Assessing Humoral and Cell-Mediated Immune Responses Induced by the Chimpanzee Adenovirus-based Plague Vaccine ChAdOx1 in a Nonhuman Primate Model

Charles Costanzo[1], Tiffany Chang[1], Xiaoying Yu[1], Emily Hendrix[2], Paul Kilgore[2], Ashok Chopra[2]
1. Department of Biostatistics and Data Science 2. Department of Microbiology and Immunology

Background

- *Yersinia pestis*, the causative agent of plague, is a deadly, highly invasive bacterium endemic in many regions globally.
- Currently classified by the WHO as a reemerging pathogen, plague outbreaks are increasing with climate change and rodent carrier range shifts.
- *Y. pestis* is classified as a Tier 1 select agent by the CDC due to its bioterrorism potential and high mortality rate.
- Dangerous multiple antibiotic-resistant strains of *Y. pestis* occur naturally or have been developed intentionally as a biothreat agent.
- As there is no FDA-approved human plague vaccine, there is a critical need for new-generation plague vaccine development.
- **This project aims to contribute to this need by assessing the cell-mediated and humoral immune responses induced by the Chimpanzee adenovirus-based plague vaccine ChAdOx1 developed by Oxford University, UK, in collaboration with UTMB in a nonhuman primate (NHP) model as this is closest to humans.**

Methods

- Blood serum samples collected from ten African green monkeys (*Chlorocebus sabaeus*) were analyzed.
- NHPs were randomly assigned to vaccine and control groups split evenly by sex.
- Enzyme-linked immunosorbent assay (ELISA) was conducted to determine Immunoglobulin G (IgG) antibody titer levels in preimmune, prime, and boost NHP sera reacting to either F1 capsular antigen or the type 3 secretion system component and effector LcrV (low-calcium-response V antigen).
- Interferon-gamma (IFNγ) ELISpot assay was conducted in triplicate to measure the total number of F1-V specific spot forming units (SFUs) per 500,000 cells from boost sera.
- F1-V is a fusion protein containing both F1 and LcrV antigens.

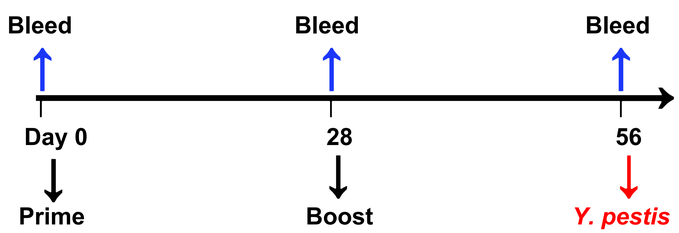

Figure 1: Immunization Scheme
Vaccinated NHPs (n = 6) were immunized intramuscularly (i.m.) with ChAdOx1 on days 0 and 28. Control NHPs (n = 4) received doses of phosphate-buffered saline i.m. on days 0 and 28. Preimmune and prime sera were collected on days 0 and 28, respectively, prior to immunization. Boost serum was collected on day 56 prior to challenge with *Y. pestis*.

Statistical Analysis

- R (Version 4.2.2) and the R packages "permuco" and "MASS" were used to run permutation tests and negative binomial regression models.
- Significant differences are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$) in each figure.
- **Humoral Immune Response:** Because the assumptions required to perform analysis of variance (ANOVA) do not hold, permutation tests for repeated measures ANOVA were used followed by pairwise comparison and adjustment for multiple testing using Bonferroni's correction.
- **Cell-Mediated Immune Response:** Negative binomial regression models were fit to determine if significant associations exist between the count of normalized F1-V specific SFUs per 500,000 cells and vaccination status, sex, and their interaction. Only the vaccination status main effect was retained in the final model due to non-significance of the sex main effect and interaction effect.

Results

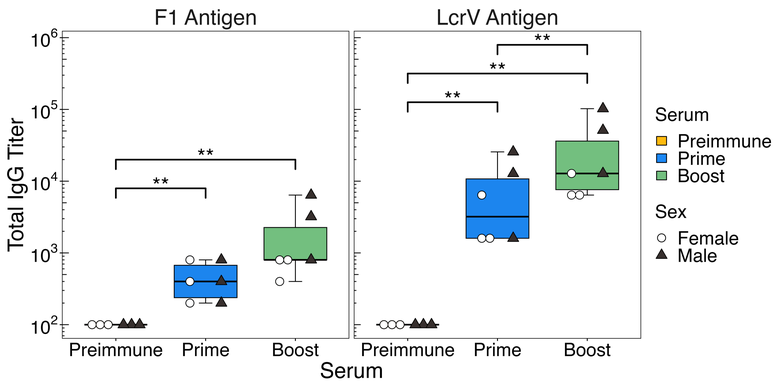

Figure 2: Statistical Analysis of Humoral Immune Response
Antigen-specific total IgG antibody titers. Each symbol represents the value for an individual NHP (n = 6) in preimmune, prime, or boost serum. Control group NHPs (n = 4) maintained titer values of 100 and are not included in this analysis. Values that are significantly different by permutation tests for one-way repeated measures ANOVA with a Bonferroni correction are indicated by bars and asterisks as follows: **, $P < 0.01$.

