JCI The Journal of Clinical Investigation

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J Clin Invest. 2011;121(12):4584-4592. https://doi.org/10.1172/JCI57173.

Review Series

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Syphilis: using modern approaches to understand an old disease

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Syphilis is a fascinating and perplexing infection, with protean clinical manifestations and both diagnostic and management ambiguities. *Treponema pallidum* subsp. *pallidum*, the agent of syphilis, is challenging to study in part because it cannot be cultured or genetically manipulated. Here, we review recent progress in the application of modern molecular techniques to understanding the biological basis of this multistage disease and to the development of new tools for diagnosis, for predicting efficacy of treatment with alternative antibiotics, and for studying the transmission of infection through population networks.

Treponema pallidum — the causative agent

Treponema pallidum subsp. *pallidum* (*T. pallidum*) is a spirochete that, while able to persist for decades in the mammalian host, is extremely fragile ex vivo. It can be cultured only transiently in vitro in rabbit epithelial cells and must be propagated in rabbits to maintain strains for laboratory examination. Consequently, genetic manipulation of this organism is not yet possible. In addition, the limited repertoire of immunological reagents available for rabbits adds to the challenges of studying immune responses to this organism.

Analysis of the *T. pallidum* genome reveals a notable absence of metabolic capability (1). *T. pallidum* has the genes encoding enzymes involved in glycolysis but lacks those related to the tricarboxylic acid cycle and the electron transport system. Similarly, it lacks most genes associated with synthesis of nucleotides, amino acids, and lipids. The genome encodes for multiple transport proteins, indicating that it may rely heavily on scavenging required compounds from its human host (1).

Despite the difficulties in working with *T. pallidum*, much has been learned about the molecular basis of syphilis pathogenesis. For each stage of infection, we review the most closely related steps of pathogenesis.

The natural history of syphilis

Primary syphilis — transmission, adhesion, local host immune response. *T. pallidum* is usually transmitted sexually through microabrasions in mucosal membranes or skin and rapidly enters the bloodstream to disseminate to other tissues. *T. pallidum* can be identified by PCR in the bloodstream of patients with all stages of syphilis, and the quantity of treponemes in blood is highest during early syphilis (2, 3). Individuals with lesions of early syphilis are most likely to transmit *T. pallidum*. While the risk of infection in exposed individuals is approximately 30% (range, 10%–80%) (4–6), inoculation studies with the Nichols strain of *T. pallidum* suggest that the intradermal ID_{50} is only 57 organisms (7). The natural history of *T. pallidum* infection is summarized in Figure 1.

To establish infection, *T. pallidum* adheres to epithelial cells and extracellular matrix components of the skin and mucosa. Several *T. pallidum* proteins mediate adherence, including TP0155 and TP0483, which bind to matrix fibronectin and to both soluble and matrix forms of fibronectin, respectively (8). TP0136, a protein identified by reactivity with primary human syphilitic sera (9), also binds to human fibronectin (10). TP0751 can bind to laminin, which has the highest concentration in the basement membrane (11–13), and to fibrinogen, a blood-clotting protein that functions to contain bacteria (13). TP0751 can also degrade laminin and fibrinogen using its zinc-dependent protease domain, which may be a means by which *T. pallidum* disseminates to surrounding tissues and the bloodstream (13).

T. pallidum replicates at the site of initial inoculation, dividing once every 30–33 hours (14, 15), inducing a local inflammatory response that results in a painless chancre approximately 3–6 weeks after initial infection. In each chancre, proliferating spirochetes are surrounded by immune cells, including $CD4^+$ and $CD8^+$ T cells, plasma cells, and macrophages, which produce IL-2 and IFN-γ cytokines, indicating a Th1-skewed response (16–21). Tissue necrosis and ulceration occur due to small vessel vasculitis, and trafficking immune cells cause a non-tender regional lymphadenopathy. Within 3–8 weeks, the chancre heals, indicating clearance of *T. pallidum* locally. However, by this time, *T. pallidum* has spread systemically to multiple tissues and organs, setting the stage for secondary syphilis.

