of concurrent viral plaques. Despite rapid cell-to-cell spread of HSV-2, infected cells are eliminated by localised CD8+ T-cells within 24 h of plaque initiation. Moreover, the extent of secondary plaque formation prior to episode termination is determined by spatial CD8 + T-cell density surrounding the site of infection.

Conclusions Genital HSV-2 utilises three kinetically distinct methods of spread to initiate and sustain prolonged shedding episodes. The extent and severity of secondary plaque formation is determined by spatial immune cell density.

04-S1.05 RNA HELICASES, P72 AND P68 AND HDAC1 INTERACT WITH HIV-1 INTEGRASE AND AFFECT VIRAL REPLICATION

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F Larguet, É Rassart, E Edouard. *Université du Québec à Montréal, Montreal, Canada*

Background Human immunodeficiency virus type-1 (HIV-1) infection is one of the leading causes of death worldwide. Current anti-HIV-1 therapy, referred as highly active antiretroviral therapy (HAART), is based on the use of combination of drugs directed against viral enzymes mainly reverse transcriptase and protease and more recently integrase. Indeed, HAART has dramatically improved the clinical course of the disease. However, the emergence of multi-drug resistant virus strains during treatment highlights the urgent need to develop novel antiretroviral drugs against new HIV-1 targets. HIV-1 is able to hijack cellular machinery for its replication through protein-protein interactions between viral and host cell factors and a rising strategy against HIV-1 infection is to inhibit key virus-cell interactions. Integrase that catalyses HIV-1 viral DNA integration into the host cell genome is currently a focus for the development of new drugs. Several cellular partners of integrase have been identified using different methods. Based on a different strategy, our study aimed to identify new integrase cellular partners.

Methods We used a biotinylated oligonucleotide derived from the viral U3 LTR end as bait to isolate integrase in a streptavidin beads magnetic separation. Proteins co-purified with integrase were analysed by mass spectrometry.

Results Interestingly, our method allowed the identification of new cellular proteins notably p72 and p68 RNA helicases and histone deacetylase 1 (HDAC1) as integrase partners in addition to proteins already reported in literature. The interaction of p72, p68 and HDAC1 proteins with integrase was confirmed by co-immunoprecipitation. In addition, specific knockdown of p72 and p68 RNA helicases and HDAC1 were shown to affect HIV-1 replication.

Conclusions Our data suggest that cellular proteins, p72 and p68 RNA helicases and HDAC1 facilitate HIV-1 replication through interaction with integrase.

04-S1.06 HPV 16 PREDICTS CLINICAL OUTCOME IN ORAL CANCER PATIENTS TREATED BY RADIOTHERAPY

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R M Apolinario, P C Lara, L A Henriquez, M Lloret, B Pinar. *Las Palmas University Hospital-Canarian Institute for Cancer Research (ICIC), Las Palmas GC, Spain*

Growing molecular and clinical evidence indicates that human papillomavirus (HPV) is involved in the aetiology of oral squamous cell carcinomas (SCCs). HPV(+) tumours appear to be clinically distinct from HPV(−) tumours, conferring improved survival outcomes for patients in oropharyngeal cancer but limited knowledge exist on oral cancer. Determination of the HPV status of tumours may assist in patient risk-stratification and ultimately guide optimum treatment. The primary aim of this study was to examine the distribution of HPV in oral SCCs as assessed in vitro amplification assays and correlated with clinical and demographic data. The secondary aim was to correlate the positivity of HPV tumours with clinical outcome in the largest series of oral cancer published with a long follow-up (up to 20 years).

Materials and Methods One hundred thirty-one invasive oral SCCs were tested for HPV using laboratory-developed PCR assays for HPV16. P53 expression, tumour angiogenesis (CD-31 staining) and proliferation (MIB-1) were also assessed by immunohistochemistry in parafine embedded tissue. Patients mean age was 58.09±10.41, median 59 (116 men and 15 women). Clinical Stage distribution was: st I:17 cases; II: 56,III:32.IV:26. Most tumours were histological grade I (39) and II (74). Patients with pathological stage I-II were refereed to surgery (65 cases) and patients with Stage III-IVA were referred to surgery and postoperative radiation therapy (66 cases). Mean radiotherapy given doses were 62.13±7.74, median 65 Gy in 1.8-2 Gy fractions. No chemotherapy was used in any case.

