Strategies for syphilis vaccine development

Lorenzo Giacani[1,2]

ABSTRACT

Research to identify a syphilis vaccine began shortly after the isolation of the first *Treponema pallidum* subspecies *pallidum* (*T. pallidum*) strain in 1912 by Nichols and Hough and the identification of several possible animal models for the infection, with the rabbit being the best one. During the century following *T. pallidum* isolation, none of the numerous immunization/challenge experiments performed with preparations ranging from whole-inactivated *T. pallidum* cells to recombinant proteins yielded an effective vaccine, and the search for a vaccine languished. Recently, however, scientific communities have experienced a resurgence in interest in developing a syphilis vaccine due to

1. the awareness that syphilis constitutes a tremendous burden for maternal health, particularly in low- and middle-income nations;
2. the improved understanding of the immunological processes leading to pathogen clearance during natural infection and of the mechanisms this pathogen developed to persist in the host;
3. the availability of a near-complete list of *T. pallidum* genes encoding putative surface-exposed antigens, which represent the most likely vaccine candidates; and, last but not least,
4. the effort made to expand the knowledge on the genetic and antigenic diversity of these vaccine candidates in strains circulating worldwide.

Thus far, the most recent vaccine designs based on a subset of the pathogen's surface-exposed antigens have provided immunized rabbits with a significant but incomplete protection upon infectious challenge. Nonetheless, the outcomes of these experiments help investigators refine strategies to achieve a formulation with the highest chances of moving from preclinical experimental settings to clinical trials. This editorial focuses on a subset of the strategies currently believed to be essential for vaccine development, namely, the improvement of our still limited understanding of the genomic diversity in *T. pallidum* strains from diverse geographical locations through the collection and isolation of modern syphilis strains and the identification of protective epitopes in potential vaccine targets by evaluating the ability of monoclonal antibodies to bind the target antigen and facilitate pathogen clearance. The use of genetic engineering of the syphilis spirochete to identify target surface proteins with an essential or near-essential role in *T. pallidum* biology to target in immunization/challenge experiments is also discussed.

Keywords: Syphilis. Vaccine development. *Treponema pallidum*. Strategies.

INTRODUCTION

Syphilis remains a significant concern for global health as it causes significant morbidity and mortality worldwide. The World Health Organization (WHO) estimated that global incidence of syphilis ranges between 5.6 and 11 million cases every year, while global prevalence is between 18 and 36 million cases[1,2]. Although most cases occur in low- and middle-income countries, syphilis rates have been on the rise for years in high-income nations, primarily among men who have sex with men (MSM), even though epidemiological trends show that the gap between MSM and the heterosexual population is reducing[3-8]. It has also been calculated that about 1.4 million pregnant women worldwide acquire syphilis in a year. Congenital syphilis (CS), due to the ability of the syphilis spirochete, *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), to cross the placenta, causes an estimated ~300,000 annual cases of fetal loss or stillbirth and ~215,000 infants born prematurely and/or with clinical evidence of infection[9-11]. In Brazil, according to Dos Santos et al.[12], the rate of acquired syphilis in 2017 was 81.4 cases per 100,000 population, representing a 561% increase compared to the reported rate of 12.3 cases per 100,000 population in 2011, while CS rates went from 2 cases per 1,000 live births in 2007 to staggering 9 cases per 1,000 live births in 2017, corresponding to a 338% increase. In the United States, the number of syphilis cases in 2020 was the highest since 2000, confirming the resurgence trend seen in the past two decades[13]. Public health initiatives to eliminate syphilis and CS led in the recent past by the Centers for Disease Control and Prevention (CDC) and WHO[14,15] have indeed contributed to reducing syphilis incidence but have not attained their intended elimination goals. The availability of an effective vaccine would significantly help global disease control.

In vitro isolation modern *T. pallidum* strains

T. pallidum strain isolation is still performed through rabbit intratesticular injection with samples as diverse as blood, lesion exudates, cerebrospinal fluid (CSF), kidney and liver biopsies, amniotic fluid, and neonatal serum[16-20]. Injections are generally performed using fresh samples to avoid pathogen cell loss due to freezing/thawing and maximize the chances of successful isolation, even though recent work demonstrated the rabbit-based isolation of new strains from cryopreserved lesion exudates[21]. The possibility of freezing samples reduces the need for the clinical collection sites to be located near a research facility capable of housing rabbits in a Biosafety Level 2 environment.

However, strain isolation using rabbits has several major disadvantages, starting with the significant cost associated with animal purchase and husbandry, veterinary care, and the need for trained personnel for rabbit monitoring. Furthermore, the animal immune response to *T. pallidum* may result in pathogen clearance if strain harvest is not performed as soon as the rabbit develops orchitis or, in the absence of it, immediately after seroconversion. In the latter case, additional passages in the animal are generally necessary to expand the strain further.