Secondary syphilis — motility, systemic host immune response, diagnosis, systemic spread. *T. pallidum* propels itself using a corkscrew-like mechanism by rotating around its longitudinal axis, using endoflagella contained within the periplasmic space between the cytoplasmic membrane and the outer membrane (22–24). *T. pallidum* traverses the tight junctions between endothelial cells (25, 26) to enter the perivascular spaces, where large numbers of treponemes and immune cells accumulate. Based on electron microscopy images of secondary syphilis skin lesions, *T. pallidum* may also use transcytosis to spread through the endothelium (27). *T. pallidum* can induce the production of MMP-1 (28), which degrades collagen and may facilitate access to and egress from the bloodstream, resulting in systemic spread.

Usually within 3 months of infection, symptoms of secondary syphilis appear. The most common clinical manifestation is a disseminated maculopapular rash. Additional symptoms may include malaise, weight loss, muscle aches, generalized lymphadenopathy, patchy alopecia, meningitis, ocular inflammation, mucous patches (localized inflammation of mucosal tissues in

Conflict of interest: Sheila A. Lukehart is receiving research support from Cempra Pharmaceuticals.

Citation for this article: *J Clin Invest.* 2011;121(12):4584–4592. doi:10.1172/JCI57173.

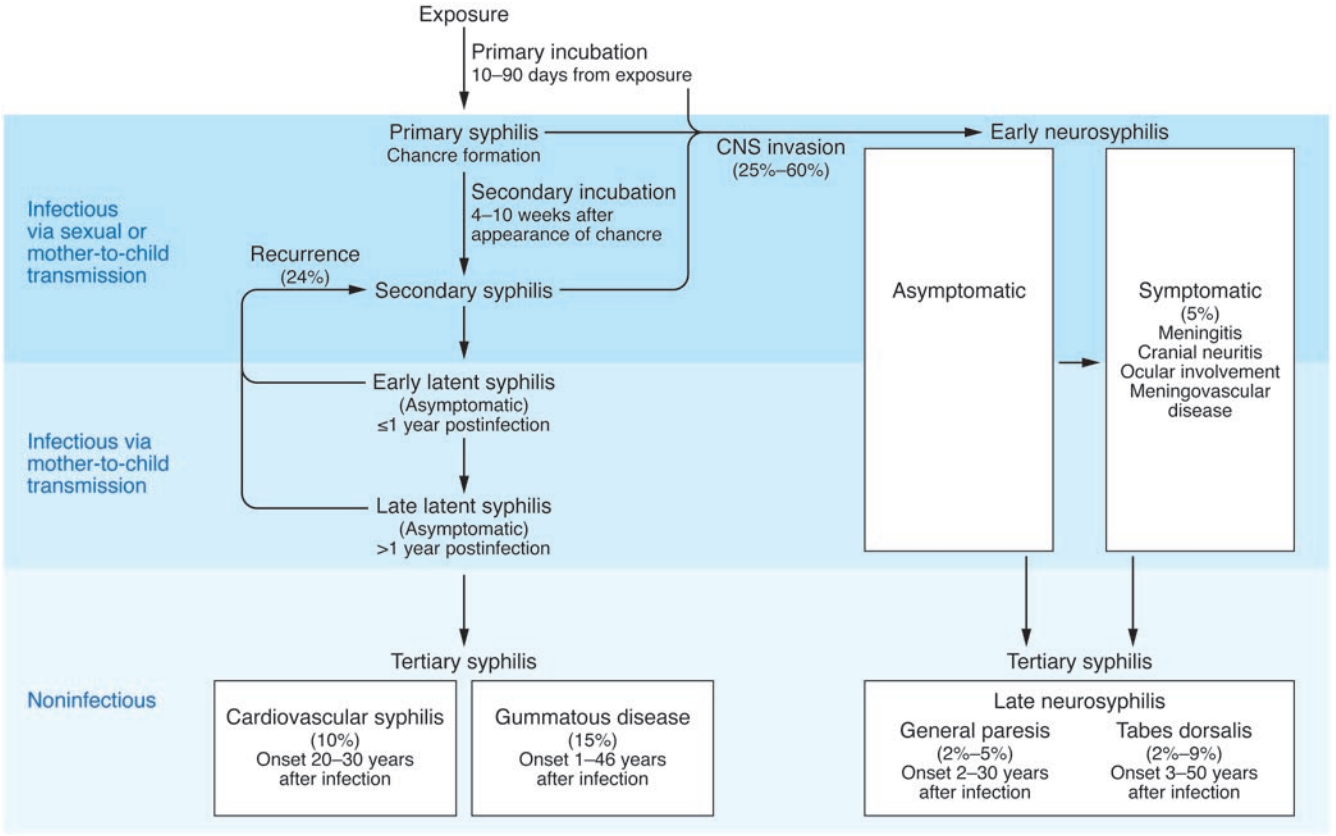

Figure 1
The natural history of untreated syphilis in immunocompetent individuals. Percentages of individuals developing to specific stages as well as time intervals are based on information in references 137, 146, and 147 (based on data from refs. 146–148).

the oral cavity and genitals), hepatitis, and gastric dysmotility (29, 30), reflecting *T. pallidum* invasion and the resulting immune cell infiltration of these tissues.