- Both prime and preimmune sera reacting to the F1 antigen have significantly different IgG antibody titers relative to preimmune serum. However, there is no significant difference in IgG titers between prime and boost serum.
- Both prime and preimmune sera reacting to the LcrV antigen have significantly different IgG titers relative to preimmune serum. There is also a significant difference in IgG titers between prime and boost serum.

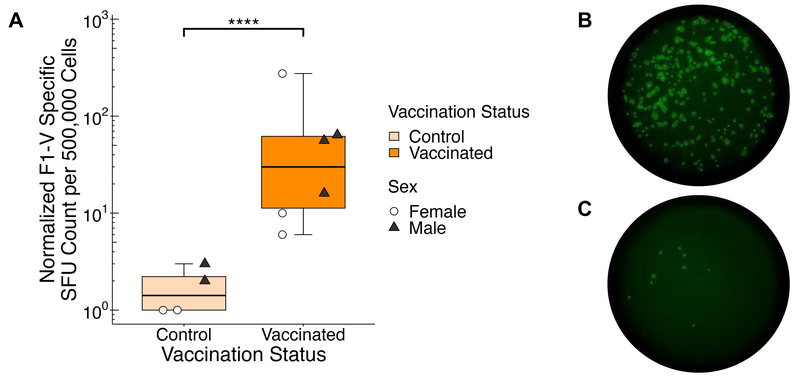

Figure 3: Statistical Analysis of Cell-Mediated Immune Response
(A) Normalized F1-V specific SFU counts per 500,000 seeded cells. Each symbol represents the count for an individual NHP (n = 10) in boost serum. Counts from three F1-V stimulated technical replicates were normalized by subtracting the mean count from two unstimulated technical replicates. Values were then averaged and rounded to the nearest integer. Negative binomial regression results show a significant association between vaccination and SFU count, indicated by a bar and asterisks as follows: ****, $P < 0.0001$. F1-V stimulated ELISpot plate from a (B) vaccinated and (C) control NHP.

- There is an estimated 40.7-fold increase in the mean count of F1-V specific SFUs in vaccinated NHPs relative to the control group, 95% CI: [8.78, 180.56].

Conclusion

- These results indicate that the ChAdOx1 vaccine elicits significant cell-mediated and humoral immune responses in nonhuman primates, which are essential for providing protection against the challenge pathogen *Y. pestis*.
- Further studies are needed to demonstrate that ChAdOx1 could be considered as an optimal human vaccine candidate.

References

- Andersson JA, Fitts EC, Kirtley ML, Ponnusamy D, Peniche AG, Dann SM, Motin VL, Chauhan S, Rosenzweig JA, Sha J, Chopra AK. 2016. New role for FDA-approved drugs in combating antibiotic-resistant bacteria. *Antimicrob Agents Chemother* 60:3717–3729. doi: 10.1128/AAC.00326-16.
- Ponnusamy D, Fitts EC, Sha J, Erova TE, Kozlova EV, Kirtley ML, Tiner BL, Andersson JA, Chopra AKC. High-throughput, signature-tagged mutagenic approach to identify novel virulence factors of *Yersinia pestis* CO92 in a mouse model of infection. *Infect Immun.* 2015;83:2065–2081. doi: 10.1128/IAI.02913-14.
- Rosenzweig JA, Hendrix EK, Chopra AK. 2021. Plague vaccines: new developments in an ongoing search. *Appl Microbiol Biotechnol* 105:4931–4941. doi: 10.1007/s00253-021-11389-6.
- Tiner BL, Sha J, Kirtley ML, Erova TE, Popov VL, Baze WB, van Lier CJ, Ponnusamy D, Andersson JA, Motin VL, Chauhan S, Chopra AK. Combinational deletion of three membrane protein-encoding genes highly attenuates *Yersinia pestis* while retaining immunogenicity in a mouse model of pneumonic plague. *Infect Immun.* 2015;83(4):1318–1338. doi: 10.1128/IAI.02778-14.

Acknowledgments

- Research reported in this poster was supported by The University of Texas Medical Branch Summer Institute in Biostatistics and Data Science (UTMB-SIBDS) NIH/NHLBI grant #R25HL161715 and NIH/NIAID grant #R01AI153524, with additional funding from Innovative Group UK, Oxford University.