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PELVIC INFLAMMATORY DISEASE SUPPLEMENT

Preclinical Testing of Vaccines and Therapeutics for Gonorrhea in Female Mouse Models of Lower and Upper Reproductive Tract Infection

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Murine models of *Neisseria gonorrhoeae* lower reproductive tract infection are valuable systems for studying *N. gonorrhoeae* adaptation to the female host and immune responses to infection. These models have also accelerated preclinical testing of candidate therapeutic and prophylactic products against gonorrhea. However, because *N. gonorrhoeae* infection is restricted to the murine cervicovaginal region, there is a need for an in vivo system for translational work on *N. gonorrhoeae* pelvic inflammatory disease (PID). Here we discuss the need for well-characterized preclinical upper reproductive tract infection models for developing candidate products against *N. gonorrhoeae* PID, and report a refinement of the gonorrhea mouse model that supports sustained upper reproductive tract infection. To establish this new model for vaccine testing, we also tested the licensed meningococcal 4CMenB vaccine, which cross-protects against murine *N. gonorrhoeae* lower reproductive tract infection, for efficacy against *N. gonorrhoeae* in the endometrium and oviducts following transcervical or vaginal challenge.

Keywords. gonorrhea; mouse; reproductive tract; PID; vaginal microbicides; antibiotics; vaccine.

Gonorrhea has a major impact on female reproductive health and the health and well-being of newborns. An estimated 87 million annual *Neisseria gonorrhoeae* infections occur globally [1], and approximately 15% of cervical infections ascend to cause pelvic inflammatory disease (PID) [2]. PID and associated postinfection complications significantly contribute to the morbidity and mortality associated with gonorrhea. Maternal gonorrhea is associated with low birth weight babies and is a significant cause of neonatal conjunctivitis in regions where routine prophylaxis at childbirth is not performed or insufficient [3]. *Chlamydia trachomatis* can also cause PID and is more common than *N. gonorrhoeae* PID in most but not all surveys [4]. PID due to *Mycoplasma genitalium* and concurrent infections with these pathogens, often with anaerobes, is also common [2]. Gonococcal salpingitis has been associated with more acute symptoms than chlamydial PID [5], a finding that is supported by a more recent comparative survey that measured PID severity based on number of hospitalizations [6].

There are currently no licensed *N. gonorrhoeae* or *C. trachomatis* vaccines; therefore, control of these infections relies on identification and treatment of infected individuals and their sexual contacts, and safe-sex counseling. While *C. trachomatis* remains susceptible to doxycycline or azithromycin, control of gonorrhea by antibiotic therapy is seriously threatened by the rapid evolution of antibiotic resistance. Currently, the recommended first-line therapy for gonorrhea in the United States is limited to ceftriaxone [7], which is given with azithromycin in many parts of the world [8]. However, *N. gonorrhoeae* susceptibility to ceftriaxone and azithromycin is decreasing worldwide, and fully resistant strains to either or both antibiotics have been isolated from treatment failures [9].

This alarming situation has ignited translational research on new prophylactic and therapeutic interventions against gonorrhea. An important step in the product development pipeline is the preclinical testing of candidate products for safety and efficacy in animal models. Thus far, experimental *N. gonorrhoeae* reproductive tract infection has only been established in chimpanzees and estradiol-treated female mice [10], and experimental murine infection is currently used to accelerate product development against gonorrhea. However, infection is limited to the cervicovaginal region [10, 11], and therefore current mouse models cannot be used to evaluate product efficacy against ascending *N. gonorrhoeae* infection. In contrast, several animal models of chlamydial upper reproductive tract (URT) infection are used to test candidate chlamydial vaccines and antibiotic efficacy against salpingitis [12–14].

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Here we describe *N. gonorrhoeae* lower reproductive tract (LRT) infection of female mice and summarize studies in which efficacy of therapeutic or prophylactic products was tested in various murine models of LRT infection. We also describe the first mouse model of sustained URT infection and discuss the need for URT infection models for testing therapeutics against PID and whether vaccines and topically applied agents can protect against ascending infection. Finally, we established the murine URT infection model described here for vaccine testing by testing a licensed meningococcal vaccine that induces cross-protection against *N. gonorrhoeae* LRT infection [15] for efficacy against murine endometrial and oviduct infection.

