Bologna, Italy

Background Chlamydia trachomatis is the most prevalent bacterial sexually transmitted infection worldwide. A strong link between serogroup/serovar and serological response has been suggested previously. This study aims to elucidate serovar specific serological responses in two independent Dutch patient groups using two serological assays.

Methods We performed genotyping of serovars in two patient groups of C. trachomatis infected patients (total n = 718). We pooled the two study populations to form one study group and within this group we analysed men and women separately. We used two commercially available ELISA kits (medac Diagnostika) to determine specific serum IgG levels.

Results Calculations of the kits to determine IgG concentrations were comparable and could therefore be pooled. We observed very significant differences when comparing the mean IgG titres of three serogroups, B, C, and I. In the female group B vs. C: p < 0.0001 (mean titres B 270.0 vs. C 8.8); B vs. I: p < 0.0001 (270.0 vs. 108.5).

Male group B vs. C: p = 0.0005 (190.2 vs. 69.6); B vs. I: p = 0.0002 (190.2 vs. 92.9); C vs. I was not significant. Serovars D and E of serogroup B induce the highest mean IgG titres compared to the other serovars in both men and women: 145.5 and 199.1 vs. ≤ 107.9 for men and 305.6 and 262.7 vs. ≤ 161.5 for women.

Conclusions This study shows a statistically significant higher serological response induced by B group serovars compared to the C and I group serovars in vivo in both men and women. This study is currently being extended with a different ethnic population and a different serological test.

P1.005 EVALUATION OF CYTOKINES AND MATRIX METALLOPROTEINASES GENES EXPRESSION IN GENITAL ORGANS AFTER VAGINAL EXPOSURE TO CHLAMYDIA MURIDARUM


1 A Marangoni, C Cavallini, C Foschi, P Nardini, R Aldini, A D’Errico, F Rosini, R Cevenini, 1University of Bologna, Microbiology, DIMES, Bologna, Italy; 2University of Bologna, Laboratory of Molecular Biology and Stem Cell Engineering, DIMES, Bologna, Italy; 3University of Bologna, FaBIt Dpt., Bologna, Italy; 4University of Bologna, DIMES, Bologna, Italy

Background Although the pathologic consequences of Chlamydia genital infection are well-established, the mechanisms leading to tissue damage are not completely understood.

Methods All the experiments were approved by the Ethical Committee of the University of Bologna. Animals used were 24 female Balb/c mice, 7 weeks old. All animals received medroxyprogesterone acetate 9 and 2 days prior the infection.

Twelve mice were infected by placing 15 µl of sucrose-phosphate-glutamic acid (SPG) buffer containing 10⁶ inclusion forming units (IFUs) of C. muridarum into the vaginal vault. Nine animals were inoculated with 15 µl of SPG containing heat-inactivated 10⁶ IFUs of C. muridarum. As controls of inflammation, 3 animals were challenged with 15 µl of SPG.

At 3, 10, and 20 days post-infection 4 infected animals, 3 animals inoculated with heat-inactivated bacteria and 1 control were sacrificed.

Genital tracts were divided into the cervical-vaginal region, uterine horns, and oviducts.

Right uterine horns and oviducts were stored in formalin and later processed for histological examinations. The remaining parts of the organs were used for RNA extraction, by using Trizol Reagent (Invitrogen), in combination with RNeasy Mini Kit (Qiagen).

cDNA was synthesised with SuperScript III RT (Invitrogen). Real-time RT-PCR was performed with SYBR Green Fast Start kit (Roche Diagnostics). Primers used to assess INF-γ, TNF-α, MMP-2, MMP-9 and GAPDH levels were from SuperArray (SABiosciences).

Results At histological examination no controls showed inflammation. On the contrary, scores of inflammation in all the organs from infected animals peaked at day 10, whereas only a single animal inoculated with inactivated bacteria showed a very mild inflammation at day 10 in its uterus.

At day 10, organs from infected animals showed significantly higher MMP-2 and MMP-9 genes expression than organs obtained from non-infected mice.

Conclusions Our study confirms the pivotal role of MMPs in the development of tissue damage.

