Extensive recombination in a population can also limit inferences about phylogenetic history. Here, we investigate the impact of recombination in the study of isolates with reduced susceptibility to cefixime (cefX; cefixime MICs ≥ 0.25 µg/ml) in the United States.

Methods We generated draft genome sequences for 242 gonococcal isolates collected by CDC’s Gonococcal Isolate Surveillance Program (GISP). These isolates comprise all 141 cefX isolates from GISP in 2009–10 and 141 susceptible isolates matched by location, collection date, and sexual orientation of the infected individual. We predicted recombinant regions and generated a maximum likelihood phylogenetic tree from core SNPs. We performed in silico MLST and NG-MAST typing, and compared phylogenies of antibiotic resistance loci to whole genome-based phylogenies.

Results Per site e/m ratios (relative likelihood that a polymorphism was introduced through recombination rather than mutation) of recent branches in the phylogenetic tree are higher and fraction of homoplasic sites much lower than for the overall tree, suggesting that extensive recombination reduces confidence in the phylogeny’s deep branches. Comparison with in silico MLST and NG-MAST reveals that traditional typing-based phylogenetic inferences, even for recent events, are confounded by recombination. Of the 21 penA alleles in this dataset, mosaic FB2 pattern XXXIV was the most common (present in 116/121 cefX isolates). We find several recombination events introducing this allele into distinct lineages, and an event within the dcs gene cluster, which includes the penA allele, associated with reversion from cefX to cefixime susceptibility.

Conclusions Genomic methods reveal the impact of recombination on phylogenetic history, spread of resistance elements, and genome evolution, and offer a superior approach to traditional typing schemes in understanding population structure and dynamics.

001.1 ASSOCIATION OF GENETIC VARIANTS WITH CHLAMYDIA TRACHOMATIS REINFECTION


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Background Up to 20% of Chlamydia trachomatis (CT)-infected patients are reinfeeted within months after treatment, suggesting some fail to develop protective immunity. Genetic determinants influencing CT reinfection risk have not been fully elucidated. Our primary research objective is to identify genetic determinants of CT reinfection. Based on previously reported associations of HLA class II alleles with CT complications, our initial investigations focus on HLA class II genes.

Methods In an ongoing prospective natural history study, CT-infected subjects are enrolled, treated with azithromycin 1 g single dose, and return for a 6-month follow-up visit for repeat CT testing using the Gen-Probe APTIMA Combo 2 assay (Gen-Probe, Inc., San Diego, CA). HLA class II alleles are resolved by a combination of PCR-based techniques. Genomic DNA is stored for further genotyping.

Results A total of 199 African American subjects have been studied to date: 90% women and median age 23. CT reinfection at follow-up was noted in 18%. Subjects with HLA-DQB1*05 more often had reinfection (20 [26%] vs. 16 [13%], P = 0.018), which remained significant after controlling for age and gender (OR 2.6, 95% CI 1.2–5.6, P = 0.012). Other HLA-DQB1 alleles were not significantly associated with reinfection (P ≥ 0.1).

Conclusion HLA-DQB1*05 was associated with CT reinfection, suggesting it could influence protective immunity. More comprehensive genotyping from larger prospectively studied cohorts should help confirm or refine this observation. Analysis of additional HLA class II genes and genes beyond the human MHC is in progress.

001.2 INNATE IMMUNITY MODULATION BY TRICHOMONAS VAGINALIS GALECTIN-BINDING GLYCOLID DOMAINS


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Background Trichomonas vaginalis is a protozoan extracellular parasite causing long-lasting and recurrent vaginitis with a wide range of symptoms and increased risk of HIV and other viral STIs. The protozoan virulence factors that subvert the mucosal immune response are poorly understood. Here we investigate the role of the ceramide-phosphadidylinositol-glycolipid core (CPI-GC) of the protozoan lipophosphoglycan (LPG), which is the major glycoconjugate on the trichomonad surface (2–3 million copies/parasite). We have previously determined that CPI-GC lacks mannose but
contains poly lactosamine repeats representing potential ligands for animal lectins called galectins, implicated in HIV pathogenesis.

**Methods** CPI-GC was isolated from *T. vaginalis* LPG by mild acid hydrolysis and C18-SepPak separation. Binding to galectin-1 and -3 (Gal-1 and -3) was determined by Biolayer Interferometry. Inflammation-related proteins and Gal-1 and 3 were measured by a multiplex immunoassay in supernatants from human cervical and vaginal epithelial cells infected with *T. vaginalis* or exposed to CPI-GC from different clinical isolates.