Although *T. pallidum* has structural similarities to classical Gram-negative bacteria, such as having outer and inner membranes and a periplasmic space, it lacks lipopolysaccharide, a potent proinflammatory glycolipid, and does not produce any known toxic proteins. Therefore, most of the symptoms and tissue damage related to syphilis are due to activation of the host inflammatory and immune responses. Exposure to whole *T. pallidum* and its lipoprotein TpN47 can induce expression of the adhesion molecules ICAM-1, VCAM-1, and E-selectin (25, 31), which are important in adhesion of immune cells to vascular endothelium for migration into sites of infected tissue. Patients with secondary syphilis have a local immune response in the skin, consisting of monocytes, macrophages, $CD4^+$ and $CD8^+$ T cells, and DCs (32–34). This proinflammatory response is due to the lipid moiety contained on the many lipoproteins of *T. pallidum* (35, 36). Early syphilis lesions transiently contain scant polymorphonuclear leukocytes (PMNs) (37), and injection of recombinant *T. pallidum* lipoproteins TpN17 (TP0435) and TpN47 (TP0574) into the dermis can induce transient infiltration of PMNs (35, 38), as well as a local enrichment of monocytes, macrophages, memory T cells, and DCs (38, 39).

The interaction of TpN47 with TLR2 on the surface of macrophages induces the production of IL-12 (40). When DCs are exposed to *T. pallidum* or purified TpN47, they release inflammatory cytokines, such as IL-1β, IL-6, IL-12, and TNF-α (41), and express maturation markers, including CD54, CD83, and MHC class II (38, 39, 41–43). *T. pallidum* lipoproteins also stimulate macrophages and DCs by binding CD14, which transmits activation signals through the TLR1/TLR2 heterodimer (40, 44, 45). The miniferritin TpF1 stimulates human monocytes to release IL-10 and TGF-β, which are key cytokines that promote Treg differentiation and may also allow long-term persistence of *T. pallidum* in the human host (46). $CD8^+$ T cells present in the skin colocalize with staining for IFN-γ, perforin, and granzyme B (32), as well as IL-17 (34). Studies of lesional skin samples from patients with secondary syphilis show that plasma cells appear later (34).

The humoral immune response produces antibodies that function in opsonization (47) and complement-mediated immobilization or neutralization (48, 49). Macrophages clear *T. pallidum* from sites of infection through phagocytosis of opsonized organism (47, 50) using both IgG and IgM antibodies (51, 52). A study using an array of 882 polypeptides predicted to be in the *T. pallidum* proteome identified 106 proteins that could induce a detectable antibody response (53). Two *T. pallidum* lipoproteins that induce high titers of antibodies are TpN17 and TpN47 (54–57), both of which are used in new enzyme and chemiluminescence immunoassay (EIA/CIA) serological tests for syphilis. Genome analysis of *T. pallidum* predicts that there are as many as 22 putative lipoproteins in the organism (1).

Measurement of antibodies is important for screening and diagnosis of syphilis. Two categories of antibodies — termed

"non-treponemal," which are directed against phospholipids, and "treponemal," which are directed against *T. pallidum* polypeptides — have been used for this purpose. The non-treponemal antibodies are detected by the rapid plasma reagin (RPR) test, the Venereal Disease Research Laboratory (VDRL) test, and the toluidine red unheated serum test (TRUST). Treponemal antibodies are detected by immunofluorescence in the fluorescent treponemal antibody-absorbed (FTA-ABS) test or by agglutination in the *T. pallidum* hemagglutination (TPHA) or *T. pallidum* particle agglutination (TP-PA) test.