Results 41 cases (31.3%) were HPV 16(+). No relation was found with age, gender, or tumour characteristics. In fact no relation was found to p53 expression, tumour proliferation or angiogenesis. 15-year DFS was 62.20% in HPV(+) patients was, compared to 37.3% in the HPV(-) group ($p<0.076$). In stage III-IV cases (treated by surgery and radiation therapy) this differences reached statistical signification (15 y DFS 72.4% vs 36.0% $p<0.020$). Similar results were found for Cause Especific Survival (15 y DFS 68.4% vs 26.2% $p<0.054$).

Conclusion These data show that the HPV status is a good predictor of DFS and survival in patients treated with radical surgery and adjuvant radiotherapy in oral carcinomas. This prognostic advantage seem to be independent of tumour proliferation, p53 status or angiogenesis. Other molecular processes could be implicated in the different response to radiotherapy.

Basic sciences oral session 2—Immunity and animal models

04-S2.01 THE HOST RESPONSE TO CHLAMYDIAL INFECTION RESULTS IN INCREASED GONOCOCCAL COLONISATION IN A NOVEL COINFECTION MODEL

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[1]A Jerse, [1]R Vonck, [2]T Darville. *[1]Uniformed Services University, Bethesda, USA; [2]University of Pittsburgh Medical Center, Pittsburgh, USA*

Objective *Neisseria gonorrhoeae* and *Chlamydia trachomatis* cause similar urogenital diseases and up to 70% of individuals with gonorrhoea also have chlamydia. Using a newly developed female mouse model of coinfection, we recently reported that higher numbers of *N gonorrhoeae* were recovered from mice with a pre-existing *Chlamydia muridarum* infection, the mouse strain of Chlamydia, compared to mice infected with *N gonorrhoeae* alone. Recent studies on the host response to *N gonorrhoeae* implicate toll-like receptor 4 (TLR4) and IL17 responses as being protective against *N gonorrhoeae*. Here we tested the hypothesis that the immune response to chlamydial infection makes the genital tract more permissive to *N gonorrhoeae*.

Methods Using an immune-targeted RT-PCR array we screened for alterations in host gene expression during chlamydial infection of BALB/c mice that may account for the observed increase in gonococcal colonisation. Mouse genital cells were collected by vaginal swab and analysed for TLR4 expression by flow cytometry. Coinfection studies were performed in BALB/cJ (TLR4 wild type) and C. C3-TLR4LPS-d/J (TLR4 mutant) mice and the number of viable chlamydiae and gonococci recovered from each group was determined by immunofluorescence using L929 cells and quantitative culture on GC agar, respectively.

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 TLR4expression in coinfected mice, markers of inflammation (TLR2, TNFα, IL-1ß, platelet activating factor receptor [Pafr], and IL-23α) 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.

O4-S2.02 SEROPREVALENCE OF NOVEL IMMUNOGENS OF CHLAMYDIA TRACHOMATIS AND THEIR CYTOKINE RESPONSE IN PBMC CELLS UNDER IN VITRO CONDITIONS

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D Saluja, A Patel, P Mishra, D Sachdev. *University Of Delhi, Delhi, India*

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 pkn1and DNA j following natural CT infection in human. The prospects of pkn1 as a Type third secretion substrate and DNA j a non surface Chlamydial protein as potential antigen, prompted us to explore the immunogenic potential of both protein.
Methods pkn, DNA j and ompA were cloned in bacterial expression vector pTrcHis. Ni+-NTA affinity chromatography was used to purify the recombinant proteins. Antigenic stretches of Pkn1, DNA j and OmpA were identified using Bcepred 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 serorecactivity 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-y levels upon stimulation of PBMC with Pkn1 and OmpA. Cytokine expression profiling (IL-1ß, TNF-α, IL-2 , IL10 and IFN-y) 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 Humoural 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.

O4-S2.03 AN ANTI-ADHESIVE APPROACH TO PREVENTION OF *C TRACHOMATIS* INFECTION

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L A Campbell, S V Zidal, D Patton, Cho-chou Kuo. *University of Washington Seattle, USA*

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.

O4-S2.04 CAN A CERVICAL BARRIER PREVENT CHLAMYDIA INFECTION IN THE PIGTAILED MACAQUE CERVICAL CHALLENGE MODEL?

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[1]D Patton, [1]Y Cosgrove Sweeney, [2]T Moench. *[1]University of Washington, Seattle, USA; [2]ReProtect, Inc, Baltimore, USA*

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.