MURINE MODELS OF GONOCOCCAL REPRODUCTIVE TRACT INFECTION

Lower Reproductive Tract Infection

Experimental *N. gonorrhoeae* infection of female mice requires the use of 17β-estradiol and antibiotics to promote long-term susceptibility. Several inbred mouse strains, transgenic mice that relieve host restrictions, and knock-out mice have been used [10]. In BALB/c mice, gonococci are seen in the vaginal lumen, cervical and vaginal tissue, and within the lamina propria, and a vaginal neutrophil influx containing intracellular diplococci occurs. Similar to that described for human cervical infections, hormonally driven cyclical fluctuations in the number of *N. gonorrhoeae* recovered from vaginal swabs are observed [11]. As seen in human infection, mice can be reinfected with the same strain and the adaptive immune response to *N. gonorrhoeae* infection is suppressed [11, 16]. Low numbers of *N. gonorrhoeae* can be transiently recovered from the endometrium, as was also observed in a model in which *N. gonorrhoeae* is transcervically inoculated into progesterone-treated or diestrus-stage mice [17]. Therefore, it is not feasible to examine the efficacy of products in preventing or treating *N. gonorrhoeae* ascending infection in these current models.

Upper Reproductive Tract Infection

N. gonorrhoeae is a human-specific pathogen and one or more host-restricted factors is likely responsible for the difficulty in establishing sustained murine *N. gonorrhoeae* URT infection. Host-restricted factors include colonization receptors, negative regulators of the complement cascade, and uptake of zinc from human calprotectin and iron from human transferrin (hTf) and lactoferrin (hLf) through specific *N. gonorrhoeae* outer membrane receptors [10, 18]. While expression of either the *N. gonorrhoeae* transferrin or lactoferrin receptor is required for urethral infection in male volunteers, neither is required for LRT infection in mice, presumably due to the greater availability of soluble iron in the lower pH of this body site and the presence of iron-laden siderophores and iron complexed to metabolites (reviewed in [11]). Due to the poor recovery of *N. gonorrhoeae* from the murine URT following vaginal inoculation, we hypothesized that *N. gonorrhoeae* may need human iron-binding glycoproteins for growth in this body site. To test this hypothesis, we administered phosphate-buffered saline (PBS) or hTf to BALB/c mice treated with sodium estrone sulfate (Premarin) using a treatment regimen that results in serum hTf levels similar to humans [19]. Mice were inoculated transcervically with 10^6 colony-forming units (CFU) of *N. gonorrhoeae* strain FA1090 and endometrial and oviduct tissue were cultured for *N. gonorrhoeae* on days 1, 5, and 7 postinoculation (Supplementary Methods). In support of our hypothesis, *N. gonorrhoeae* was recovered from the endometrium and oviducts of 42%–54% and 21%–50% of hTf-supplemented mice, respectively, at these time points, but not from PBS-treated mice (Figure 1A and 1B).

To determine whether hTf would support ascending infection, we inoculated groups of hTf-supplemented mice transcervically or vaginally with 10^6 CFU of *N. gonorrhoeae* strain FA1090. We found the same percentage (65%) of mice with positive