Traditionally, *T. pallidum* infection has been diagnosed using a non-treponemal screening test, with reactive results confirmed using treponemal serologic tests. Rapid point-of-care tests (58), EIAs (59), and CIAs (60, 61) have been developed that detect anti-treponemal IgM and IgG antibodies, usually to recombinant *T. pallidum* proteins. The EIA/CIA tests can be automated, which has led some large laboratories in the United States to use revised syphilis screening algorithms beginning with a treponemal test. Positive tests are subsequently confirmed with a non-treponemal test, and discordant sera must be retested with a traditional treponemal test. One disadvantage of these newer tests is that they cannot distinguish between recent and remote syphilis, or between treated and untreated infection. In addition, because the new EIA/CIA tests are more sensitive than the fluorescence or agglutination tests, many sera that are reactive in the EIA/CIA tests are nonreactive in the confirmatory non-treponemal tests, particularly in low-risk populations such as pregnant women (62). These results have led to concerns about the specificity of the antigens used in these tests for syphilis infection. Indeed, a published study (63) reports that persons with periodontal disease carry oral treponemes that can be detected with monoclonal antibodies to the same TpN47 antigen used in many EIA/CIA tests. Persons with periodontal disease have detectable antibodies to this and other *T. pallidum* antigens. Additional related concerns regarding screening with automated treponemal tests include increased health care and public health costs caused by follow-up of unconfirmed EIA/CIA screening.

In the rabbit model, despite the presence of functional antibodies, passive immunization with immune serum fails to provide protective immunity against *T. pallidum* infection (64), demonstrating that cellular immunity is also required for protection. The link between humoral and cellular immunity in humans is indicated in studies of human PBMCs exposed in vitro to *T. pallidum*: internalization of treponemes by macrophages is facilitated by human syphilitic serum, leading to secretion of TNF-α, IL-6, and IL-1β by macrophages and resulting in IFN-γ production by NK cells, NK T cells, and T cells (65). After most *T. pallidum* have been cleared in the rabbit infection model, a few organisms remain and are able to resist macrophage ingestion even in the presence of immune serum (66), suggesting this subpopulation may be able to avoid opsonic antibody, persisting to cause latent or later stages of infection.

Early CNS invasion and neurological involvement. While CNS involvement of syphilis infection is classically considered as the tertiary stage of infection, invasion of the nervous system by *T. pallidum* and neurosyphilis occur within days or weeks of infection. Neurosyphilis is diagnosed by clinical manifestations (see below) as well as by cerebrospinal fluid (CSF) abnormalities such as elevated white blood cell (wbc) count, elevated CSF protein, or reactive CSF-VDRL test. Many affected patients may be asymptomatic in spite of the presence of abnormal CSF.

While most patients with CNS infection appear to control or clear CNS infection by *T. pallidum*, the factors underlying the subsequent development of symptomatic neurosyphilis in some patients are not known. Symptomatic and asymptomatic neurosyphilis are more common when the serum RPR titer is 1:32 or greater regardless of HIV status, or in HIV-infected individuals when the peripheral blood $CD4^+$ T cell count is 350 or fewer cells/μl (67–70).

Symptoms of early neurosyphilis may occur during or following the primary or secondary stages of syphilis, especially in HIV-infected individuals (71) and include meningitis (headache, fever, and stiff neck), visual changes (blurred vision, loss of vision, photophobia, and other signs of ocular inflammation), hearing changes or loss, and facial weakness. Some studies indicate that HIV-infected individuals may have more significant symptoms of neurosyphilis (72), and HIV-infected individuals who have symptomatic neurosyphilis have more severe CSF abnormalities (70, 73). Treatment of HIV-infected patients with antiretroviral therapy (ART) decreases the chance of developing neurosyphilis by 65% (70), suggesting that immune reconstitution with ART may result in an improved local immune response against *T. pallidum* and better control of the infection.

Diagnosis of asymptomatic neurosyphilis is complicated by the fact that none of the CSF measures currently used is very sensitive (CSF-VDRL) or specific (CSF wbc, CSF protein). In addition, concurrent HIV infection itself may cause an elevated CSF wbc count or protein concentration. A recently described adjunct diagnostic marker for neurosyphilis is the B cell chemokine CXCL13 (74).

Latent and tertiary syphilis — antigenic variation and persistence. Despite a host immune response that results in effective local clearance of *T. pallidum* from primary and secondary lesions, treponemes persist in many tissues without causing clinical signs or symptoms. This is termed the latent stage. While *T. pallidum* may seed the bloodstream intermittently during the latent stage and thus infect a developing fetus during pregnancy, sexual transmission is rare.

