Results Tp's BamA (TP0326) is the central component of a hybrid BAM system in which POTRA1–5 interacts with the DUF domain of TamB (TP0325). Although Tp lacks lipopolysaccharide, its genome encodes a nearly complete LPS transporter apparatus. The LptD ortholog (TP0515) contains a large unstructured C-terminal domain, which models LptE-like inside the β -barrel. Tp encodes four 8-stranded β -barrels, each containing positively charged extracellular loops that could contribute to pathogenesis. Surprisingly, Tp encodes five FadL orthologs, all of which display features (i.e., hatch domain and NPA motifs) that are characteristic of Gram-negative FadLs. Three (TP0548, TP0859 and TP0865) have α -helical extensions that could interact with periplasmic components. The Tp genome encodes Mac and RND efflux pumps that presumably achieve combinatorial diversity via co-expression of four paralogous OMFs. Lastly, we confirmed the bipartite membrane topology of Tprs using SAXS to solve solution structures for MOSPN domains of TprK and MOSP.

Conclusions The Tp Nichols OMPeome provides a structural framework to (i) elucidate Tp's enigmatic parasitic strategies, (ii) identify targets of natural immunity, and (iii) interrogate candidate vaccinogens.

001.5 ANTIGENIC VARIATION IN A PRIMATE MODEL OF PERSISTENT MYCOPLASMA GENITALIUM INFECTION

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Background *Mycoplasma genitalium* persists in the genital tract for months despite the presence of specific antibody that reacts with the immunodominant MgpB and MgpC adherence proteins. MgpB and MgpC undergo antigenic variation via recombination between the mgpBC expression site and archived variable sequences present in nine MgPar sites located around the genome. The objective of this study was to determine if antigenic variation occurs in our pig-tailed macaque model of persistent cervical infection, if variants arise coincident with the appearance of antibodies specific for MgpB and MgpC, and if variation correlates with persistence. **Methods** MG was cultured from persistently infected primates over 18 weeks. Whole genome sequencing was performed on these MG cultures using Nanopore sequencing technology then compared to the MG reference genome and to in vitro passaged bacteria.

Results Antigenic variants arose in the variable regions of MgpB and MgpC in infected primates but not during in vitro passage in broth. Variants were abundant in primates with persistent infection, and dynamic with different variants predominating at different time points. The sequence changes observed were consistent with reciprocal exchange of variable regions between the expression site and MgPars. Antigenic variants were not detected in a primate that cleared the infection after four weeks, suggesting that variation is required for persistence.

Conclusion Antigenic variation of the MG MgpB and MgpC proteins occurs frequently in vivo and is associated with persistence in the genital tract of female pig-tailed macaques. Future work will measure antibody reactivity to specific variable and conserved regions within the MgpB and MgpC proteins to correlate the appearance of antibody to specific sequences with clearance from the genital tract.

001.6 A CHLAMYDIA TRACHOMATIS INCLUSION MEMBRANE PROTEIN BLOCKS INTERFERON GAMMA STIMULATED IMMUNITY IN HUMANS AND NONHUMAN PRIMATES

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Background Epithelial cells that are stimulated with interferon gamma (IFN γ) carry out a potent immune response termed cell-autonomous immunity. While *Chlamydia trachomatis* is susceptible to murine cell-autonomous immunity, it evades this response in humans. The virulence factors underlying this evasion are unknown. A better animal model is needed to evaluate the importance of this defense pathway against *C. trachomatis* infection.

Methods We leveraged a novel library of *C. trachomatis* x *C. muridarum* chimeras to identify candidate virulence factors. We assayed chimeras and follow-up mutants for their ability to evade inclusion ubiquitination in IFN γ -primed human cells. Growth restriction assays were performed in human cells in the presence or absence of IFN γ . We isolated primary fibroblasts from a macaque skin biopsy to test whether a similar response is induced by IFN γ -stimulation in the nonhuman primate (NHP) model.

Results The inclusion membrane protein CT135 is required for evasion of cell-autonomous immunity. Organisms lacking a functional copy of this gene failed to block ubiquitin recruitment to their inclusions in IFN γ -primed human and NHP cells. This ubiquitination was associated with a severe growth defect in vitro. Retrospective analysis of a NHP study revealed an inoculum containing a CT135 mutant had poor fitness in vivo. We clonally isolated the mutant and confirmed it was susceptible to cell-autonomous immunity.

Conclusion A historic paucity of genetic tools has hindered the identification of virulence factors that facilitate *Chlamydia trachomatis* pathogenesis. By leveraging a novel library of mutants, we identified a virulence factor that facilitates chlamydial subversion of cell-autonomous immunity in human and NHP cells. While this *C. trachomatis* gene is nonfunctional in the mouse model, our reevaluation of a previous study suggests it is required for in vivo survival in NHP. This work underscores the importance of NHP as model organisms to study chlamydia immunity.

001.7 AT LONG LAST: GENETIC ENGINEERING OF TREPONEMA PALLIDUM SUBSP. PALLIDUM, THE SYPHILIS SPIROCHETE

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Background The recently described method for in vitro cultivation of the syphilis agent, *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), paved the way to transformation experiments to genetically engineer this pathogen. The demonstration that genetically modified syphilis treponemes are attainable would represent a milestone in syphilis research, destined to revolutionize our approach to understand the mechanisms behind *T. pallidum* success as a pathogen. Here,

for the first time in syphilis research, we describe a transformation protocol that successfully replaced the *tpaA* (tp0009) pseudogene in the SS14 *T. pallidum* strain with a kanamycin resistance (*kanR*) cassette.