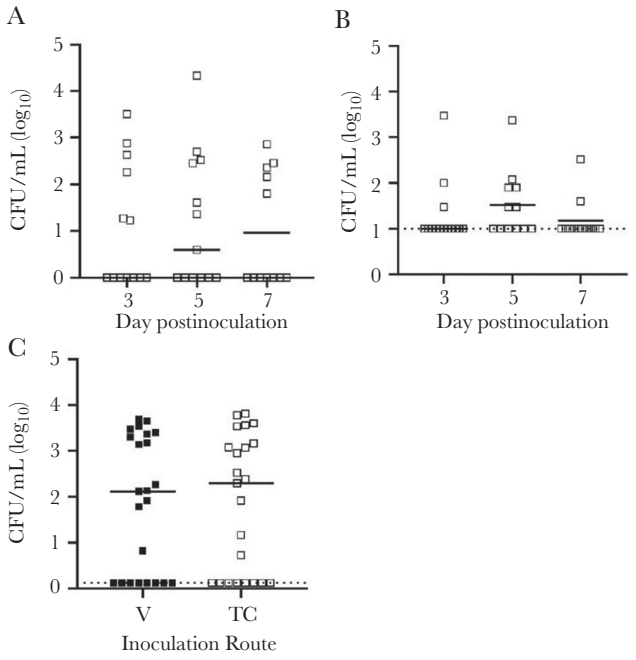

Figure 1. Administration of hTf to female mice supports *Neisseria gonorrhoeae* infection of the upper reproductive tract. Recovery of *N. gonorrhoeae* from the (*A*) endometrium or (*B*) oviducts of hTf-supplemented female BALB/c mice on days 3, 5, and 7 after transcervical inoculation with 10^6 CFU of *N. gonorrhoeae* strain FA1090. The percentage of mice with positive endometrial cultures ranged from 41% to 54%; oviduct cultures were positive in 21% (day 3), 50% (day 5), and 17% (day 7) of mice. No positive cultures were obtained from control mice given phosphate-buffered saline instead of hTf. Data shown are from 3 experiments combined with a total n = 12–14 mice/time point. *C*, Recovery of *N. gonorrhoeae* from the endometrium of hTf-supplemented female mice on day 5 after vaginal or transcervical inoculation with 10^6 CFU of strain FA1090. The percentage of mice with positive endometrial cultures in each group was 65%, with no statistical difference in the percent colonized or the number of CFU recovered (n = 23 mice/group, 2 experiments combined). Abbreviations: CFU, colony-forming units; hTf, human transferrin; TC, transcervical; V, vaginal.

endometrial cultures 5 days after bacterial inoculation in each group (Figure 1C), with no difference in the number of viable gonococci recovered, which ranged from 10^1 to 10^4 CFU/mL of endometrial scrapings among culture-positive mice. We conclude that the availability of hTf breaks at least one host restriction barrier in the URT by providing a usable iron source, and that this model may be a useful in vivo system for testing the efficacy of therapeutic products in clearing URT infection and of vaccines and vaginally delivered prophylactic products against ascending infection.

PRECLINICAL TESTING OF PRODUCTS AGAINST GONORRHEA

A summary of published preclinical studies in which murine infection models have been used to test candidate antibiotics, immunotherapies, vaginal microbicides, probiotics, and vaccines against gonorrhea is shown in Table 1. The status of each of these classes of interventions with respect to product development for gonorrhea and the strengths and limitations of protocols used in these studies are presented below. We also demonstrate the use of the URT infection model to test vaccine efficacy against *N. gonorrhoeae* ascending infections.

Therapeutic Products

Antibiotics

The urgent call for new treatments for gonorrhea has resulted in renewed efforts to develop novel anti-infectives against *N. gonorrhoeae* [35, 36]. Three new antimicrobials have been tested in phase 2 or 3 clinical trials, and several others are under investigation [8, 35], some of which show efficacy in murine LRT infection models (Table 1). Two different murine models have been established for antibiotic testing. In the standard in vivo efficacy protocol utilized by our laboratory, BALB/c mice are infected with *N. gonorrhoeae* for 2 days after which the test compound, vehicle control, and positive control (ceftriaxone [CRO] for CRO^S strains and gentamicin for CRO^R strains) are administered. The clearance rate and bioburden are measured over 8 consecutive days by quantitative vaginal culture [37].

A commercially available model offered by Eurofins Panlabs Taiwan uses estradiol-treated, ovariectomized (Ov-) BALB/c mice to omit the need to stage mice prior to estrogen treatment. In this model, test or control antibiotics are administered 2 hours after vaginal inoculation with *N. gonorrhoeae*, and terminal vaginal washes are cultured at 24 hours and 7 days posttreatment [23]. Advantages to this protocol include reduced cost due to fewer culture time points and the use of water-soluble estradiol as opposed to a slow-release formulation. Other differences between the 2 protocols are the interval between bacterial inoculation and treatment, and the use of intact versus Ov-mice, the latter of which do not exhibit an inflammatory response to *N. gonorrhoeae* infection, most likely due to the absence of progesterone [11]. This latter characteristic may affect the efficacy of bacteriostatic compounds that may need neutrophils or other innate immune effectors to facilitate clearance. In contrast, intact BALB/c mice exhibit an inflammatory response that occurs around 3–5 days after test antibiotics are given. C57BL/6 mice do not have an inflammatory response to *N. gonorrhoeae* infection [11], and could be tested in comparison to evaluate the importance of inflammation-associated effectors in antibiotic-mediated clearance. Tissue pharmacokinetic (PK) differences between estradiol-treated Ov-mice and intact mice should also be considered when comparing data from the 2 models. Ov-mice have a thin vaginal mucosa and take longer to respond to estradiol with respect to thickening of the vaginal epithelia that occurs under estrogen-driven cell proliferation. In contrast, intact mice have a fully stratified columnar epithelium within 4–5 days after estradiol treatment is initiated.

