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 rabbits, while able to persist for decades in the mammalian host, is extremely fragile ex vivo. It can be cultured only transiently in vitro in rabbits, and rapidly enters the bloodstream while able to persist for decades in the mammalian host, is extremely fragile ex vivo. It can be cultured only transiently in vitro in rabbits, and rapidly enters the bloodstream. While able to persist for decades in the mammalian host, is extremely fragile ex vivo. It can be cultured only transiently in vitro in rabbits, and rapidly enters the bloodstream. It can be cultured only transiently in vitro in rabbits, and rapidly enters the bloodstream. It can be cultured only transiently in vitro in rabbits, and rapidly enters the bloodstream.

The genome of *T. pallidum* encodes for multiple transport proteins that 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).

*Treponema 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 adenopathy. 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, dissemination, 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

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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 lipo-protein 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 antitreponemal 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 for syphilis infection. Indeed, a published study (63) reports that many sera that are reactive in the EIA/CIA tests are nonreactive in the VDRL test, and the toluidine red unheated serum test. Reactive results must be confirmed by the treponemal IFA/CIA tests. Persons with periodontal disease carry oral treponemes that can persist untreated in infection. In addition, because the new EIA/CIA 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.
The increasing incidence of syphilis may be due to high risk behaviors over the past 5 years in both African American men and women (92). 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 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 azithromycin are alternatives to parenteral penicillin and macrolides, such as erythromycin and azithromycin. Tetracycline and doxycycline require multiple doses daily for 2–4 weeks, reducing the 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.
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, tprG, and tprJ) determined by restriction fragment length polymorphism (RFLP) analysis (126). T. pallidum subtypes with a range of 2 to 21 RFLP 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 tpo548 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.

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