**Results** CPI-GC activated NF-kB and upregulated cFos, COX-2, IL-8, MIP-3β, IL-6, IL-1β and VEGF in a MEK1/2 dependent manner. In addition, IL-6, ICAM-1 and VEGF up-regulation was mediated by p38 while IL-8 and MIP-3β were ERK 1/2 mediated. CPI-GC from different clinical isolates varied in their ability to bind Gal-1 and Gal-3, which were constitutively expressed by vaginal and cervical epithelial cells and released at higher levels in the extracellular space during exposure to live trichomonads and CPI-GC. CPI-GC from all isolates invariably reduced levels of the natural microbiode SLPI. Mutant trichomonads that failed to bind Gal-1 and Gal-3 showed higher proinflammatory activity suggesting a role for the CPI-GC–galectin binding in suppressing innate immune responses.

**Conclusion** Interventions targeting CPI-GC or restoring the balance of natural immune defence represent a promising strategy for preventing adverse outcomes from *T. vaginalis* infection.

**REGULATORY T CELLS IN PERIPHERAL BLOOD AND CEREBROSPINAL FLUID OF SYPHILIS PATIENTS WITH AND WITHOUT NEUROLOGICAL INVOLVEMENT: A COMPREHENSIVE AND COMPARATIVE STUDY**

**Background** Syphilis, a sexually transmitted disease caused by spirochetal bacterium Treponema pallidum, can progress to affect central nervous system, causing neurosyphilis. While many neurosyphilis patients may be asymptomatic, some patients can develop severe neurological and psychiatric symptoms. Accumulating evidence suggest that skin lesions and clinical symptoms of early syphilis patients result from host immune and inflammatory responses. However, very little is known about the immune components in neurosyphilis.

**Methodology/Principal Findings** In the present study, we performed a comprehensive and comparative analysis of regulatory T cells (Tregs) between 102 neurosyphilis patients and 431 syphilis patients without neurological involvement. We found secondary and serofast patients had increased Treg percentage, suppressive function and TGF-β levels in peripheral blood compared to healthy donors and serum Rapid Plasma Reagin (RPR) titers were positively correlated with Treg numbers in these patients. Neurosyphilis patients had higher Treg frequency in peripheral blood than those of syphilis patients without neurological involvement. Importantly, CD4+ T cells were increased and predominated in cerebrospinal fluid (CSF) of both asymptomatic and symptomatic neurosyphilis patients. Interestingly, a significant decrease in CSF CD4+ CD25 high Treg percentage was observed in symptomatic neurosyphilis patients compared to those of asymptomatic neurosyphilis patients, which may be associated with low CSF TGF-β levels.

**Conclusions** Our findings suggest that neurologically progresses in syphilis patients may be associated with an enhanced systemic Treg response and an increased local CD4+ T cell infiltration. A decrease in Treg frequency in CSF of symptomatic neurosyphilis patients indicates that immune-mediated tissue damage might be involved in the development of neurological symptoms.

**BLOOD TRANSCRIPTIONAL PROFILING OF WOMEN WITH CHLAMYDIA TRACHOMATIS IDENTIFIES A PELVIC INFLAMMATORY DISEASE (PID) SIGNATURE**

**Objective** Most women with Chlamydia trachomatis (CT) infection are asymptomatic, while ~3% progress to pelvic inflammatory disease (PID) within two weeks of untreated infection. The identification of biomarkers that predict development of PID would aid in identification of women at risk for complications of infertility and ectopic pregnancy. The specific aim of this study was to identify a whole blood transcript signature for acute PID due to chlamydial infection.

**Methods** We performed gene expression microarrays using whole blood from 79 women who had a gynecologic exam, and cervical and endometrial microbiologic testing. Samples were divided into five groups: Group 1, women with acute PID who were CT+ at endometrium (PID+, CT+, and E+); Group 2, asymptomatic women who were CT+ at endometrium (PID-, CT+, E+); Group 3, asymptomatic women who were CT+ at cervix (PID-, CT+, E+); Group 4, asymptomatic women who were CT- at cervix and endometrium (PID-, CT-, E+); Group 5, women with symptoms of PID who were negative for CT or other sexually transmitted pathogens (PID+, STI-, E-).

**Results** We identified a transcript signature that discriminated women with chlamydial PID from all other groups. Pathway analysis revealed that the chlamydial PID signature contained genes from interferon response pathways. Gene transcription in a subset of women with chlamydial endometrial infection clustered with women with chlamydial PID.

**Conclusions** Our study raises the possibility that transcriptional biomarkers with potential as diagnostic and prognostic tools can be identified to combat chlamydial reproductive tract disease in women.