Results Prior to *N gonorrhoeae* inoculation, mice with a pre-existing chlamydial infection had decreased expression of TLR4 and antimicrobial peptide (CRAMP, SLPI) genes. Consistent with the finding of decreased TLR4 expression in coinfecting mice, markers of inflammation (TLR2, TNF α , IL-1 β , platelet activating factor receptor [PafR], and IL-23 α) were up-regulated only in mice infected with *N gonorrhoeae* alone. A significantly lower percentage of TLR4-expressing epithelial cells was detected in vaginal swabs from chlamydia-infected wild-type mice prior to gonococcal challenge, and importantly, chlamydial infection did not enhance *N gonorrhoeae* infection of TLR4 mutant mice.

Conclusions These data suggest the host response to chlamydial infection creates an environment that is less protective against gonococcal infection by down-regulating the expression of TLR4 and antimicrobial peptides. This work therefore further illuminates the basis of this interesting consequence of coinfection and may also help direct the development of immunomodulatory therapies against this common coinfection and its consequences on reproductive health.

04-S2.02 SEROPREVALENCE OF NOVEL IMMUNOGENS OF CHLAMYDIA TRACHOMATIS AND THEIR CYTOKINE RESPONSE IN PBMC CELLS UNDER IN VITRO CONDITIONS

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Background Chlamydia trachomatis (CT) is an obligate intracellular parasite which causes STD and trachoma. Despite major research into Chlamydial pathogenesis and host immune responses, immuno-protection has been hampered by the incomplete understanding of protective immunity in the genital tract. Characterised vaccine candidates in general have shown variable efficacy ranging from no protection to partial protection. It is therefore a research priority to identify novel Chlamydial antigens that may elicit protective immune responses.

Objectives The goal of the present study was to assess the seroprevalence to pkn1 and DNA j following natural CT infection in human. The prospects of pkn1 as a Type III secretion substrate and DNA j as a non surface Chlamydial protein as potential antigen, prompted us to explore the immunogenic potential of both proteins.

Methods pkn1, DNA j and ompA were cloned in bacterial expression vector pTrcHis. Ni²⁺-NTA affinity chromatography was used to purify the recombinant proteins. Antigenic stretches of Pkn1, DNA j and OmpA were identified using Bepred web server, designed for identification of subunit vaccine candidate by Bioinformatics Centre of IMTECH Chandigarh, India. To validate the bioinformatics based analysis, sera of human patient were used to determine seroreactivity of pkn1 and DNA j proteins. OmpA was used as a positive control during the study.

Results Present study showed a high seroprevalence of antibodies against Pkn1 and OmpA ($p < 0.001$) in sera of humans infected with CT. While, no antibodies were observed for DNA j. Our studies have shown an association between release of TNF- α and IFN- γ levels upon stimulation of PBMC with Pkn1 and OmpA. Cytokine expression profiling (IL-1 β , TNF- α , IL-2, IL10 and IFN- γ) of Human PBMCs in response to Pkn1 stimulation demonstrate for the first time that Pkn1 is a novel immunodominant Chlamydial antigen that is capable of influencing both Th1 and Th2 immune responses by stimulating the release of both Humoral and Cell-mediated regulatory cytokines.

Conclusions Our study demonstrated strong serological responses to Pkn1 and major outer membrane in natural human infection suggesting the role of pkn1 in immune response. Studies are in

progress to check how the immunomodulation by Pkn1 alters host-pathogen interactions.

04-S2.03 AN ANTI-ADHESIVE APPROACH TO PREVENTION OF C TRACHOMATIS INFECTION

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Background We have reported that the major outer membrane of *C trachomatis* is glycosylated and the glycan is a high mannose oligosaccharide. Cumulative studies have demonstrated that the glycan is important in attachment and infectivity through binding to the mannose receptor (MR). Glycan removal decreases infectivity in vitro and in vivo and simultaneous administration of mannan, a ligand of the MR, abrogates *C trachomatis* infection in a mouse model of pneumonitis. Thus, we are investigating the feasibility of an anti-adhesive therapy to prevent *C trachomatis* infection by identifying oligosaccharides that are effective in inhibiting infection in vitro and testing their efficacy in a mouse model of genital tract infection.

Methods HeLa 229 cell monolayers were pretreated with serial concentrations of oligosaccharides prior to infection with *C trachomatis*. Neutralisation of infectivity was scored as >50% inhibition. For animal experiments, 8-week-old Swiss Webster female mice were primed with subcutaneous injections of Depo-Provera 1 week prior to challenge. Subsequently, mice were inoculated intravaginally with carbohydrates or PBS ($n=5$ mice per group) 30 min prior to infection with *C trachomatis*. Vaginal swab samples were obtained at 24, 48, and 72 h. post-infection, at peak times of shedding. Statistical significance was determined by the Student's t test.

Results Carbohydrates have been tested in vitro in hapten inhibition experiments against three serovars (D, E, F) most frequently isolated from genital tract infection. At the highest concentration tested, 4-nitrophenyl- α -D-mannopyranoside inhibited infectivity by 91%–92%; α -D-mannose-PAA from 77 to 93%; hen ovalbumin by 85%–89%; mannan by 77%–83%; and the high mannose fraction prepared from ovalbumin by 59%–98%. To determine efficacy in vivo, Swiss Webster mice were inoculated with different concentrations of inhibitors. Of those tested thus far, shedding of organism was significantly decreased ($p < 0.05$). The maximum inhibitions observed were: 4-nitrophenyl- α -D-mannopyranoside (86%), α -D-mannose-PAA (81%), mannan (93%), and the high mannose fraction from ovalbumin (94%).

Conclusions These preliminary studies suggest the potential feasibility for developing an “anti-adhesive therapy” as an alternate topical microbicide approach to prevent *C trachomatis* genital tract infection.

04-S2.04 CAN A CERVICAL BARRIER PREVENT CHLAMYDIA INFECTION IN THE PIGTAILED MACAQUE CERVICAL CHALLENGE MODEL?

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Background Numerous microbicides that inactivate *Chlamydia trachomatis* in vitro have failed to prevent transmission of this pathogen in the pigtailed macaque cervical challenge model. Since *C trachomatis* replicates in endocervical columnar epithelium but not in the squamous vaginal epithelium, we tested whether a cervical barrier would improve protection when used in conjunction with an otherwise non-protective microbicide.