH INSTITUTE] AND HERMAN STAATS [DUKE UNIVERSITY])

Based on the evidence presented in the workshop, it was clear to this discussion group that an efficacious gonococcal vaccine would likely need to induce a mucosal immune response. Also noted in the workshop was the availability of the estradiol-treated mouse model of infection. Adjuvant experts indicated that advancements in adjuvant development would allow testing of a variety of antigen/adjuvant combinations for induction of protective immunity. These activities may also contribute to defining correlates of protection. Discussions of adjuvant selection also led to a debate on routes of vaccine administration. Almost all currently licensed vaccines are delivered parenterally; there was consensus that a parenteral vaccine would be easier to evaluate and license than mucosal tissue immunization, and experts recommended that adjuvant testing begin with those that are administered parentally and induce mucosal immunity and then move on to adjuvants that may require immunization of mucosal tissue such as by intranasal administration. Experts also cautioned that just because one adjuvant does not work with a particular antigen, that does not necessarily mean that the antigen is ineffective; it may just mean that another adjuvant may need to be utilized. Similarly, different adjuvants may prove most efficacious in combination with different antigens.

BREAKOUT SESSION: ANIMAL AND HUMAN STUDIES/MODELS (CHAIRS: CAROLYN DEAL [NATIONAL INSTITUTE OF ALLERGY AND INFECTION DISEASES] AND ANN JERSE [UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES])

The main goals of animal and human studies/models are to provide information on host-pathogen interactions, to determine potential parameters of protective immunity, and to provide in vivo systems for testing of preventative measures such as vaccination. The panel reflected upon what was discussed earlier in the workshop regarding the conclusion that the mouse model of gonococcal infection, though imperfect, will undoubtedly prove to be an important tool in vaccine testing and design, although the model would clearly benefit from refinement (e.g., use of knock-in mice) and from harmonization of protocols among different laboratories. Support was also voiced for the idea brought up in session 1 that the human model of gonococcal infection should be utilized in modified phase I/II safety and efficacy studies for the evaluation of advanced vaccine candidates. The members of the panel also suggested that the field of investigation of gonococcal vaccines would be well served with more natural history studies, particularly if they can inform as to the mechanism of immunity. In particular, a study could be built into existing gonorrhea screening programs and locations that would enroll initial nucleic acid amplification test (NAAT)-positive patients who are called back for treatment. These patients would consent to sampling (e.g., using vaginal/cervical swabs and blood), and a second NAAT could dif-
ferrate two groups: those that controlled their infection and those still infected. Careful analysis of the humoral, cellular, and tissue immune responses may identify correlates of protection and/or candidate vaccine antigens.

BREAKOUT SESSION: BIOINFORMATICS, ANTIGEN SELECTION, AND ANTIGEN VARIABILITY (CHAIRS: DAVID TREES [U.S. CENTERS FOR DISEASE CONTROL AND PREVENTION] AND MARTIN MAIDEN [UNIVERSITY OF OXFORD, UNITED KINGDOM])

It was agreed that publicly accessible databases of annotated representative whole-genome sequencing (WGS) data that employ common nomenclatures are a priority. Such databases have a major role to play in the choice of novel vaccine antigens and in understanding the variations of these antigens; in functional studies, including proteomics and structural biology; and in investigations into the evolution and spread of antimicrobial resistance. Novel “omics” technologies, such as transcriptome sequencing (RNA-seq) for expression studies and PacBio sequencing to determine the methylene, present opportunities to link genome sequencing with functional studies, especially in conjunction with well-characterized isolate collections such as the Gonorrhea Isolate Surveillance Project (GISP) collection. For these opportunities to be realized, it will be necessary for existing and novel bioinformatics platforms (e.g., the European Nucleotide Archive [ENA] and the Short Read Archive [SRA]; Bionumerics 7; PubMLST.org; NCBI; Sanger) to have interoperability and to work to the same quality standards.

A number of approaches can be used to exploit WGS for the development of vaccine candidates; such approaches can include systematic searches for conserved vaccine antigens (“reverse vaccinology”), examination of the distribution and nature of variation for the development of vaccines with a cocktail of antigens, and candidate antigen approaches. In all these approaches, the intellectual property position is important. Such vaccines may target disease, colonization, transmission, or infection, and knowledge of the natural history of the gonococcus will be important in designing vaccines that target appropriate stages. Expression studies will be of particular importance here, as will knowledge of human biology, including immunity and behavior likely to promote transmission.

The group discussion concluded with agreement that immediate and achievable action points included ensuring database interoperability and data sharing; defining gonococcal core and pan genomes; taking advantage of technologies such as PacBio sequencing; developing a “decision tree” for the assessment of genome-derived vaccine antigens; and, importantly, integration of open-access genomic data with good-quality provenance and phenotype data, including relevant epidemiological data. The usefulness of proteomics in antigen discovery was also discussed.

BREAKOUT SESSION: IN VITRO ANALYSIS AND IN VITRO CORRELATES (CHAIRS: MARIAGRAZIA PIZZA [GlaxoSmithKline] AND MICHAEL APICELLA [UNIVERSITY OF IOWA])

In vitro assays are routinely employed or developed by the gonococcal research community for the study of N. gonorrhoeae pathogenesis and immune responses. These include functional bactericidal, opsonophagocytic, and immunolabeling assays and assays/studies in which the gonococcus interacts with human cervical or urethral cells. Many of these analyses allow in vitro gauging of vaccine performance along with potential identification of novel vaccine antigens. The breakout panel identified a clear need for the harmonization of in vitro assays in order to maximize the efficiency of gonococcal vaccine development. Fulfilling this need would include the availability of an agreed-upon set of assays needed for vaccine evaluation and of strains, reagents, and protocols that can be freely accessed. These efforts would allow direct comparisons of candidate vaccines among researchers, which would expedite the process of identifying vaccine candidates that should enter the development pipeline. Other parameters, such as N. gonorrhoeae growth conditions, should be consistent as well, and efforts should be made to further develop in vitro models of infection that can be used to correlate vaccine efficacy or identify new candidate vaccines (e.g., “organ on chip” models).

CONCLUSION AND RECOMMENDATIONS

This workshop allowed the gonococcal research community, industry representatives, public health advocates, and government agency staff to work together to define the current status of gonorrhea vaccines and to identify gaps and challenges to allow development to move forward. Improvements in collaboration and acceleration of the vaccine development process could be enhanced by broader access to in vitro assays, reagents, and animal models with the goal of harmonizing selected protocols across laboratories to ensure that the members of the research community are striving to achieve the same set of goals. Over the last several years, NIAID has supported select preclinical services and improved access to common reagents (http://www.niaid.nih.gov/labsandresources/resources/ translational/Pages/clinical Preclinical.aspx). Efforts to refine existing animal models to better mimic human infection should also continue, and dialog should continue on the potential and feasibility of leveraging the experimental human male infection model for phase I/II clinical trials to test candidate vaccines and/or define correlates of protection. Finally, clinical studies to develop a better understanding of the natural history of infection will assist in better defining host responses to infection and determine whether the immunosuppressive pathways predicted from studies performed with human immune cells or animal models occur during human infection. It is hoped that these proceedings could be used as a guide for future high-quality and thematically integrated research projects on gonococcal vaccine design and testing.

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