To better establish the murine model used in our laboratory for antibiotic efficacy studies, we recently conducted PK studies for 2 extended-spectrum cephalosporins: CRO and cefixime (CFM) [37]. CFM, which is given orally, was the treatment of choice prior to the emergence of unacceptable levels of resistance. This decision left CRO, which is injectable, as the only remaining recommended first-line therapy for gonorrhea, the doses of which have been increasing as susceptibility to CRO among isolates decreases [9]. We found a clear dose response in CRO and CFM plasma levels in conditioned mice given increasing single doses of either antibiotic, and by identifying the lowest single dose with 100% efficacy against an ESC^S strain, we defined the therapeutic times (time above the free minimum inhibitory concentration [MIC]; fT_{MIC}) as 23.6 hours and 36.8 hours, respectively. PK modeling data accurately predicted the failure of single doses of CRO against the CRO^R strain and were useful in designing an effective multidosing regimen, with the only effective treatment regimen having a predicted therapeutic time of 22.9 hours [37]. The observed PK/PD relationships for CRO in mice reflects that reported in humans, for which in vivo efficacy against a CRO^S strain requires doses that yield an fT_{MIC} in excess of 20–24 hours [38].

PK modeling data are valuable for designing treatment regimens for test compounds that are driven by the fT_{MIC} and for developing adjunctive therapy strategies [39]. Whether PK predictions for clearance of murine LRT infection are the same for URT infection is not known but should be addressed based on human data. For example, levels of different cephalosporins and cephamycin antibiotics in uterine and fallopian tube tissues differed in women who had similar plasma levels of these antibiotics [40]. Furthermore, while plasma ceforanide and cefazolin levels were similar in women undergoing prehysterectomy prophylactic antibiotic therapy, endometrial ceforanide levels exceeded the MIC_{90} for *Escherichia coli* while cefazolin levels were below the MIC_{90} in 50% of myometrial and 67% of endometrial samples [41]. It should be noted that

Table 1. Published Efficacy Trials Using Murine *Neisseria gonorrhoeae* Genital Tract Infection Models for Therapeutic and Prophylactic Products Against Gonorrhea

Product Type	Model Description	Study Outcome	Citation
Antibiotics			
Aminomethyl spectinomycin 1950 and 2324 and spectinomycin	BALB/c mice, LRT	5 daily doses of all compounds significantly cleared infection by a multidrug resistant *N. gonorrhoeae* strain compared to vehicle control; results were comparable to gentamicin	[20]
RPE	BALB/c mice, LRT	RPE (5 doses for 5 days) significantly reduced the number of *N. gonorrhoeae* recovered over time compared to vehicle control; a trend for faster clearance of infection was observed	[21]
Acylaminooxadiazole (trans-translation inhibitor) MBX-4132	BALB/c mice, LRT	A single oral dose of MBX-4132 significantly cleared infection by a multidrug resistant *N. gonorrhoeae* strain compared to vehicle control; results were comparable to gentamicin	[22]
Topoisomerase inhibitor REDX05931	Ovariectomized BALB/c mice, LRT	Dose-dependent decrease in number of *N. gonorrhoeae* recovered at 1 h and 24 h posttreatment compared to vehicle control; highest dose of REDX05931 resulted in no recovery of *N. gonorrhoeae* after 24 h and no or low numbers of *N. gonorrhoeae* after 7 days	[23]
Immunotherapeutics			
fH-IgG1 Fc chimeric protein	BALB/c mice, LRT Hu FH/C4BP Tg BALB/c mice, LRT $C1q^{-/-}$, $C6^{-/-}$ and wild-type C57BL/6 mice, LRT	Daily vaginal delivery of the fH-IgG1 Fc chimera to *N. gonorrhoeae*-infected mice significantly reduced infection duration and bioburden	[24, 25]
C4BP-IgM fusion protein	Hu FH/C4BP Tg BALB/c mice, LRT	Daily vaginal delivery of a C4BP-IgM fusion protein to mice infected with C4BP-binding *N. gonorrhoeae* strains significantly reduced infection duration and bioburden	[26]
Vaginal microbicides			
Acid-buffering agents ACIDFORM PRO-2000, BufferGel	BALB/c mice, LRT	ACIDFORM and PRO-2000 significantly reduced infection rates compared to PBS; BufferGel showed significant activity compared to PBS	[27]
Sulfated and sulfonated polymers CarraGuard [poly]sodium 4-styrene Sulfonate (T-PSS) Ushercell	BALB/c mice, LRT	Significantly reduced infection rates compared to treatment with PBS or vehicle control	[27]
Cellulose acetate phthalate	BALB/c mice, LRT	Significantly reduced infection rates compared to treatment with PBS but not vehicle control when given as a gel or embedded in sponges	[27]
Probiotics			
Lactobacillus crispatus	BALB/c mice, LRT	Precolonization of mice with H_2O_2-producing *L. crispatus* did not alter duration of *N. gonorrhoeae* infection or colonization load	[28]
Neisseria elongata	BALB/c mice, LRT	*N. elongata* significantly cleared *N. gonorrhoeae* infection when coinoculated with *N. gonorrhoeae* into mice; a transformation-deficient *N. gonorrhoeae* mutant was not susceptible to *N. elongata* in vitro or in vivo	[29]
Vaccines			
N. gonorrhoeae OMV-QuilA	BALB/c mice, LRT	IN immunization; accelerated clearance of homologous strain	[30]
N. gonorrhoeae nOMV-microencapsulated IL-12	BALB/c mice, LRT	Vaginal immunization; accelerated clearance of homologous and heterologous strains	[31]
2C7 multiantigenic peptide, (MAP1) - MPL	BALB/c mice, LRT	IP immunization; accelerated clearance and reduced recovery of *N. gonorrhoeae*	[32]
2C7 multi-antigenic octa-peptide, (MAP1) - MPL	BALB/c mice, LRT	IM immunization; accelerated clearance and reduced recovery of *N. gonorrhoeae*	[33]
rMetQ-CpG	BALB/c mice, LRT	SC, IN, IN immunization; accelerated clearance of *N. gonorrhoeae* from lower reproductive tract	[34]
4CMenB	BALB/c mice, LRT	IP or SC immunizations; significantly accelerated clearance of *N. gonorrhoeae* LRT infection and reduced the bioburden.	[15]
4CMenB	hTf-supplemented BALB/c mice, URT	SC immunization; significantly lower percentage of immunized mice with vaginal, endometrial and oviduct cultures following transcervical or vaginal challenge with *N. gonorrhoeae*	This report

