001.2 PYROCOCCUS FURIOSUS THIOREDOXIN AS A PLATFORM TO EXPRESS EXTRACELLULAR LOOPS OF TREPONEMA PALLIDUM OUTER MEMBRANE PROTEINS

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10.1136/sextrans-2021-sti.49

Background Development of a vaccine is a necessary step towards curtailing the global syphilis epidemic. Towards this end, our group has utilized bioinformatic and biophysical methods to characterize *T. pallidum*'s repertoire of outer membrane proteins (OMPs): the two 'stand-alones', BamA and LptD, and the *T. pallidum* repeat (Tpr), 8-stranded β -barrel, outer membrane factor (OMF) for efflux pump, and FadL paralogous families. We have hypothesized that antibodies against extracellular loops (ECLs) of OMPs are responsible for the opsonic and protective activities of immune rabbit serum (IRS). We used *Pyrococcus furiosus* thioredoxin (PfTrx) as a scaffold to present ECLs for assessment of reactivity with syphilitic sera and quantitation of circulating ECL-specific B-cells. To develop this system, we focused on BamA/ECL4 which we previously have shown is immunogenic and a target for opsonic antibodies.

Methods PfTrx was engineered to express BamA-ECL4 (PfTrx-BamA/ECL4) with a N-terminal His6- and C-terminal Avi-tags for Ni-NTA purification and in vivo biotinylation, respectively. Reactivity of IRS and human syphilitic sera (HSS) with PfTrx-BamA/ECL4 was assessed by immunoblotting. To determine the accessibility of the BamA/ECL4 epitope, a pull-down assay was performed by incubating PfTrx-BamA/ECL4 or PfTrx with IgG from IRS or normal rabbit serum (NRS) immobilized on protein G agarose beads. BamA/ECL4-specific IgG+ B-cells were identified by flow cytometry using PfTrx-BamA/ECL4 conjugated with streptavidin (SP)-Alexa Fluor 647 and SP-Brilliant Violet-421. PfTrx was conjugated to SP-APC-Cy7 to exclude non-specific binding to scaffold.

Results IRS and HSS recognized PfTrx-BamA/ECL4 but not PfTrx. PfTrx-BamA/ECL4 was pulled down by IgG from IRS but not NRS, while PfTrx was not pulled down by either. 1.66% of circulating IgG+ B-cells were PfTrx-BamA/ECL4-specific.

Conclusion When used in conjunction with OMP structural models, PfTrx-ECL is a promising platform for identification of antigenic ECLs and isolation of ECL-specific B-cells for the generation of anti-ECL monoclonal antibodies with opsonic activity.

001.3 AN INTEGRATIVE OMICS APPROACH TO UNDERSTANDING HOST CELLULAR RESPONSES TO TREPONEMA PALLIDUM

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10.1136/sextrans-2021-sti.50

Background *Treponema pallidum* ssp. *pallidum* (Tp), the causative agent of syphilis, is a highly invasive pathogen that moves throughout the body via the bloodstream and invades organs and tissues to cause the serious sequelae associated with sexually transmitted and congenitally acquired syphilis infections. The endothelium represents a crucial barrier for Tp dissemination, and we hypothesized that Tp alters endothelial structure and signalling. In this study, we apply an integrative omics approach to characterize transcriptomic, proteomic, and immune secretomic responses of endothelial cells to Tp.

Methods Endothelial cells were grown in vitro and exposed to viable Tp or a background control lacking the pathogen. For transcriptomic analysis, RNA was harvested from the endothelial cells and sequenced via RNAseq. For proteomic analysis, experimental and control samples grown using stable isotope labeling by amino acids in cell culture (SILAC), total protein was harvested and analyzed via orbitrap fusion tandem mass-spectrometry (LC-MS/MS). For immune secretomic analysis, supernatant from endothelial cells co-incubated with Tp was harvested and analyzed for cytokine content via cytometric bead arrays (CBA) using flow-cytometry.

Results RNAseq studies identified genes within immune signalling pathways that are differentially regulated in response to Tp. Proteomic analysis identified several pathways involved in cellular structure and modulation, interleukin signalling, and intracellular signalling. Data from CBA experiments further indicate the ability of Tp to alter endothelial cytokine production and secretion. These data give a novel multi-faceted look into the role and response of the endothelium during Tp infection.

Conclusion These studies represent a novel systems-level approach to understanding the molecular mechanisms behind the establishment and persistence of Tp infection. Further, they provide insight into how Tp may induce host cellular changes to enable barrier traversal and immune cell recruitment, and in this way provide information that is relevant to syphilis vaccine design.

001.4 THE GLOBAL TREPONEMA PALLIDUM OMPEOME: A STRUCTURAL PLATFORM FOR DECIPHERING STEALTH PATHOGENICITY AND DEVELOPING A SYPHILIS VACCINE WITH WORLDWIDE EFFICACY

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10.1136/sextrans-2021-sti.51

Background The outer membrane (OM) of *Treponema pallidum* (Tp) serves as the interface between the syphilis spirochete and its obligate human host and also is key to developing a vaccine with worldwide efficacy. We used structural modeling and bioinformatics to delineate the repertoire of OM proteins (OMPs) in the Tp Nichols strain. The Tp 'OMPeome' consists of two 'stand-alone' proteins (BamA and LptD) involved in OM biogenesis and four paralogous families involved in influx/efflux of small molecules: 8-stranded β -barrels, long-chain fatty acid transporters (FadLs), OM factors (OMFs) for efflux pumps, and Tp repeat protein (Tprs).

Design/Methods Three-dimensional structural OMP models were generated with I-TASSER, Phyre2, and trRosetta. SAXS was used to solve solution structures for TprK and *T. denticola* MOSP, the Tpr family parental ortholog.