04-S1.02 MOLECULAR CLONING AND EXPRESSION OF HYDROGENOSOMAL MALATE DEHYDROGENASE OF *TRICHOMONAS VAGINALIS*

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Background Trichomoniasis, a sexually transmitted disease caused by*Trichomonas vaginalis*, is associated with adverse pregnancy outcomes, and increased risk of HIV acquisition. Malate dehydrogenase (MDH), which catalyses the interconversion of malate to oxaloacetate, has a pivotal role in the survival and pathogenicity of this amitochondrial protozoan. The objective of this study was to clone and express Malate dehydrogenase gene of*T vaginalis*, and analyse the biological function of this hydrogenosomal enzyme.
Methods The MDH gene from a clinical isolate of*T vaginalis* was amplified by PCR, and cloned into pET101/D-TOPO vector with a C-terminal 6XHis tag. Positive clones were screened and identified by restriction endonuclease digestion and sequence analysis. The plasmid pET101/D-MDH was then transformed into E.coli BL21 (DE3) to express after IPTG induction. The expression product further analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. The recombinant protein was purified with Ni-NTA agarose under native conditions. Western blot, using antibody raised against whole cell*T vanginalis*, was performed to determine the immunogenicity of purified recombinant protein.
Results The recombinant plasmid pET101/D-MDH was constructed successfully. High homology (98%) of nucleotide sequence was revealed between the cloned MDH and the corresponding gene. The recombinant protein showed a high expression level when induced with 1 mM IPTG at 37° C for 4 h. SDS-PAGE analysis showed that the recombinant MDH protein with the correct molecular weight (about 60 kDa) was expressed in E.coli BL21 (DE3). Western blotting revealed that the purified recombinant protein was specifically recognised by sera from mice infected with whole cell*T vaginalis*.
Conclusions A prokaryotic expression system of*T vaginalis* Malate dehydrogenase gene has been established successfully. The immunogenicity of the recombinant protein has been tested. The present study shows that the recombinant MDH is specific and suitable for use as an antigen for detecting anti-*Trichomonas vaginalis* IgG antibodies. Our work has established a good foundation for future studies on*T vaginalis* vaccine construction.

04-S1.03 DEFINING THE IN VITRO FUNCTIONS OF MONOCLONAL ANTIBODIES DEVELOPED TO THE *HAEMOPHILUS DUCREYI* TRIMERIC AUTOTRANSPORTER DSRA

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Background The DsrA protein of*Haemophilus ducreyi*, the etiological agent of the genital ulcer disease chancroid, is a multifunctional outer membrane trimeric autotransporter (TA) and a virulence factor. Most*H ducreyi* strains, which are grouped in two classes (I and II), express a DsrA protein. Although DsrA proteins from both classes of*H ducreyi* strains share little identity in their N-terminal functional passenger domain, both are involved in serum resistance and function as adhesins to the extracellular matrix (ECM) proteins fibronectin (Fn) and vitronectin (Vn), as well as to fibrinogen (Fbg) and HaCat keratinocytes.
Methods Monoclonal antibodies (mAbs) to DsrA were developed by immunising mice with full-length recombinant DsrA from class I strain 35 000HP (DsrAI). Western blots were used to determine the specificity of the mAbs and their ability to recognise multimers of DsrA. Whole-cell binding ELISAs were used to examine the capacity of the mAbs to bind the surface of*H ducreyi*. The domains and the shortest nominal peptides of DsrA recognised by those mAbs were identified using a library of truncated DsrA proteins expressed in a dsrA mutant, a peptide library representing full-length DsrAI, as well as Surface Plasmon Resonance and mutagenesis studies. mAbs were tested for bactericidal activity and their ability to block binding of Fn, Vn and Fbg by*H ducreyi* using bactericidal assays and whole cell blocking assays, respectively.
Results Anti-DsrAI mAbs bound monomers and dimers of the DsrA protein from several class I*H ducreyi* strains but not the DsrAII protein. mAb 1.82 bound native DsrAI protein at the surface of a panel of class I*H ducreyi* isolates, but not the H .ducreyi strains that cause cutaneous chancroid. The DsrA protein from these strains was recognised by mAb 4.79, along with the DsrA protein from strain 35 000HP. Both 1.82 and 4.79 bound with high affinity to peptides in the N-terminal region of the DsrA translocator domain and the shortest nominal epitope for 1.82 is MEQNTHNINKLS. In whole cell blocking assays, mAb 1.82 partially blocked binding of Fn and Fbg by class I*H ducreyi* strain 35 000HP.
Conclusions 1.82 and 4.79 are DsrAI-specific mAbs that recognise multimers of DsrA and bind the surface of intact*H ducreyi*. Both mAbs bind an epitope in the N-terminal region of the translocator domain of DsrAI with high affinity. mAb 1.82 has modest Fn and Fbg blocking activity.

04-S1.04 RAPID SPREAD OF HERPES SIMPLEX VIRUS-2 IN THE HUMAN GENITAL TRACT

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Background Genital herpes simplex virus-2 (HSV-2) infection consists of shedding episodes that correlate with transmission and genital lesion formation. Episodes are heterogeneous in terms of viral production and duration.
Methods Using quantitative PCR data from 14 685 daily genital swabs performed daily in 531 persons, we summarised 1020 episodes according to their frequency, duration, peak HSV DNA copy number, first and last swab copy number, expansion and decay slopes, and morphology. We designed competing dynamical stochastic mathematical models and attempted to fully reproduce result ranges for each of these episode features. The models assumed the possibility of concurrent, spatially distinct plaques of viral infection and immunologic response.
Results Our spatial model reproduced all kinetic features of the empirical shedding data. The model and parameter set that achieved best fit highlights three distinct mechanisms of viral spread. First, HSV-2 is constantly dispersed from neurons to epidermal cells throughout the genital tract at a rate of ~100 HSV DNA copies/day. Approximately 75 times each year, a neuron-derived viral particle infects an epidermal cell, initiating an infectious plaque of epidermal cells and a detectable shedding episode. Second, within each plaque, cell-to-cell spread of HSV-2 particles occurs extremely rapidly between epidermal cells. One cell can produce 105 cell-associated HSV genomic copies/day, with 3% of viral particles infecting a neighbouring cell each day. In large plaques, >105 cells may be infected within ~12 h producing up to 109 HSV DNA copies/ml. Third, cell-free viruses, despite being 3000-times less infectious than cell-associated viruses, decay more slowly and initiate spatially distinct secondary plaques. We use video simulations to display that episodes longer than 3 days, consist of dozens