Abbreviations: C4BP, C4b-binding protein; fH, factor H; hTf, human transferrin; IgG, immunoglobulin G; IM, intramuscular; IN, intranasal; IP, intraperitoneal; LRT, lower reproductive tract; OMV, outer membrane vesicle; PBS, phosphate-buffered saline; RPE, resorufin pentyl ether; SC, subcutaneous; Tg, transgenic; URT, upper reproductive tract.

the PK of antibiotics may differ during pregnancy and several weeks afterwards due to changes in renal function and changes such as increased uterine weight, blood volume, extracellular fluid, and endometrial blood flow, which may result in poor antibiotic perfusion in the uterus [42]. The effect of the menstrual cycle on antibiotic bioavailability in the reproductive tract has not been investigated.

It is not clear whether antibiotic resistance in *N. gonorrhoeae* has impacted the successful treatment of PID, which is treated with a combination of a broad-spectrum antibiotic plus doxycycline [43]. Published assessments of treatment efficacy against endometrial and fallopian tube infections are limited [43]. Of note, Walker et al [44] compared the efficacy of doxycycline given in combination with cefotetan or cefoxitin in 108 women with acute salpingitis due to *C. trachomatis*, *N. gonorrhoeae*, anaerobes, and facultative aerobes, or a combination thereof. Clinical cure was documented in 51 of 54 women in each treatment group. All 6 patients whose treatment failed had positive cultures for *N. gonorrhoeae* and facultative/anaerobic bacteria, and not for *C. trachomatis*, suggesting the therapies were only 94% effective against *N. gonorrhoeae* PID. This study predates the emergence of *N. gonorrhoeae* strains with reduced susceptibility or resistance to the ESCs, which begs the question of how effective recommended therapies are for *N. gonorrhoeae* PID in this era of antibiotic resistance.

