Abstracts

P08 - Chlamydia infections

**P08.01** CHARACTERISING CD4+ AND CD8+ T-CELL RESPONSES BY INTRACELLULAR CYTOKINE STAINING IN WOMEN WITH AND WITHOUT CHLAMYDIA TRACHOMATIS REINFECTION

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**Background** Chlamydia trachomatis (CT) infection is the most prevalent bacterial sexually transmitted infection worldwide and, untreated, can lead to significant reproductive morbidity. Unfortunately, the prevalence remains high in the United States and no effective CT vaccine exists, in part because of an inadequate knowledge of immunological responses to CT infection in humans and, specifically, no correlates of protective immunity to guide vaccine studies. In animal studies, IFN-γ and/or TNF-α producing CD4 cells are known to mediate protection against C. trachomatis. The objective of this study was to characterise the T-cell mediated immune responses to C. trachomatis in humans with and without CT reinfection at a follow up clinic visit.

**Methods** In an ongoing study, peripheral blood mononuclear cells (PBMCs) are collected from CT-infected women at the time of treatment and stimulated in vitro with C. trachomatis antigens MOMP or PGP3, then fixed and permeabilized. The percentage of CD4+ and CD8+ T-cells expressing either IFN-γ or TNF-α is then assessed using intracellular cytokine staining (ICCS) and flow cytometry. Women return at 3- and 6-months for repeat genital chlamydia screening. Cytokine-specific ICCS responses are then compared in women with versus without subsequent CT reinfection at follow-up to assess for possible correlates of protection.

**Results** To date, we have performed ICCS on 44 women who have completed the study. Compared with women who had CT reinfection at a follow-up visit (n = 14), women without CT reinfection (n = 30) had higher baseline positive CD8+ TNF-α responses (p = 0.042). There was no significant association of IFN-γ and/or TNF-α CD4 responses with CT reinfection risk.

**Conclusions** Our preliminary findings reveal that a TNF-α-producing CD8+ T-cell response appears to correlate with a decreased risk for CT reinfection, suggesting a possible role in protective immunity. CD4 T-cell responses have not significantly differed in women with versus without CT reinfection.

**Disclosure of interest statement** Nothing to declare.

**P08.02** CHLAMYDIA TRACHOMATIS CAUSES MITOCHONDRIAL DAMAGE IN KERATINOCYTES

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**Introduction** Chlamydia trachomatis causes two different sexually transmitted diseases: genital discharge (GD) and lymphogranuloma venereum (LGV). Apart from differences in tissue tropisms, little is known about differences in pathogenicity to the cells they infect, especially keratinocytes, the primary target of infection for LGV chlamydia.

**Methods** Human keratinocytes (HaCaT cells), one LGV (serovar L2) and one GD (serovar E) isolate were used for all experiments with uninfected cells as the negative control. Experiments were performed at 37 and 33°C. For transmission electron microscopy (TEM) cells grown on Thermaxx coverslips within 24-well plates were infected (MOI = 0.25) and incubated for various time-points over 48 h. The diameter of 15 mitochondria were measured in uninfected and infected cells at 1, 36 and 48 h post-infection using image analysis software. For the MTT assay cells grown in 96-well plates were infected (MOI = 0.25) and incubated for various time-points over 48 h. Median mitochondrial diameter was compared using Kruskal-Wallis nonparametric ANOVA with Dunn’s post-test. Percentage mitochondrial activity was compared using two-tailed paired T test. P ≤ 0.05 was considered significant.

**Results** The GD isolate alone caused mitochondrial swelling, cristae rearrangement and a pale mitochondrial matrix at 1, 36 and 48 h post-infection at 37°C but not 33°C. Median mitochondrial diameter was 430 nm in uninfected cells; 477 nm, 470 nm and 1221 nm after 1, 36 and 48 h post-infection respectively for serovar E; and 343 nm, 420 nm and 392 nm after 1, 36 and 48 h respectively for L2. Mitochondrial diameter was significantly larger in serovar E-infected cells 48 h post-infection compared to uninfected cells (P < 0.001) or L2-infected cells (P < 0.01). The MTT assay indicated a significant (P = 0.0373) reduction in mitochondrial activity upon infection with serovar E, but not serovar L2 at 37°C.

**Conclusion** C. trachomatis of the GD biovar but not the LGV biovar causes transient mitochondrial swelling and a decrease in mitochondrial activity in infected keratinocytes at 37°C.

**Disclosure of interest statement** This study is supported by the National Research Foundation of South Africa. The authors have no conflict of interest to declare.

**P08.03** MINING GENOME OF CHLAMYDIA TRACHOMATIS TO IDENTIFY VACCINE CANDIDATES

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**Introduction** Chlamydia trachomatis, an obligate intracellular parasite, is a major cause of genital infections in human. The pathogen is sexually transmitted and responsible for serious reproductive and other health problems. Despite the availability of antibiotic therapy, infection rates are increasing worldwide. This is mainly due to the asymptomatic nature of most infections and the lack of effective screening programs. Vaccination is therefore considered to provide the best means of controlling chlamydial infection. Attempts to vaccinate with whole-cell vaccine or with purified chlamydial proteins eliciting CD4+ T cells and/or antibodies have failed as they provide only partial protection in animal models. Thus, identification of protective antigens that could be used either as an alternative to those already characterised or in combination with them is a high priority in chlamydial vaccine development.