An alternative to sample inoculation into rabbits is offered by the cell culture system suitable for continuous *in vitro* propagation of *T. pallidum* used in our laboratory for testing of antibiotics to broaden the repertoire of therapeutic options available for syphilis and to

[1]University of Washington, Division of Allergy and Infectious Diseases, Department of Medicine – Seattle, United States of America.
[2]University of Washington, Department of Global Health – Seattle, United States of America.

generate *T. pallidum* knockout mutants[22-26]. This system has yet to be applied to *T. pallidum* isolation from clinical samples. Recently, however, we demonstrated that strain isolation is possible using this cultivation system and treponemes obtained after performing needle aspiration of experimental primary lesions, using either fresh or frozen/thawed specimens[27]. Although preliminary, our study suggests that combining the cultivation system for *T. pallidum* and properly collected and stored samples from a patient could effectively lead to isolating new *T. pallidum* strains without the need for rabbit inoculation. For laboratories already culturing *T. pallidum*, strain isolation using this approach would require minimal additional time and labor. Laboratories with tissue culture capabilities could implement this technique after acquiring a tri-gas incubator and a steady supply of nitrogen to achieve a microaerophilic environment that prevents *T. pallidum* from dying of oxidative stress. Soon, we will apply this procedure to clinical specimens, such as aspirates of secondary disseminated lesions collected after surface disinfection, to avoid sample contamination with the skin flora.

Given that to date only a handful of laboratories worldwide have implemented the *in vitro* cultivation system for *T. pallidum* and that strains that are likely more valuable for vaccine development are those circulating in South America, Sub-Saharan Africa, and South-East Asia, it would be desirable that key laboratories in countries in these regions develop the ability to cultivate the syphilis spirochete. This will create a very valuable repository of strains that could be used for genome sequencing and to challenge immunized animals in preclinical studies to evaluate the degree of protection a vaccine formulation achieves against diverse strains.

Genetic diversity of *T. pallidum* and vaccine development

Syphilis reverse vaccinology began with the elucidation of the Nichols strain genome in 1998[28]. Examples of vaccine candidates that were identified and tested thanks to the application of bioinformatics analyses to find putative surface-exposed antigens include conserved regions of the *T. pallidum* repeat antigen K (TprK; encoded by the *tp0897* gene), the conserved amino-terminal portion of the TprC-I antigens (encoded by the *tp0117*, *tp0131*, *tp0316*, and *tp0620* genes), and the Tp0751 adhesin. Immunization with Tpr-based peptides was shown to attenuate early lesion development and reduce the treponemal burden at injection sites following challenge[29-31], while Tp0751-immunized animals had significantly reduced *T. pallidum* dissemination to distant organs after infection[31,32]. Vaccine formulations based on Tpr and Tp0751 antigens constitute the hub of a two-pronged approach to vaccine development that exploits the ability of these antigens to attenuate early manifestations (hence reducing the chances of transmission) and inhibit dissemination, which is associated with the most serious manifestations of the infection[31]. At the same time, these early works highlighted the importance of sequencing genomes of syphilis strains to define the overall degree of genetic diversity in these genes. As complete and partial genomes from *T. pallidum* strains kept accumulating[33-35], it became clear that substantial diversity characterizes many *T. pallidum* genes-encoding surface-exposed proteins. These genes have often been challenging to elucidate through whole-genome sequencing (WGS) because of the presence of repetitive sequences, particularly in the Tpr-encoding genes. Several ongoing initiatives are addressing the need for more (and complete) *T. pallidum* genomes. Genomes can now be sequenced directly from patient samples, thanks to the use of enrichment probes that can specifically capture the pathogen's DNA and separate it from the host genome[33] or specific genome amplification before high-throughput sequencing[36]. These approaches not only eliminate the need for *in vitro* or rabbit propagation of a strain (unless an isolate is wanted) but also make possible the collection of samples from the most diverse geographical areas, particularly those where syphilis is endemic. Indeed, most *T. pallidum* genomes available today originate from Europe, North America, and Australia, and sampling in African, South American, and Asian countries must be increased. Furthermore, in addition to collecting swabs of syphilis genital and anal lesions, samples such as oral swabs (even in the absence of an obvious lesion) or saliva[37,38] have proven to be relatively good sources of treponemal cells for sequencing. These findings expand the range of collectible samples where enough *T. pallidum* DNA can be present (200–1,000 genomes)[33] to perform WGS. Upon performing WGS, deposition of reads, assembled genomes, and non-identifying patient-associated clinical data in public repositories will enable more researchers to participate in vaccine development. Although it is possible that syphilis vaccine will need to be tailored to the genetic pedigree of the strains circulating in an area, evidence that there are only two clades (Nichols and SS14) of this pathogen circulating worldwide and that *T. pallidum* strains continue to share more than 99% of their genomic identity (except for the hypervariable *tprK* gene) will greatly facilitate vaccine research.

