Determining the efficacy of a ChAdOx1-vectored Chlamydia vaccine

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Abstract

Chlamydia trachomatis is the causative agent of chlamydia, the most common sexually transmitted bacterial infection worldwide[1], which is estimated to infect 336 million people globally[1]. These infections are often asymptomatic and can lead to serious sequelae like pelvic inflammatory disease, pregnancy complications, and infertility, making a vaccine urgently needed[2]. We have developed a vaccine against the mouse pathogen *C. muridarum* that uses a modified chimpanzee adenovirus, to express the candidate antigen chlamydial protease-like activity factor (ChAdOx1.CPAF). Our preliminary data revealed that high-dose intramuscular ChAdOx1.CPAF prime-boost immunization reduced the amount of infectious *Chlamydia* 10-fold over the course of infection in mice. As part of our ongoing NIH U19-funded Chlamydia Vaccine Initiative (CVI), we further determined that ChAdOx1.CPAF is effective at reducing burden in C57BL/6 mice (P=0.0448). Further, we demonstrated that IFN-γ producing CD4 T cells are a main correlate of protective immunity against *Chlamydia* in mice, while CD8 T cells are less essential. These data suggest future vaccines should elicit high frequencies of IFN-γ producing CD4 T cells to reduce *Chlamydia* burden and protect against oviduct disease.

Methods

Immunogenicity:
- Two weeks post boost, mice were euthanized from immunized groups for CPAF-specific T cell responses (ELISpot/ICS)

Challenge:
- Mice were intravaginally infected with *C. muridarum* (CM006) 30 days post boost
- Infection was monitored by quantifying infection forming units (IFUs) in lower genital tract swabs
- Mice were euthanized day 42 post-challenge for assessment of oviduct dilatation and CPAF-specific T cell responses (ICS)

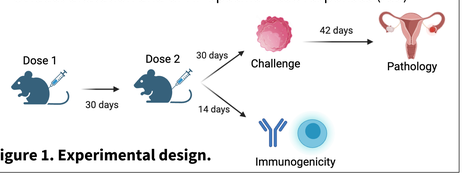

Figure 1. Experimental design.

References & Acknowledgments

(1) Newman, L., et. al. (2015). Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PLOS ONE, 10(12). https://doi.org/10.1371/journal.pone.0143304

(2) Centers for Disease Control and Prevention. (2021, January 25). Chlamydia at a glance. Centers for Disease Control and Prevention. Retrieved January 30, 2023, from https://www.cdc.gov/std/statistics/prevalence-2020-at-a-glance.htm

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Project Goals

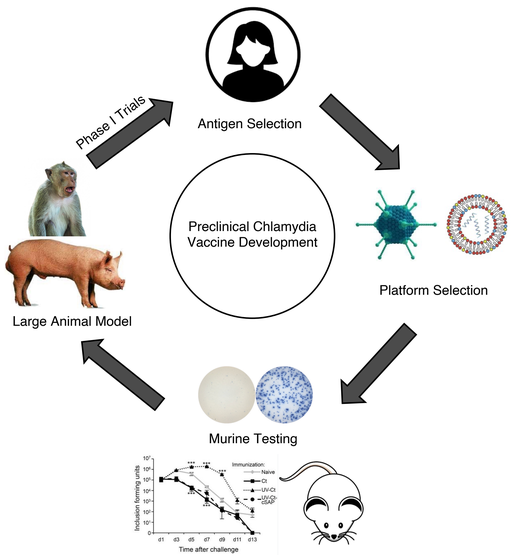
As part of our ongoing NIH U19-funded CVI, this project will further determine the efficacy of ChAdOx1.CPAF vaccination in C57BL/6 mice and identify the cellular components driving protection using mice deficient in CD4 T cells or CD8 T cells.

Hypothesis

IFN-γ producing CD4 T cells are the main correlate of protective immunity in ChAdOx1.CPAF immunized mice.

Background

- *Chlamydia trachomatis* is the most common sexually transmitted bacterial infection in the world[1] and in the United States[2].
- Chlamydia is estimated to infect 336 million people globally.[1]
- These infections are often asymptomatic and can lead to serious sequelae like pelvic inflammatory disease, pregnancy complications, and infertility, making a vaccine urgently needed.[2]
- Our preliminary data revealed that i.m. ChAdOx1.CPAF prime-boost immunization reduced the amount of infectious Chlamydia by 1-log over the course of infection in C57BL/6 mice (data not shown).
- Our preliminary data also revealed that CD8 T cells are the primary producers of IFN-γ in intramuscular ChAdOx1.CPAF prime-boost immunized C57BL/6 mice (Figure 2).
- Determining the cell types driving protective vaccine responses are critical for designing and optimizing an effective human vaccine.



Results

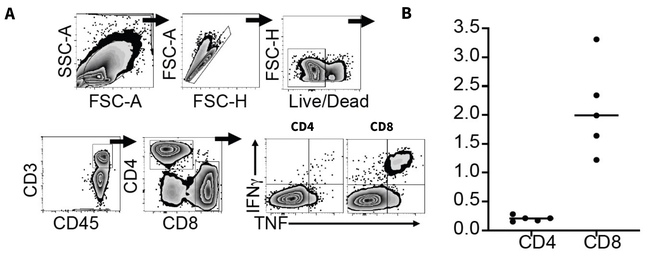


Figure 2. Gating strategy and CPAF-specific CD4 and CD8 T cell responses by Intracellular Cytokine Staining (ICS). C57BL/6 (n=5) mice were immunized with ChAdOx1.CPAF following the schedule in Figure 1. ICS for IFN-γ and TNF-α was performed on splenocytes. (A) Gating strategy. (B) Frequency of cytokine producing T cells.

