

A PROMISING MUCOSAL PROTEIN VACCINE AGAINST TUBERCULOSIS



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BACKGROUND

Tuberculosis (TB) represents a significant challenge to global health, particularly in endemic countries where the burden of the disease is the highest. Multistage vaccines represent an innovative advancement in the fight against TB. In order to achieve multistage protection against *Mycobacterium tuberculosis* (*Mtb*) infection, we investigated the host response to 85A *Mtb* antigen (Ag85A), the most expressed protein during the initial stages of TB infection, and Rv2626c *Mtb* antigen, a protein expressed under hypoxic conditions. To do this, we analyzed the potential of both recombinant proteins to be employed as mucosal vaccines. Our group has previously reported that individuals with latent tuberculosis infection (LTBI) produce significantly higher levels of IFN- γ against Rv2626c as compared to patients with active TB and healthy donors, suggesting that this protein induces cellular immunity in individuals during the latency stage of *Mtb* infection (D. Peña et al. 2015). Therefore, we proposed to test Rv2626c as a vaccine candidate. On the other hand, the immunogenic capacity of Ag85A has been widely reported (M. Karbalaei Zadeh Babaki et al. 2017), and for this reason we included this antigen (Ag) in our study.

Unlipidated outer membrane protein 19 (U-Omp19) is a mucosal adjuvant that stimulates immune cells and is also capable of inhibiting host gastrointestinal and endosomal proteases (ML. Darriba. 2021). These two main properties may explain the adjuvant activity of U-Omp19: it protects co-delivered Ags from degradation; increases the half-life of the Ags and promotes their arrival at the induction sites. Therefore, U-Omp19 would enhance the adaptive immune response induced by co-administered Ags.

AIM

➤ To study the protective capacity of the protein vaccine candidate administered with mucosal adjuvant U-Omp19 by intranasal and sublingual routes.

METHODS

Protein vaccine: BALB/c mice were initially immunized with Rv2626c (5 μ g) and Ag85A (5 μ g) recombinant proteins together with the U-Omp19 adjuvant by the sublingual (sl) or intranasal (in) routes (three doses every 7 days) and after 4 weeks the animals were challenged with the pathogenic H37Rv *Mtb* strain by intratracheal inoculation. Thirty days post infection, the spleen and lungs were aseptically removed and *Mtb* CFU counting was performed. Mice were immunized with PBS or BCG (*M. bovis* BCG Danish – 5x10⁴ CFU) as negative and positive controls, respectively.

MVA vaccine: BALB/c mice were initially immunized using two doses of MVA2626c (1x10⁵ CFU) together with the U-Omp19 adjuvant delivered by sl or intramuscular (im) routes. Ten days after the last immunization, splenocytes were obtained and stimulated with recombinant Rv2626c or CD4⁺T cell-specific Rv2626c peptides. Then, IFN- γ production was measured by ELISA or Flow cytometry. Moreover, a group of experimental mice was challenged with the H37Rv *Mtb* strain and afterward, CFUs were determined in the spleens and lungs. Mice were immunized with PBS or BCG (*M. bovis* BCG Danish – 5x10⁴ CFU) as negative and positive controls respectively.