Immunotherapies

A novel strategy being developed by Ram and colleagues makes use of human complement regulatory proteins (factor H [hFH] or C4BP-binding protein [hC4BP]) that bind the *N. gonorrhoeae* surface to downregulate complement activation. Two therapeutics have been developed (Table 1), one of which genetically fuses human IgG1 Fc to the 3 C-terminal domains of hFH that lack complement-inhibiting activity, but bind sialylated *N. gonorrhoeae*. The resultant Fc-mediated complement-dependent bactericidal activity clears murine LRT infection in wild-type and human FH/C4BP transgenic mice, but not in mice that lack C1q or C6 [24, 25]. A similar strategy fuses the *N. gonorrhoeae* binding domains of hC4BP to IgM, which competes with hC4BP and activates the classical pathway, resulting in clearance of LRT infection in hFH/C4BP-expressing transgenic mice [26].

Prophylactic Strategies

Vaginal Microbicides and Multipurpose Prevention Technologies

The use of topically applied broad-spectrum microbicides or anti-infectives is an attractive strategy for preventing sexually transmitted infections in females. Animal models play an important role in the preclinical evaluation of these products, including detection of toxicity by the candidate compound, excipients, or commonly used preservatives that can lead to increased susceptibility to pathogens [45, 46]. Formulated products that exhibited efficacy against gonorrhea in the murine LRT infection model are listed in Table 1.

Several topically applied formulated compounds have been tested in clinical trials with protection against human immunodeficiency virus (HIV) as the primary arm [47]. Improper and inconsistent use reduces the efficacy of this approach against HIV and other sexually transmitted infections; technological advances to improve efficacy include multipurpose prevention technologies that incorporate contraceptives and anti-infectives into slow-release devices such as vaginal rings [47]. Preclinical testing of vaginally applied compounds, however, remains a logical first step before incorporation of a compound into a slow-release device. To test the efficacy of vaginally applied compounds against PID, animal models of ascending infection rather than models based on transcervical inoculation are needed. Examples would be the use of *Chlamydia muridarum* or *Chlamydia cavaie*, which unlike *C. trachomatis* ascend from the vagina to the URT in mice and guinea pigs, respectively [12], and the newly developed *N. gonorrhoeae* URT infection model we describe here.

Probiotics

Much work has been done to develop H_2O_2-producing *Lactobacillus* sp. as a probiotic product, particularly for bacterial vaginosis [48]. Epidemiological studies also show an association between H_2O_2-producing lactobacilli and a reduced risk of gonorrhea [49], although studies with H_2O_2-producing *Lactobacillus crispatus* in the murine LRT infection model did not show inhibition of *N. gonorrhoeae* by *Lactobacillus*-produced H_2O_2 (Table 1) [28]. Recently, the commensal *Neisseria elongata* was shown to kill *N. gonorrhoeae* in vitro and during murine LRT infection [29] (Table 1). The inhibitory factor is *N. elongata* DNA, which is a novel mechanism that could be developed for translational use.

Vaccines

Gonorrhea vaccine development is challenged by a poor understanding of protective immune responses and phase and antigenic variation of several *N. gonorrhoeae* surface molecules. Nonetheless, much recent progress has been made in this area, and several protein subunit and *N. gonorrhoeae* outer membrane vesicle (OMV) vaccines are in the discovery stage of vaccine development [10]. Epidemiological evidence that meningococcal OMV-containing vaccines reduce the risk of gonorrhea has advanced gonorrhea vaccine development towards clinical trials [50]. Candidate vaccines with efficacy against *N. gonorrhoeae* in the murine LRT infection model are listed in Table 1, and include the OMV-based meningococcal vaccine 4CMenB (Bexsero; Glaxo-Smith-Kline) [15].

No vaccine has been tested for efficacy against *N. gonorrhoeae* URT infection, and to explore this question we tested 4CMenB in the hTf-supplemented mouse

model. We first established the infectious dose (ID_{80}) for *N. gonorrhoeae* strain F62 when inoculated transcervically into mice equal to the age of challenge after immunization. Based on these results, we chose a dose of 10^5 CFU and the day 7 time point to test the efficacy of 4CMenB against URT infection (Supplementary Figures 1 and 2). Groups of BALB/c mice were immunized with 4CMenB or alum subcutaneously as described [15], and challenged with *N. gonorrhoeae* transcervically or vaginally (Supplementary Methods). As previously observed [15], a robust serum antibody response that cross-reacted with F62 OMVs was detected (Supplementary Figure 3), and significant protection against LRT infection occurred based on the shorter duration and lower number of *N. gonorrhoeae* recovered from vaginal swabs over time from 4CMenB-immunized mice compared to the alum control group (Figure 2). URT cultures also revealed a significant reduction in the bioburden and the percentage of mice with culture-positive endometrial and oviduct tissue, in 4CMenB-vaccinated mice compared to alum controls. Finally, the route of bacterial challenge did not impact the vaccine efficacy as measured by these parameters (Figure 3).