The accumulation of multiple genome sequences will also benefit the *in silico* structural analyses of the putative vaccine candidates to identify which regions of the antigen have the highest probability of being surface exposed. With one exception[39], no conclusive experimental data exist on the structure of *T. pallidum* vaccine candidates. Although for many of these molecules the level of homology with other bacterial proteins is sufficient to generate high confidence models using bioinformatic tools[40], there is an ongoing debate concerning the structure of Tpr antigens, mainly because the structure that is inferred from functional assays[41,42] differs significantly from that generated *in silico*[43]. Therefore, refining the structural models for all *T. pallidum* putative vaccine candidates is a pivotal step for vaccine development.

Monoclonal antibodies to pinpoint protective epitopes

Although not all, most of the antigens currently considered as vaccine candidates for syphilis are surface-exposed integral β-barrel outer membrane proteins (OMPs). These proteins range in size from 24 kDa for the 8-stranded β-barrel *T. pallidum* OmpW homolog Tp0126[44] to ~112 kDa for the Tp0515 protein, known as the *T. pallidum* LptD homolog. Structural predictions suggest that only a small portion of all these OMPs is surface exposed, mainly corresponding to the loops that connect adjacent β-sheets embedded in the outer membrane, which are necessarily exposed at the host-pathogen interface. It is plausible to assume that protective epitopes associated with these proteins would map to a subset of these loops that represent the most likely targets for opsonic

antibodies. A current strategy to identify protective epitopes to use instead of full-length *T. pallidum* OMPs, which are difficult to express, purify, and maintain in aqueous solutions, involves the isolation of monoclonal antibodies (mAbs) following animal immunization with a vaccine candidate and their testing using *in vitro* phagocytosis assays to assess their ability to opsonize *T. pallidum*. Upon the identification of a sufficient number of such epitopes, chimeric constructs based on protein scaffolding (e.g., viral-like particles) or chimeric concatemers that are amenable to large-scale production could provide a vaccine design able to transition into clinical trials.

Genetic engineering to identify essential surface antigens

We recently reported the first successful genetic engineering experiment of *T. pallidum*. In this initial work, we eliminated a *T. pallidum* pseudogene to provide a proof of concept that knockout mutants could be derived. Our many attempts to ablate the OMP-encoding *tprK* open reading frame, however, were constantly unsuccessful, which led to the hypothesis that *tprK* is an essential gene of the syphilis spirochete. This hypothesis is also indirectly supported by the evidence that high-throughput *tprK* sequencing never yielded a variant carrying an early termination due to the introduction of a stop codon or a frameshift mutation, despite the continuous recombination events that occur to create variability in this gene[45-47]. A vaccine design based on a surface-exposed antigen that mediates crucial functions in *T. pallidum* biology could be more valuable than a design based on a nonessential gene, whose function could be redundant. Work to define the "essential" repertoire of surface-exposed antigens and their function using functional genomics approaches might also accelerate vaccine development. To this end, the development of novel genetic tools such as inducible systems to attain knockdown mutants and transposon mutagenesis is highly desirable.

Concluding remarks

This short article highlighted some of the strategies currently seen as pivotal for syphilis vaccine development. Additional strategies and topics of equal importance that are not discussed here include

1. the choice of adjuvant, which necessarily must be approved for human use and foster a Th1 response to facilitate phagocytosis of opsonized *T. pallidum* cells[48-52];
2. platforms to deliver the protective antigens or epitopes, including RNA-based vaccines;
3. the level of protection that must be associated with vaccination;
4. the possibility that vaccination might interfere with some treponemal diagnostic tests; and
5. populations that should be given priority for vaccination.

Although syphilis testing and treatment are available and affordable, and the threat of antibiotic resistance is only marginal for the syphilis spirochete, syphilis control has remained challenging, and the years of life lost due to congenital transmission are significant[53]. A vaccine that can reduce syphilis incidence, particularly congenital infection, could make a substantial difference in public health and, in particular, maternal health.

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Conflict of interest

The author declares no conflicts of interest.

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Address for correspondence
LORENZO GIACANI
Harborview Medical Center 325 – 9th Ave – University of Washington
Seattle WA 98105, United States of America
Postal Code: 98104
E-mail: giacal@u.washington.edu
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