RESULTS

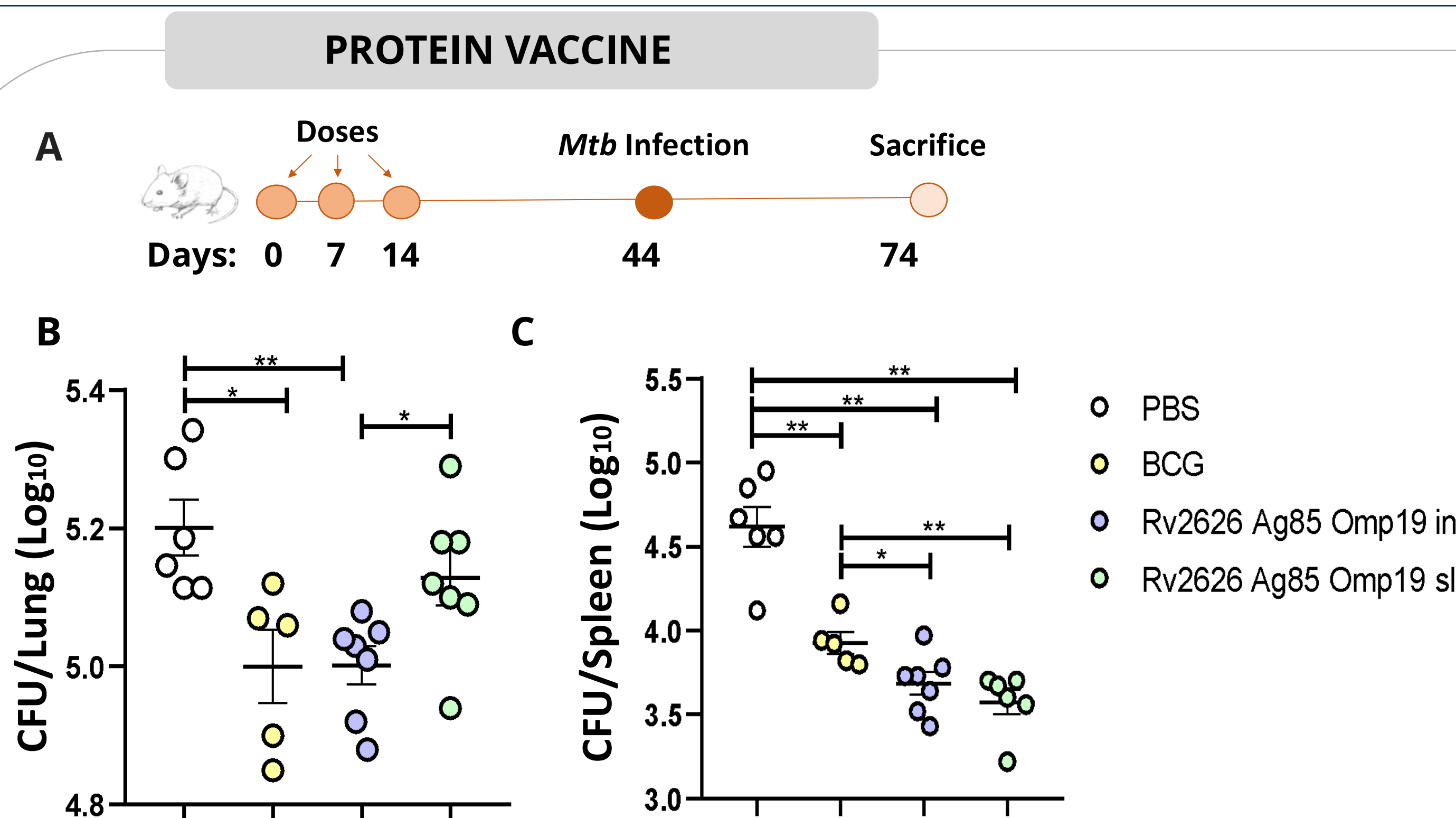


Figure 1. (A) Experimental design. CFUs in lungs (B) and spleens (C) were analyzed by culturing tissue homogenates, thus determining the number of bacteria in them. Each point represents the Log CFU/organ for each animal. Comparisons among groups were determined by Mann Whitney test. $p < 0.05$ was considered statistically significant. * $p < 0.05$ and ** $p < 0.01$.

The Rv2626c+Ag85A+U-Omp19 vaccine preparation administered by the intranasal route induced significant higher protection in mice lungs as compared to the sublingual way.