We conclude that immunization with 4CMenB accelerates clearance of *N. gonorrhoeae* from both the lower and upper reproductive tracts in a murine model that better mimics human infection by providing a host-restricted iron source. The similar levels of vaccine-mediated protection in mice that were challenged transcervically versus vaginally demonstrates that vaginal challenge, which better mimics ascending infection in humans, can be used in vaccine challenge studies. The demonstrated efficacy of 4CMenB against *N. gonorrhoeae* ascending infection is encouraging; whether this finding is predictive of vaccine efficacy in humans is not yet known.

SUMMARY AND RESEARCH GAPS

Research on new therapeutic and prophylactic interventions against gonorrhea has grown in response to the threat of untreatable gonorrhea. Murine infection models have accelerated gonorrhea product development and a new model of

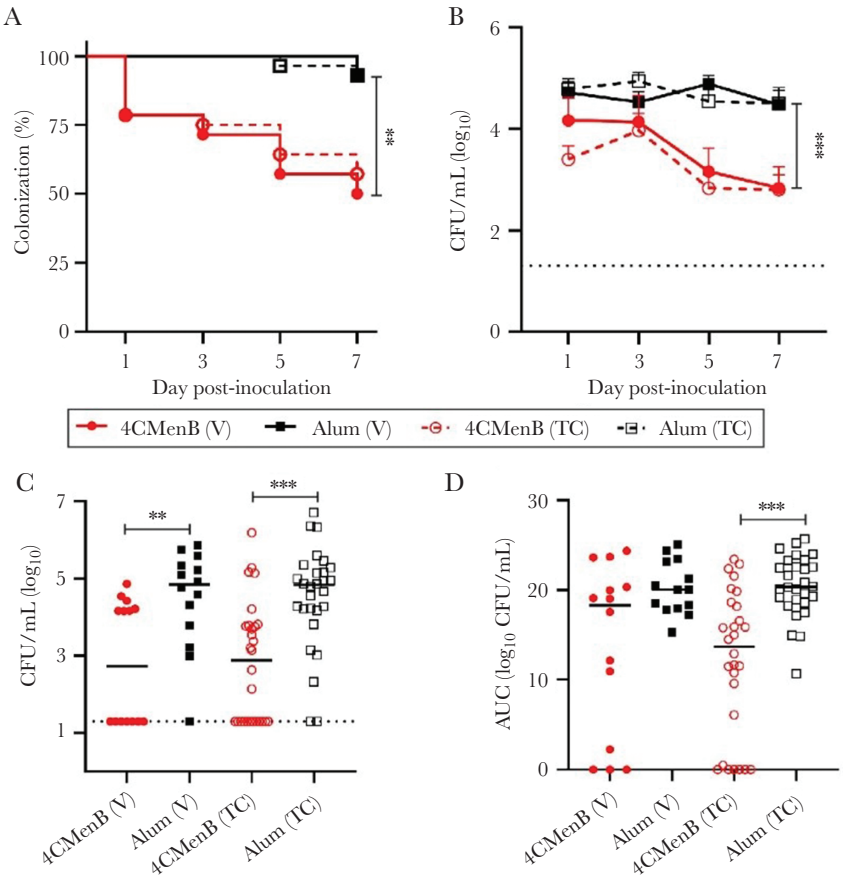

Figure 2. 4CMenB accelerates clearance and reduces the bioburden in the lower reproductive tract in hTf-supplemented mice following vaginal or transcervical challenge. Mice were immunized 3 weeks apart with 250-µL doses of 4CMenB (red) or alum (black) by the subcutaneous route. Three weeks after the final immunization, mice were supplemented with hTf and challenged with *Neisseria gonorrhoeae* strain F62 vaginally (solid symbols, n = 14 mice/group) or transcervically (open symbols, n = 28 mice/group). *A*, Percentage of culture-positive mice over time. *B*, Average CFU/mL of a single vaginal swab suspension. *C*, CFU/mL recovered from individual mice on day 7 postchallenge. *D*, Total bioburden over 7 days expressed as area under the curve. ** $P < .01$, *** $P < .0001$. Abbreviations: CFU, colony-forming units; hTf, human transferrin; TC, transcervical; V, vaginal.