P1.006 THE EFFECTS OF SYPHILIS ON CD4 CELL COUNTS AND PLASMA HIV-1 VIRAL LOADS AMONG PATIENTS WITH HIV-SYPHILIS CO-INFECTION


I Levy, Y Maor, V Litchovsky, G Rahav. Sheba Medical Center, Ramat Gan, Israel

Background Concomitant syphilis and human immunodeficiency virus (HIV) infection is increasingly frequent in industrialised countries. We examined the effect of active syphilis on CD4 counts and plasma HIV-1 viral loads.

Methods All patients with syphilis-HIV coinfection treated at the Sheba medical centre between 2007 and 2012 were included in the study. Patients were divided to those with early or secondary syphilis and to those with latent syphilis (early and late). CD4 cell counts and plasma HIV-1 viral loads were measured before, during and following syphilis treatment.

Results 17 patients were included: all were men having sex with men, 11 (65%) were treated with ART: 5 with primary syphilis, 4 patients with secondary syphilis, 2 patient with early latent and 6 patients with latent syphilis of unknown duration (rrp > 1:52), one of them with neurosyphilis. Median CD4 cell count significantly dropped during syphilis by 25%: from 575 (246–828) before syphilis diagnosis to 415 (195–630) cells/mm³ during syphilis (p = 0.0002). After penicillin treatment it rose back to 621 (250–1579) (p = 0.01).

Plasma VL in 6 of the patients that did not receive ART increased during syphilis, although this rise was not statistically significant.

Conclusions Syphilis was associated with a transient decrease in the CD4 cell count and with an increase in VL in HIV-infected men; This increase in VL, although statistically non significant, may explain at least partially the increased risk of HIV transmission in HIV patients not treated by ART that are co infected with syphilis.

P1.007 IMMUNE-RESPONSE IN LESIONAL SKIN OF SECONDARY SYPHILIS


M Cusini, D Fanoni, S Ramoni, E Berti. Dermatology Unit - Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy

Background It is well known that tissue immune response plays an important role in the pathogenetic mechanisms of several bacterial and viral infections. Several studies focused on the cutaneous host immune-response during infections by mycobacteria, HIV, HHVs, HCV and HSVG, while few data are known about cell-mediated immunity in skin syphilitic lesions.

Methods By an immunohistochemistry technique, we characterized the cutaneous inflammatory infiltrate in 5 cases of secondary syphilis, using a large panel of monoclonal antibodies (MAbs) and a specific polyclonal antibody against Treponema pallidum (TP) antigen.
**Poster presentations**

**P1.008 INTRASTRAIN GENETIC HETEROGENEITY IN TREPONEMA PALLIDUM SSP. PALLIDUM**


D Cejkova, M Strouhal, D Smajs. Masaryk University Brno, Brno, Czech Republic

**Background** Next-generation sequencing techniques, besides determination of complete genome sequences and interstrain variability, can assess variability within individual bacterial strains. Here we examined intrastrain heterogeneity in two strains of T. pallidum ssp. pallidum, the agent of syphilis.

**Methods** To ascertain intrastrain heterogeneity within Nichols and SS14 strains, whole-genome sequencing Illumina reads were used. Individual reads were mapped to complete genomes using Borrows-Wheeler Aligner. To determine the allele frequency of mapped reads for every genome position, the SAMtools package and Perl script were applied and heterogenous positions showing at least six independent reads calling for an alternative allele were further examined. To distinguish true variants from sequencing errors, each heterogenous position was visually evaluated.

**Results** Altogether, 17 and 31 intrastrain variable positions were found in the unique regions of Nichols and SS14 genomes, respectively. Only 2 positions in each strain were located in intergenic regions. The variable positions of the Nichols and SS14 strains altered 16 and 29 genes, respectively, while four genes (coding for DNA repair mechanism and motility were found. Neutral selection was detected mainly in genes coding for hypothetical proteins and genes encoding proteins involved in cell structure and cell processes, negative selection was predominantly found in genes encoding components of general metabolism, transport and translation. Neutral selection was detected mainly in genes encoding hypothetical proteins and genes encoding proteins involved in general metabolism.

**Conclusions** Our results, showing that syphilis is mainly composed by abundant plasmocytes and CD8+ lymphocytes in absence of dendritic Langerhans cells, suggest the presence of a characteristic immune-disregulation in the skin affected from TP infection. Further studies on a larger number of patients are needed in order to better clarify the exact immunological mechanisms in skin syphilitic lesions.

