Abstract

Background: *Chlamydia trachomatis* is the most prevalent sexually transmitted bacterial infection with 129 million new cases globally in 2018. Chlamydial infection can lead to serious sequelae in women when the pathogen ascends to the upper genital tract, such as pelvic inflammatory disease and infertility. Interferon-gamma producing CD4+ T cells (Th1 cells) are critical for resolution of infection and protection against reinfection. To better understand how Th1 cells protect against chlamydial infection, we developed a T cell receptor (TCR) transgenic mouse (TP1) with CD4+ T cells that recognize a single antigen in both human *C. trachomatis* strains and *Chlamydia muridarum* that naturally infected mice. The objectives of this study were to (i) determine if TP1 cells primed by live-attenuated *C. muridarum* (CM972) reduced cervical burden and protected mice against the development of oviduct hydrosalpinx after virulent *C. muridarum* (CM001) cervicovaginal challenge and (ii) to determine the specific chlamydial antigen recognized by TP1 cells. We hypothesized that TCR alpha knockout ($TCR\alpha^{-/-}$) mice with adoptively transferred TP1 cells primed by CM972 immunization would exhibit similar protection from burden and oviduct pathology as observed in wild-type CM972-immune mice upon challenge with virulent CM001.

Methods: Wild-type mice, $TCR\alpha^{-/-}$ mice, and $TCR\alpha^{-/-}$ mice with adoptively transferred TP1 cells ($TCR\alpha^{-/-}$ + TP1) were infected via intravaginal (IVAG) inoculation. Six weeks later, these three groups and wild-type naïve group were infected IVAG with virulent CM001. Cervical CM001 burden was monitored following primary (CM972) and secondary (CM001) infections. Gross oviduct pathology was determined based on the frequency of hydrosalpinx following CM001 challenge. Splenocytes isolated from CM972-immune $TCR\alpha^{-/-}$ + TP1 mice were stimulated with the chlamydial antigens PmpF, MOMP, OmcB, PmpG, CPAF, and Hsp60 and the numbers of IFN-γ producing cells were determined by ELISpot.

Results: $TCR\alpha^{-/-}$ mice with adoptively transferred TP1 cells that were immunized with CM972 had significantly reduced CM001 burden over the course of infection by 1.7 $\log_{10}$ compared to wild-type naïve mice after challenge. Despite reduced burden, immunity delivered by memory TP1 cells did not prevent oviduct hydrosalpinx. However, in wild-type CM972-immune mice, burden was reduced by 2.52 $\log_{10}$ (correct?), and oviduct hydrosalpinx was reduced. Antigen screening revealed that TP1 cells were specific for polymorphic membrane protein F (PmpF), an adhesin important for chlamydial entry.

Conclusion: PmpF-specific TP1 cells primed by CM972 infection were not as effective at reducing burden or preventing oviduct pathology, compared to wild-type CM972-primed polyclonal T cells. However, memory TP1 cells reduced bacterial burden by 1.7-logs compared to mice with naïve wild-type cells. This result indicates that PmpF could play an integral role in the design of an efficacious *C. trachomatis* vaccine. Future studies will determine the specific PmpF epitope recognized by TP1 cells and determine the efficacy of a recombinant PmpF vaccine in pre-clinical studies.