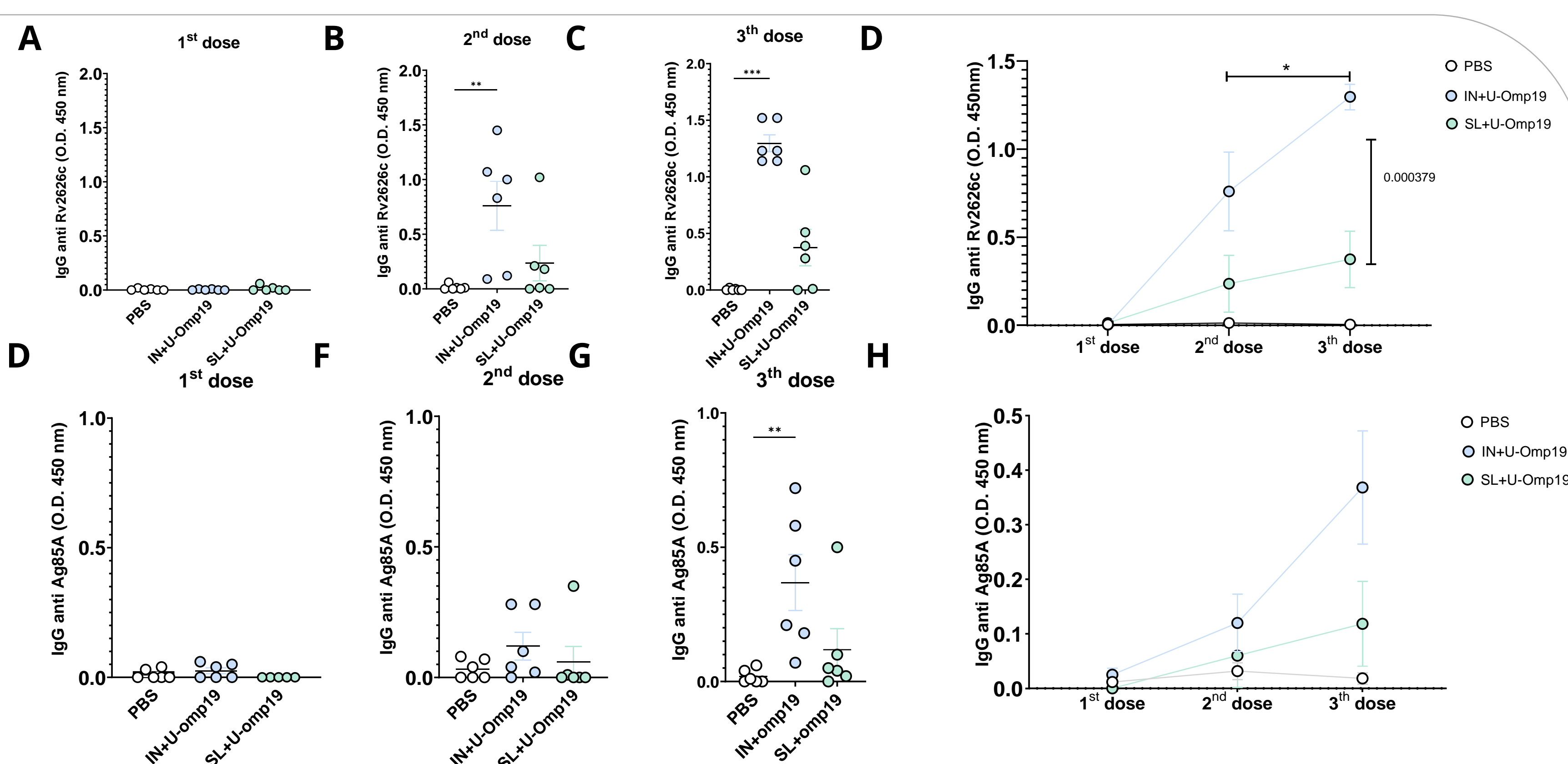


Figure 2. IgG antibody responses to Rv2626c and Ag85A immunization in mice as determined by ELISA. O.D. at 450 nm was measured to determine the antibody titers of IgG. A-D: IgG anti Rv2626c, E-H: IgG anti-Ag85A. Statistical analyses were performed using the Wilcoxon test (*) (G and H) and comparisons among groups were determined by Mann Whitney test. $p < 0.05$ was considered statistically significant. * $p < 0.05$ and ** $p < 0.01$.

Increasing antibody titers of IgG anti-Rv2626c and IgG anti-Ag85A were observed in Rv2626c+Ag85A IN+U-Omp19 and Rv2626c+Ag85A SL+U-Omp19 after all three immunizations.