How can *T. pallidum* "escape" immune detection to cause persistent and later stages of infection? Recent evidence suggests that *T. pallidum* organisms may be able to evade the acquired immune response by antigenic variation of bacterial surface proteins, consistent with the resistance to phagocytosis of those select treponemes that survive bacterial clearance of the primary lesion (51). Antigenic variation is well described in related spirochetes that cause relapsing fever (*Borrelia hermsii*) and Lyme disease (*Borrelia burgdorferi*), each of which also has a multistage clinical course (75, 76).

Although *T. pallidum* has few integral outer membrane proteins (23, 24, 77, 78), bioinformatic approaches have identified several candidates, including members of the family of 12 *T. pallidum* repeat (Tpr) proteins (79, 80). Among Tpr family members, TprK is the best studied. A strong antibody and T cell immune response is elicited against TprK (81, 82), and immunization with recombinant TprK provides partial immunity against infectious challenge (80, 83). Antibodies raised against recombinant TprK can opsonize *T. pallidum* for phagocytosis by macrophages in vitro (80). TprK sequences differ substantially between and within individual strains (84–86), and this diversity is localized to seven discrete variable regions of the protein, which are predicted to be surface exposed. TprK sequence diversity accumulates following development of acquired immunity in the rabbit model (86, 87). Molecular studies of *TprK* show that new variants arise by segmental gene conversion, with the new sequences coming from a large repertoire of "donor sites" located elsewhere on the chromosome

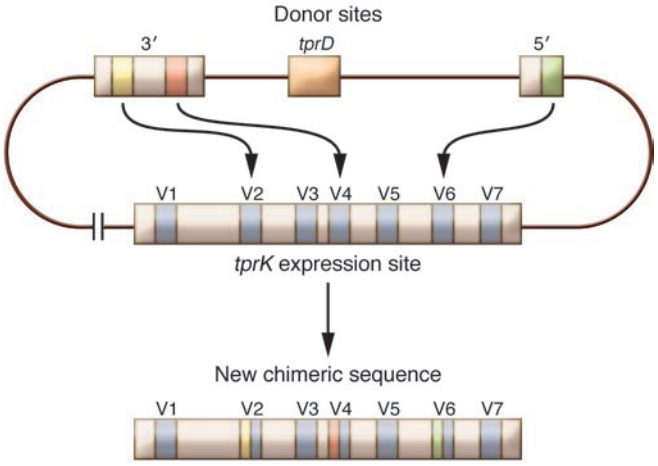

Figure 2
Gene conversion as the mechanism of antigenic variation of TprK in *T. pallidum*. Variant DNA segments located adjacent to the *tprD* gene non-reciprocally recombine with the variable regions (V1-V7) of the *tprK* gene in the expression site to generate new TprK mosaic proteins.

(ref. 88 and Figure 2). The resulting changes in exposed variable regions of TprK enable the organism to evade antibody binding and opsonophagocytosis (89). These TprK variant treponemes survive clearance and persist during chronic latent infection.

In some individuals, chronic latent infection can reactivate to cause tertiary syphilis, which occurs years to decades after initial infection and can affect multiple organs. In a retrospective study of patients from Oslo in the pre-antibiotic era, approximately one-third of patients with untreated latent syphilis developed tertiary syphilis (90). Manifestations may include gumma, cardiovascular syphilis, and tertiary neurosyphilis. In the modern antibiotic era, tertiary syphilis is rarely seen, perhaps due to inadvertent syphilis treatment with antibiotics prescribed for other infections.

HIV and syphilis

Since the beginning of HIV/AIDS epidemic, there has been a high rate of HIV-1 (HIV) coinfection among syphilis patients. In 2002, the CDC reported that 25% of primary and secondary syphilis cases occurred in persons coinfected with HIV, and the incidence rate of syphilis in HIV-infected persons was 77 times greater than in the general population (91). The number of cases of early syphilis has continued to rise over the past decade in all geographic regions, particularly in men who have sex with men (MSM), and over the past 5 years in both African American men and women (92). While the increasing incidence of syphilis may be due to high risk behaviors (93–95), higher rates of syphilis and HIV coinfection may also be due to immunological and bacteriological factors.

The primary chancre can facilitate acquisition and transmission of HIV by disrupting mucosal and epithelial barriers (96). In addition, the influx of immune cells to syphilis lesions increases the number of cellular targets available for HIV infection and the proximity of HIV-infected cells to transmit virus to the partner. *T. pallidum* itself and *T. pallidum* lipoproteins increase the expression of CCR5, the chemokine receptor expressed on macrophages and DCs that acts as a coreceptor for HIV entry into $CD4^+$ cells (33, 97).