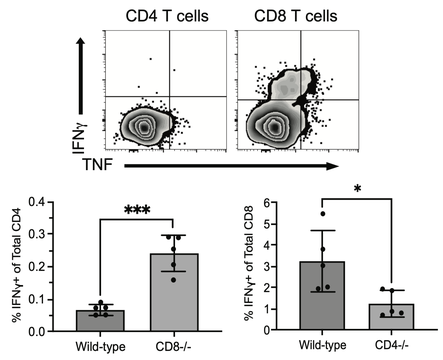


Figure 4. ChAdOx1.CPAF immunization elicits greater IFN-γ+ CD4 T cell responses in CD8 deficient mice compared to wild-type mice and greater IFN-γ+ CD8 T cell responses in wild-type mice compared to CD4 deficient mice. Representative cytokine-positive responses (top). Frequency of CPAF-specific CD4 (left) and CD8 (right) T cell IFN-γ responses in wild type, CD8-/-, and CD4-/- mice (n=5/group) determined by ICS. Mean with SD depicted. Significance determined by student's t-test. ***p=0.0002, *p=0.021

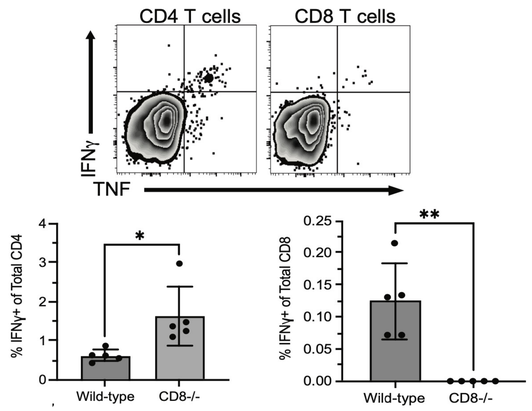


Figure 6. CM006 infection leads to greater IFN-γ+ CD4 T cell responses in immunized CD8 deficient mice compared to immunized wild-type mice. Comparison of IFN-γ+ CD4 T cell responses in immunized wild-type and CD8-/- mice before and after challenge (left) and comparison of IFN-γ+ CD8 T cell responses in immunized wild-type mice before and after challenge (right).

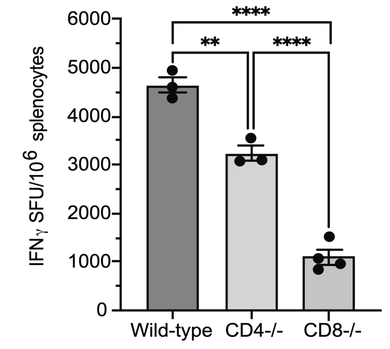


Figure 3. ChAdOx1.CPAF immunization elicits more IFN-γ+ T cells in wild-type mice than in CD4 or CD8 deficient mice. CPAF-specific IFN-γ+ T cell responses from wild-type and immunodeficient C57BL/6 mice determined by ELISpot. Mean with SD depicted. Statistical significance determined by one-way ANOVA.

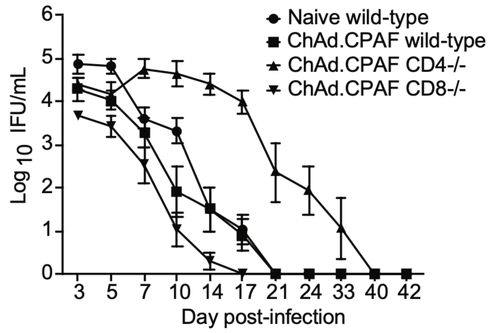


Figure 5. Immunized CD8 deficient mice cleared infection faster with reduced burden compared to wild-type immunized mice. PBS immunization represents negative control. IFUs were quantified in lower genital tract swabs 3-41 days post infection. Statistical significance determined by two-way RM ANOVA with post-hoc Tukey test. *p=0.0448 for wild-type immunized versus naive (-0.29 log); ****p<0.0001 for CD4-/- immunized versus wild-type immunized (1.14 log); ***p=0.0004 for CD8-/- immunized versus wild-type immunized (-0.44 log). ****p<0.0001 for CD8-/- immunized versus naive (-0.74 log). ****p<0.0001 for all other comparisons.

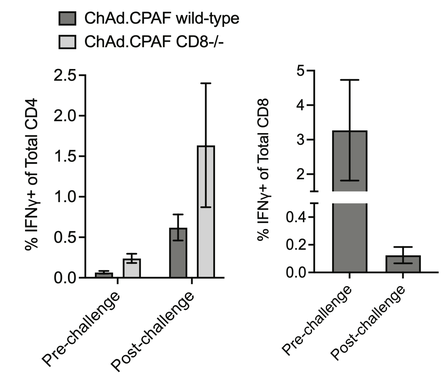


Figure 7. CM006 infection selectively boosts vaccine-elicited CD4 T cells in wild-type mice. Comparison of IFN-γ+ CD4 T cell responses in immunized wild-type and CD8-/- mice before and after challenge (left) and comparison of IFN-γ+ CD8 T cell responses in immunized wild-type mice before and after challenge (right).

Conclusions

- ChAdOx1.CPAF is immunogenic in wild-type C57BL/6 mice and elicits a CD8 dominant T cell response.
- CD8 deficiency leads to significantly greater CPAF-specific CD4 T cell responses after immunization.
- ChAdOx1.CPAF immunization reduced bacterial shedding by 1-log and shortened duration of infection in wild-type C57BL/6 mice.
- CD4 T cells are necessary for *C. muridarum* clearance in ChAdOx1.CPAF immunized C57BL/6 mice.

Future Directions

We are pursuing vaccine platforms that elicit high frequencies of IFN-γ producing CD4 T cells with the goal of reducing chlamydia burden and protecting against oviduct pathology.