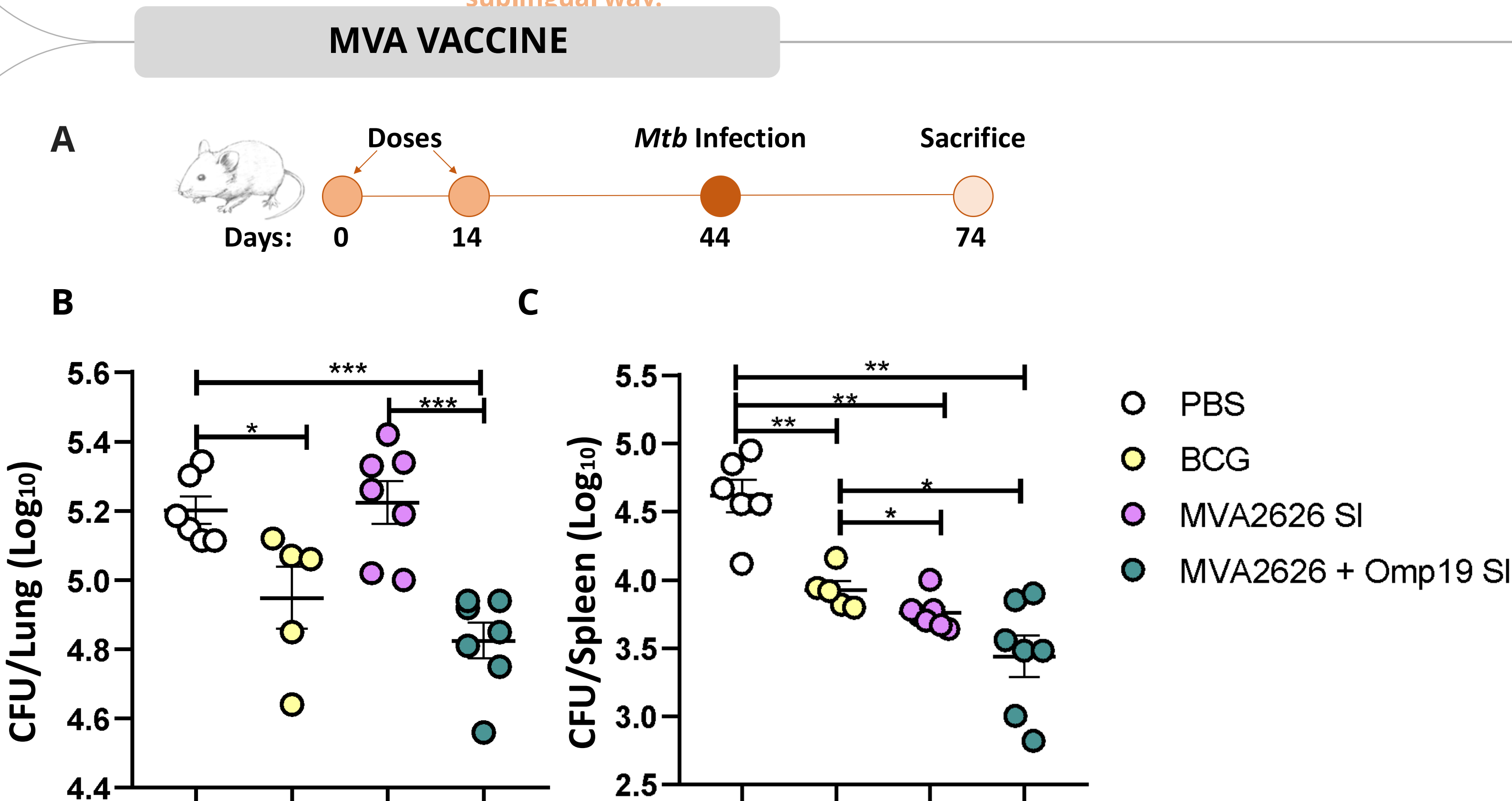


Figure 3. (A) Experimental design. CFUs in lungs (B) and spleen (C) from the animals were analyzed by culturing lung or spleen homogenates, and determining the number of bacteria. Each point represent the Log CFU/organ for each animal. Comparisons among groups were determined by Mann Whitney test. $p < 0.05$ was considered statistically significant. * $p < 0.05$ and ** $p < 0.01$.

Although sublingual administration of MVARv2626c preparation did not confer protection against H37Rv *Mtb*, the incorporation of U-Omp19 as an adjuvant significantly decreased the number of CFUs in mice lungs.