It is not clear whether HIV coinfection worsens clinical manifestations of early syphilis or neurosyphilis. However, clinical and CSF abnormalities consistent with neurosyphilis are more common in HIV-infected individuals with $CD4^+$ T cell counts less than or equal to 350 cells/ml (67, 69, 98). HIV-infected individuals with neurosyphilis have higher CSF HIV RNA concentrations, suggesting there may be an interaction between syphilis and HIV in the CNS (99).

While the long-term consequences of syphilis infection on the HIV-infected individual's prognosis are not known, one prospective study suggests that despite transient increases in $CD4^+$ T cell counts and viral loads, syphilis did not appear to affect HIV progression (100). *T. pallidum* coinfection may have a deleterious impact on the immunologic and virologic status in HIV-infected persons, which may improve with syphilis treatment, although these data are conflicting (101–104).

Treatment

For more than 50 years, parenteral penicillin has been used successfully to treat individuals with syphilis, with clinical resolution and prevention of sexual transmission. Thus, it remains the treatment of choice for syphilis, and no penicillin-resistant strains have yet been documented. Benzathine penicillin G (BPG), a depot form, is used for standard treatment of syphilis, and aqueous penicillin is used for persons with recognized neurosyphilis. Unlike aqueous penicillin, BPG does not cross the blood-brain barrier to reach *T. pallidum* that may have invaded the CNS. This is of particular concern for HIV-infected persons with syphilis.

Patients with HIV have increased rates of serological failure of syphilis treatment (105–107), and viable *T. pallidum* have been isolated from the CSF of HIV-infected patients following standard treatment for syphilis (105, 106, 108). In addition, HIV-infected individuals with neurosyphilis are less likely to achieve normal CSF laboratory values (70, 109) and may take longer to resolve CSF abnormalities following treatment, compared with HIV-uninfected individuals (70, 109, 110). Effective ART has been associated with a reduction in the rate of serologic failure for syphilis (70).

Despite these concerns, the CDC currently recommends that HIV-infected individuals undergo the same BPG treatment for syphilis as HIV-uninfected individuals, and that evidence of neurosyphilis (i.e., CSF examination) not be sought in patients without neurological signs or symptoms regardless of HIV status (92). The long-term repercussions of these recommendations for CSF examination are unclear at this time.

Alternative oral antibiotic treatments. Macrolides, such as erythromycin and azithromycin, and tetracycline antibiotics, such as tetracycline and doxycycline, are alternatives to parenteral penicillin in non-pregnant penicillin-allergic patients. Tetracycline, doxycycline, and erythromycin require multiple doses daily for 2–4 weeks, reducing likelihood of patient compliance. In contrast, azithromycin provides a single-dose oral alternative to parenteral BPG for early syphilis. Early syphilis has been successfully treated with a single 1- to 2-g dose of oral azithromycin (111–116) with efficacy equal to that of BPG.

Unfortunately, with increased use of azithromycin for many infections, there has been an alarming rise in the prevalence of macrolide-resistant *T. pallidum*. The first macrolide-resistant strain of *T. pallidum* (Street strain 14) was isolated in 1977 (117, 118), and no other such resistant strains were reported until 2004. Street strain 14 is highly resistant to both erythromycin and azithromycin (117, 119) by virtue of an adenine-to-guanine (A→G) mutation at the position cognate to A2058 in the *E. coli* 23S rRNA gene

