Evaluation of a Novel *Treponema pallidum* Proteomic Array to Improve Understanding of Syphilis Immunology

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Background: Syphilis, caused by the spirochete *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), continues to be a significant concern for global health. A better understanding of syphilis immunology could be among the keys to devise novel syphilis control strategies and stem the spread of this serious infection. Here, we focused on understanding differences in humoral reactivity to selected *T. pallidum* antigens in patients diagnosed with their first-ever syphilis episode and in patients with active syphilis but also with a history of previous infection. As for other diseases, sustained antibody level to protective antigens could lower the risk of reinfection, and identification of differences in reactivity to antigens in these groups of patients could lead to the identification of antigens to be tested as possible vaccine candidates.

Methods: We developed a novel proteomic array carrying 14 *T. pallidum* recombinant proteins selected among the pathogen's most highly expressed genes and/or putative surface antigens to identify seroreactive proteins and to determine if individuals with and without prior syphilis infection are differentially reactive to these antigens. Reactivity was assessed via enzyme-linked immunosorbent assay (ELISA) using sera from 58 patients collected at diagnosis. All these patients were diagnosed with early latent syphilis and medical records indicated that 36 had at least one previous episode of syphilis prior to sample collection, while 22 did not have history of previous infection. Differences in reactivity were analyzed using ANOVA with significance set at $p<0.05$.

Results: Serum samples from syphilis-naïve patients diagnosed with early latent syphilis showed significantly higher reactivity to the Tp0548, TprJ, and TprL antigens compared to samples from syphilis diagnosed with early latent syphilis but with a history of previous infection. All these antigens are annotated as putative *T. pallidum* outer-membrane proteins (OMPs).

Conclusions: Given that immunity to OMPs is pivotal for pathogen clearance though opsonophagocytosis, a decrease in serum reactivity against key OMPs could facilitate patient re-infection. Therefore, our study identifies possible vaccine candidates for syphilis that should be tested to evaluate their ability to to generate protective immunity to this serious infection.