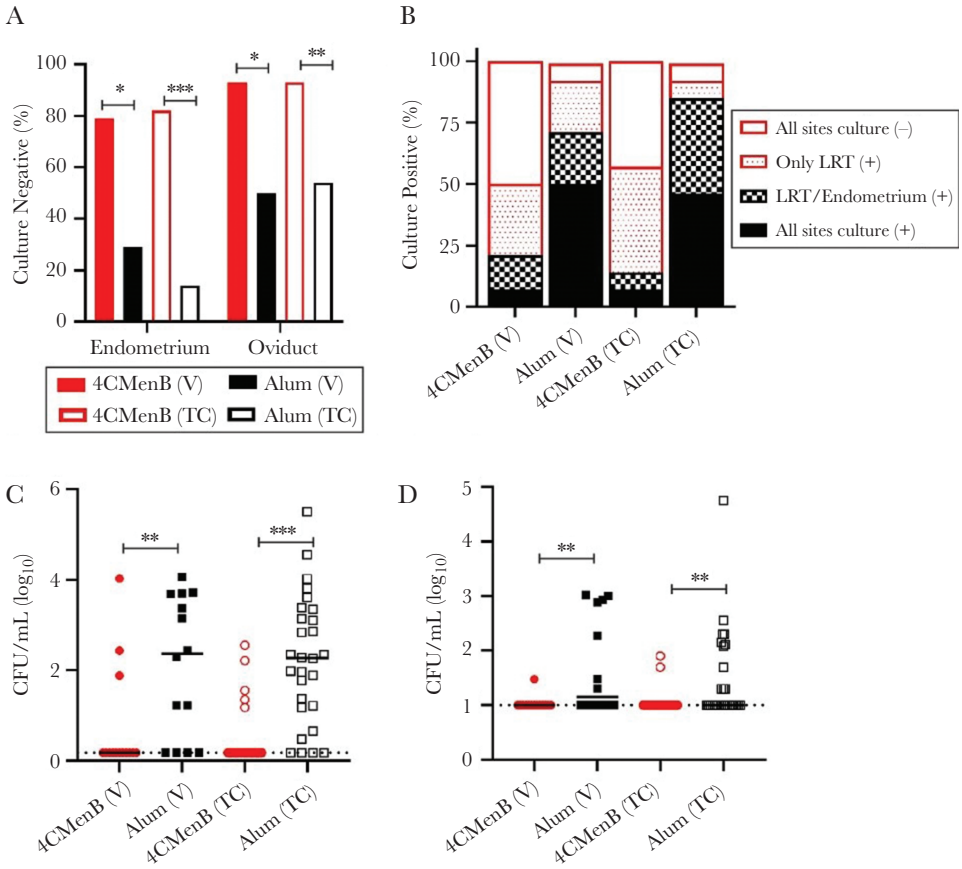

Figure 3. 4CMenB demonstrates in vivo efficacy against endometrial and oviduct colonization in hTf-supplemented mice. Mice were immunized 3 weeks apart with 250-µL doses of 4CMenB (red) or alum (black) by the subcutaneous route. Three weeks after the final immunization, mice were supplemented with hTf and challenged with *Neisseria gonorrhoeae* strain F62 vaginally (n = 14 mice/group) or transcervically (n = 28 mice/group). *A*, Percentage of mice that had *N. gonorrhoeae* recovered from endometrial or oviduct tissue on day 7 postinoculation. *B*, Percentage of mice with F62 recovered at all 3 sites (LRT, endometrium, and oviducts; solid black), LRT and endometrium only (black and white checkered), LRT only (red stippled), or culture negative (white) on day 7 postinoculation. CFU per mL recovered on day 7 postinoculation from (*C*) endometrial scrapings and (*D*) oviduct tissue. * $P < .05$, ** $P < .01$, *** $P < .0001$. Abbreviations: CFU, colony-forming units; hTf, human transferrin; LRT, lower reproductive tract.

N. gonorrhoeae URT infection should allow in vivo testing of products against PID. Characterization of immune responses and infection-induced histopathology in this model is on-going. Continued refinement of this model by alleviating additional host restrictions may better mimic PID in humans and yield interesting insights on site-specific differences in host factors. Other research gaps include the absence of *N. gonorrhoeae* rectal and pharyngeal animal infection models for translational research and *N. gonorrhoeae/C. trachomatis* URT coinfection models to examine product efficacy against *N. gonorrhoeae* in the context of *N. gonorrhoeae/C. trachomatis* coinfection for developing dually active interventions.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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