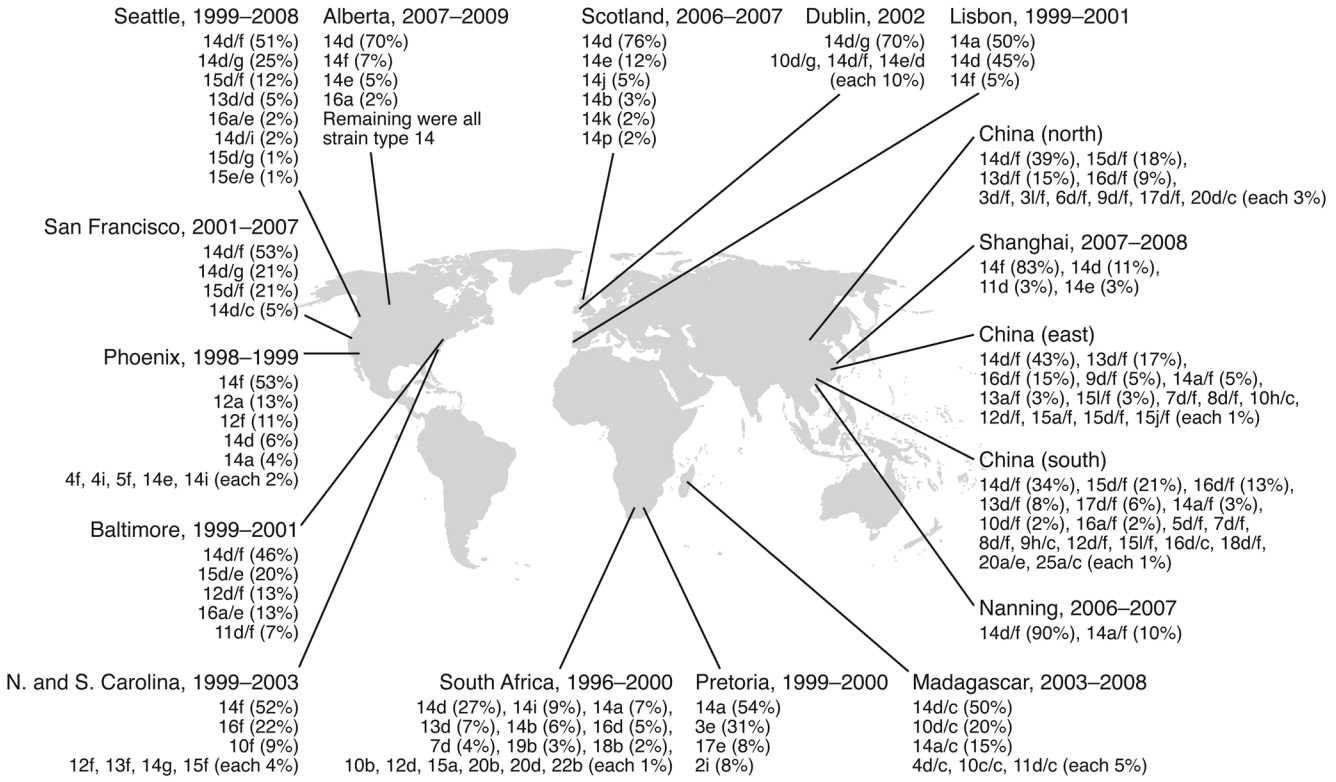

Figure 3
T. pallidum strain types identified throughout the world. The strain type information, years of collection, and the frequency of each strain type from each location are based on information in references 126, 127, 129–131, 133–135, 149, and 150.

(120). Clinical failures following azithromycin treatment in San Francisco triggered examination of *T. pallidum* strains from several geographical regions, revealing the presence of the A2058G mutation in 11%–88% of *T. pallidum* samples (121, 122). In San Francisco and Seattle, the prevalence of this mutation has increased steadily over time (121–123). In 2009, a new mutation, A2059G, was identified in the 23S rRNA gene in *T. pallidum* recovered from a patient from the Czech Republic who failed treatment with spiramycin, a macrolide antibiotic (124); this mutation also confers resistance to erythromycin, azithromycin, and spiramycin. Zhou and colleagues reported treatment failure in 132 patients who received azithromycin for syphilis in Shanghai from 2001 to 2008 (125), but the proportion of patients infected with *T. pallidum* harboring the A2058G or A2059G mutations was not reported. Three published trials of successful azithromycin treatment of syphilis in Africa have led some to conclude that macrolide-resistant strains are not present in Africa. Of note, patients were enrolled in these studies in 1994–1997, 2000–2003, and 2000–2007 (114–116), either before or very early after recognition of macrolide-resistant strains elsewhere in the world. Although there are no recent data from Africa on macrolide resistance, the absence of it does not imply the absence of resistant strains.

Although a global surveillance program for macrolide resistance mutations has not been developed for *T. pallidum*, the scattered reports from many continents suggest that these resistant strains are widespread, and strong caution is advised in contemplating the use of azithromycin for treating syphilis.