**P1.009 ANALYSIS OF SIMPLE SEQUENCE REPEATS IN THE GENOMES OF PATHOGENIC TREPONEMAL STRAINS**


M Strouhal, M Zobanikova, D Cejkova, I Petrosova, P Pospisilova, B Staudova, G M Weinstock, D Smajs. Masaryk University Brno, Brno, Czech Republic; Washington University in St. Louis, Saint Louis, MO, United States

**Background** Simple sequence repeats (SSR) are repetitive sequences of 1–6 base pairs in DNA which account for genotypic switching (phenotypic variation) through mechanism of polymerase slippage. This mechanism is common in pathogenic bacteria with reduced genome.

**Methods** Mistfinder v2.0.9 was used to identify SSR sequences in the genomes of five strains of Treponema pallidum subsp. pallidum (agent of syphilis); three strains of T. p. subsp. pertenue (agent of yaws); the Bosnia A strain of T. p. subsp. endemicum (agent of endemic syphilis); the Cuniculi A strain of T. pallidus subsp. pallidum (agent of rabbit syphilis); and the Fribour-Blanc strain (symian isolate genetically close to agent of yaws). Analysis of Solexa data and subsequent cloning approach were used for determination of intrastrain variability.

**Results** Sequence data analysis of 11 treponemal genomes revealed high degree of variability in guanine/cytosine homopolymeric tracts (G/C-regions) among pathogenic treponemal strains. Altogether, 120 G/C-regions (containing > 7 nucleotides) were found, from which 53 regions showed no variability, 26 represented nucleotide substitution differences, and 41 contained variable numbers of G/Cs. From these 41 regions, 25 were located in intergenic regions and 16 affected ORFs. Interestingly, variable regions were located upstream or within genes coding for Tpr proteins, potential virulence factors, and hypothetical proteins. Furthermore, 50 regions were specific for Cuniculi A strain, 5 regions differentiated pallidum strains from other strains, and 3 regions were specific for pertenue strains. Moreover, additional variability within treponemal strains was found in 54 G/C-regions.

**Conclusions** Inter- and intrastrain variability of G/C-regions may play a significant role in pathogenesis of treponemal diseases. Further experimental studies are needed to verify this hypothesis.

**P1.010 COMPARATIVE EVOLUTIONARY ANALYSES OF NINE TREPONEMA PALLIDUM AND TREPONEMA PARALUISCUNICULI STRAINS**


P Krecmerova, D Smajs. Masaryk University Brno, Brno, Czech Republic

**Background** Pathogenic uncultivable treponemes include the agents of syphilis (T. pallidum ssp. pallidum, TPA), yaws (T. pallidum ssp. pertenue, TPE) and endemic syphilis (T. pallidum ssp. endemicum, TEN). Treponema paralucniciuli (TPE) causes rabbit syphilis. Pathogenic treponemes are highly clonal bacteria showing minimal genetic variability in the genome sequence of individual strains.

**Methods** Five TPA strains (Nichols, SS14, Chicago, Mexico A and DAL-1), three TPE strains (CDC-2, Samoa D and Gauthier) and a one TPC strain Cuniculi A were analysed in this study. All possible combinations of gene alignments were tested on type of selection by Z-test using modified Nei-Gojobori method based on Jukes-Cantor model. Complete deletion was used as a gap treatment and transition/transversion ratio was set to 0.85. Results were considered significant at 5% level.

**Results** A total of 22 genes were found under positive selection in at least one comparison between treponemal strains. Negative and neutral selection was found in at least one combination for 238 and 206 genes, respectively. While positive selection was identified in genes encoding putative virulence factors, proteins involved in cell structure and cell processes, negative selection was predominantly found in genes encoding components of general metabolism, transport and translation. Neutral selection was detected mainly in genes encoding hypothetical proteins and genes encoding proteins involved in general metabolism.

**Conclusions** Positively selected genes are candidates for important treponemal virulence factors while negatively selected and conserved genes are likely to encode essential genes. Genes under neutral evolution may indicate genome regions that could be lost during adaptation of pathogen to its host.