Principal findings A suicide vector was constructed using the pUC57 plasmid backbone. In the vector, the *kanR* gene was cloned downstream of the *tp0574* gene promoter. The *tp0574*prom-*kanR* cassette was then placed between two 1-Kbp homology arms identical to the sequences upstream and downstream of the *tpaA* pseudogene. To induce homologous recombination of the arms into the *T. pallidum* chromosome, with resulting integration of the *kanR* cassette, in vitro-cultured SS14 strain spirochetes were exposed to the engineered vector resuspended in a transformation buffer and let recover for 24 hours before adding kanamycin-containing selective media. Integration of the *kanR* cassette was demonstrated by qualitative PCR, droplet digital PCR (ddPCR) and whole genome sequencing (WGS) of transformant treponemes propagated in vitro and in vivo. ddPCR analysis of RNA and mass spectrometry confirmed expression of the *kanR* message and protein in treponemes propagated in vitro. Moreover, *tpaA* knockout (*tpAko*-SS14) treponemes grew in kanamycin concentrations that were 64 times higher than the MIC for the wild-type SS14 (wt-SS14) strain and in infected rabbits treated with kanamycin.

Conclusion We demonstrated that genetic manipulation of *T. pallidum* is attainable. This discovery will allow the application of functional genomics to study syphilis pathogenesis and improve syphilis vaccine development.

001.8 CONTEMPORARY SYPHILIS IS CHARACTERISED BY RAPID GLOBAL SPREAD OF PANDEMIC TREPONEMA PALLIDUM LINEAGES

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Background Syphilis is an important sexually transmitted infection caused by the bacterium *Treponema pallidum* subspecies *pallidum*. The last two decades have seen syphilis incidence rise in many high-income countries, yet the evolutionary and epidemiological relationships that underpin this are poorly understood, as is the global *T. pallidum* population structure.

Methods We assembled a geographically and temporally diverse collection of clinical and laboratory samples, performing direct sequencing on the majority, and combining these with 133 publicly available sequences to compile a dataset comprising 726 *T. pallidum* genomes. We analysed the resulting genomes using detailed phylogenetic analysis and clustering.

Results We show that syphilis globally can be described by only two deeply branching lineages, Nichols and SS14. We show that both of these lineages can be found circulating concurrently in 12 of the 23 countries sampled. To provide further phylodynamic resolution we subdivided *Treponema*

pallidum subspecies *pallidum* into 17 distinct sublineages. Importantly, like SS14, we provide evidence that two Nichols sublineages have expanded clonally across 9 countries contemporaneously with SS14. Moreover, pairwise genome analysis showed that recent isolates circulating in 14 different countries were genetically identical in their core genome to those from other countries, suggesting frequent exchange through international transmission pathways. This contrasts with the majority of samples collected prior to 1983, which are phylogenetically distinct from these more recently isolated sublineages. Bayesian temporal analysis provided evidence of a population bottleneck and decline occurring during the late 1990s, followed by a rapid population expansion a decade later. This was driven by the dominant *T. pallidum* sublineages circulating today, many of which are resistant to macrolides.

Conclusion Combined we show that the population of contemporary syphilis in high-income countries has undergone a recent and rapid global expansion. This dataset will provide a framework for future characterisation and epidemiological investigation of syphilis populations.

Vaginal microbiota

002.1 CERVICOVAGINAL MICROBIOTA SPECIES DISTINCTLY MODULATE THE IMMUNOMETABOLIC MICROENVIRONMENT IN A HUMAN THREE-DIMENSIONAL CERVICAL MODEL

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Background Bacterial vaginosis-associated bacteria (BVAB) have been linked to gynecologic and obstetric sequelae, including an increased risk of STI acquisition and pre-term birth. However, there is a fundamental gap that exists in understanding the function of these microorganisms in the local microenvironment that contribute to disease. Hence, our objective was to identify immunometabolic signatures of cervicovaginal microbiota species in the context of cervical epithelium that can relate to clinical findings.

Methods Human three-dimensional (3D) cervical epithelial cell models were infected under anaerobic conditions with *Gardnerella vaginalis*, *Prevotella bivia*, *Atopobium vaginae*, *Sneathia amnii*, a polymicrobial community of BVAB, or health-associated *Lactobacillus crispatus*. Cell culture supernatants were collected 24 h post infection and analyzed using multiplex cytometric bead arrays and ultrahigh-performance liquid chromatography-mass spectroscopy.

Results *Lactobacillus* and BVAB effectively colonized the surface and crevices of human 3D cervical model visualized by scanning electron microscopy. Immunoproteomics analysis (28 targets) revealed that *A. vaginae*, *S. amnii* and polymicrobial community exert the greatest proinflammatory potentials, whereas *G. vaginalis* and *P. bivia* mostly altered epithelial barrier targets. *S. amnii* also induced proteins related to cellular stress and angiogenesis. The metabolomics analysis yielded 418 known metabolites. Random Forest analysis of metabolic profiles highlighted excellent prediction (93.75%) of infections. Furthermore, *A. vaginae*, *S. amnii* and the polymicrobial community profiles clustered