Molecular strain typing of *T. pallidum*

The ability to uncover important information about networks of transmission of syphilis infection and, particularly, to understand the development and spread of antibiotic-resistant strains requires a method for differentiating one strain of *T. pallidum* from another. Pillay and colleagues developed a *T. pallidum* typing method based on (a) determination of the number of 60-bp repeats in the acidic repeat protein (*arp*) gene and (b) sequence differences in the *Tpr* subfamily II genes (*tprE* [*tp0313*], *tprG* [*tp0317*], and *tprJ* [*tp0621*]) determined by restriction fragment length polymorphism (RFLP) analysis (126). *T. pallidum* subtypes with a range of 2 to 21 ARP repeats and 7 different RFLP patterns, designated *a*–*g*, have been described (126). This method for subtype designation has been applied to patient samples taken from chancres, condyloma lata, mouth scrapings, ear lobe scrapings, blood, CSF, and laboratory-passaged *T. pallidum* isolates from diverse geographic areas (126–134). Epidemiological studies of strain types in San Francisco and Seattle in the last decade showed that most are subtype 14d (123, 135), which may suggest a linked sexual network, while other studies indicate variation in the distribution of predominant strain types by location in the United States and worldwide (refs. 127–131, 133, 134, and Figure 3). Addition of a third gene to the typing scheme increased discriminatory power (123, 135). Subtyping using the *tp0548* gene demonstrated that the predominant 14d/f strain was replaced by the 14d/g strain in Seattle during the period from 1999 to 2008 (135). This recognition would not have been possible with the two-target typing method.

Molecular analysis can also be used to determine whether macrolide-resistant *T. pallidum* represents a single strain or whether resistance has spontaneously arisen in multiple strains. The A2058G mutation was found in molecularly separate strains in Seattle, suggesting that resistance mutations are arising spontaneously, rather than representing a single strain spreading throughout a population. Resistant strains were more likely to be found in patients who had received macrolide antibiotics in the previous 12 months (123, 136).

Strain typing may also be used to identify strains that are associated with particular clinical outcomes. Clinical descriptions from the pre-antibiotic era (137) and studies in the rabbit infection model suggest that some strains are more prone to neuroinvasion (138). A recent study of *T. pallidum* showed that patients infected with strain type 14d/f had a higher rate of neurosyphilis compared with patients infected with other strain types (135).

Vaccine development and prevention

Although highly effective treatment is available for syphilis, currently, there is an epidemic of syphilis in China and increasing incidence in the United States and Europe. The best hope for control of syphilis is development of a vaccine that prevents both disease and transmission. Attempts have been made over many decades to produce a successful syphilis vaccine by immunizing rabbits with whole killed or attenuated *T. pallidum* (reviewed in refs. 139 and 140). Only one immunization study, using multiple intravenous doses of gamma-irradiated *T. pallidum*, demonstrated complete protection against infectious challenges in the rabbit model (141). This protocol was very cumbersome and expensive, and impractical to test in humans. Immunization with recombinant *T. pallidum* antigens can stimulate production of an immune response in the rabbit model, resulting in only partial protection, with significantly attenuated lesion development but no sterile immunity (80, 83, 142–145). The discovery of antigenic variation in TprK makes the development of a protective vaccine even more formidable. However, studies are underway to test the ability of a cocktail of conserved regions of *T. pallidum* antigens to confer protection in the rabbit model.

Conclusion

Syphilis is one of the oldest recognized sexually transmitted infections, and despite the availability of inexpensive and effective therapy, the incidence is increasing in many parts of the world. *T. pallidum* is a challenging infectious agent to study because of its inability to be cultured or genetically manipulated, its physical fragility, and its outbred animal model. Despite these challenges, development of highly discriminating molecular methods for strain differentiation will provide insights into the transmission of this infection through populations, perhaps suggesting new ways to target intervention activities. In addition, our knowledge of the molecular pathogenesis of syphilis has expanded vastly during the past decade, especially with respect to understanding the host immune response to *T. pallidum*. Work on this fascinating organism continues to focus on understanding its ability to evade host immune responses, which may ultimately lead to the development of a successful vaccine.

Acknowledgments

The authors thank Christina Marra and Lauren Tantalo for critical reading of the manuscript and Lorenzo Giacani for helpful discussions. We apologize to those authors whose work we were unable to cite because of space limitations. This work was supported by U.S. Public Health Service grants R01 AI63940, R01 AI42143, and R03 AI094122 (to S.A. Lukehart) from the NIH. E.L. Ho was supported by University of Washington STD and HIV Research Training Grant T32 AI007140.

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