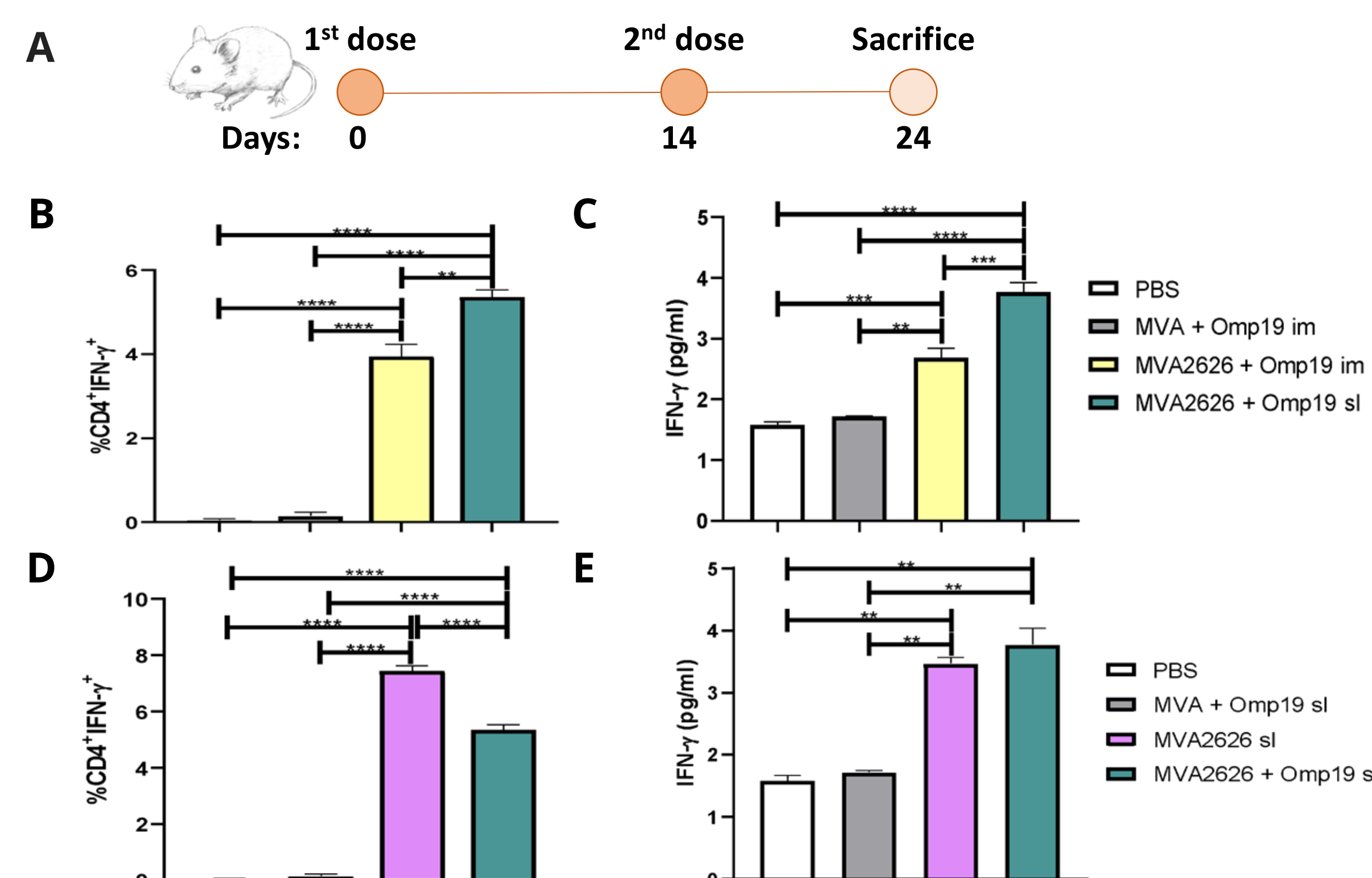


Figure 4. (A) Experimental design. IFN- γ production was determined by flow cytometry. (B and D) Each bar represents the mean (PBS subtracted) of the %CD4⁺IFN- γ ⁺ cells \pm SEM and (C and E) IFN- γ production was determined by ELISA. Each bar represents the mean of IFN- γ production (PBS subtracted) \pm SEM. Comparisons among groups were determined by one-way ANOVA followed by Turkey multiple comparison test. $p < 0.05$ was considered statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

The MVARv2626c+U-Omp19 vaccine formulation administered by the sublingual route significantly enhances the immune response of the host to Rv2626c. Less, the presence of U-Omp19 in the formulation significantly increased the percentage of CD8⁺IFN- γ ⁺ cells but markedly decreased the percentage of CD4⁺IFN- γ ⁺ cells.

DISCUSSION/CONCLUSION

➤ Our results show that diverse mucosal routes of vaccine administration induce different responses in the mice host.

➤ We found that the levels of antibodies directed against Rv2626c and Ag85A are higher in the groups that received U-Omp19, which also exhibited lower numbers of *Mtb* CFUs, indicating that the adjuvant could contribute to the efficacy of vaccines administered through mucosal routes against *Mtb* infection.

➤ The MVA2626c candidate vaccine preparation administered via the sublingual route did not confer protection against *Mtb* infection. However, the MVARv2626c+U-Omp19 candidate vaccine formulation administered by the sublingual route significantly enhanced the immune response of the host to Rv2626c.

➤ Overall, our studies open new strategies for the development of more effective and accessible vaccines in the fight against this disease.