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Novel Tuberculosis (TB) Oral Vaccine Candidate: Enhancing Mucosal Immunity with Recombinant Secretory IgA (SIgA) in Goat Milk

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BACKGROUND

Mucosal vaccines are designed to mimic the natural route of infection, which is crucial for pathogens like *Mycobacterium tuberculosis*. By delivering vaccines intranasally or orally, these vaccines can stimulate immune responses directly at mucosal surfaces, where the pathogen first encounters the host (1).

Evaluating IgA responses in mucosal vaccine development is crucial for enhancing local immunity and blocking pathogen entry (2,3).

To develop a mucosal vaccine, we are investigating chimeric secretory IgA (sIgA) as a vaccine carrier, which is produced through mammary gland transduction. Our approach, which targets three key TB-related epitopes—Ag85B (active TB), alpha crystalline (latent TB), and RpfE (reactivation TB), is intended to boost mucosal immunity, and the vaccine can be easily administered via milk.

Aim: The primary objective is to evaluate the IgA response, key indicator of mucosal immunity.

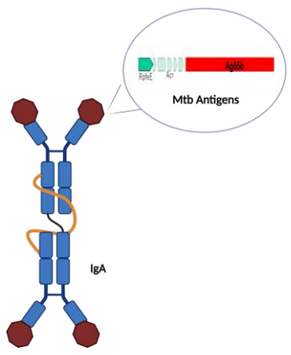

Figure 1. Schematic illustration of current platform. (Patent number: PI2021000909)

PROJECT OVERVIEW

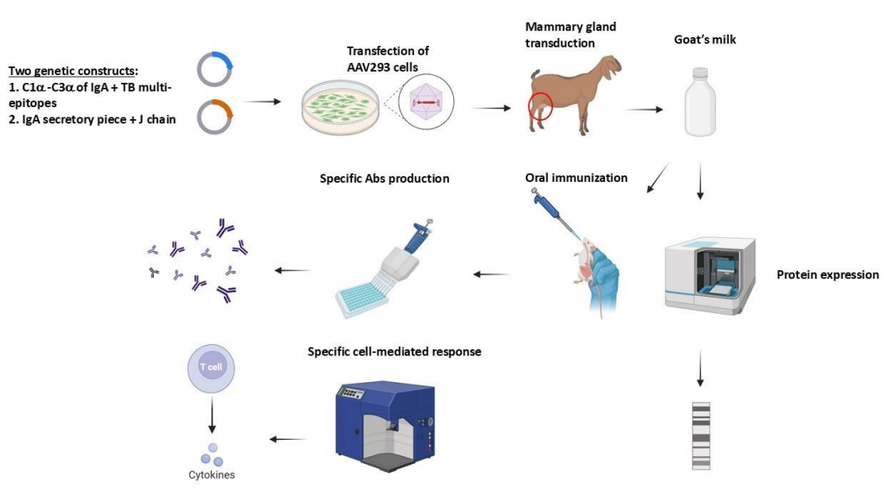

Figure 2. Research overview.

METHODS

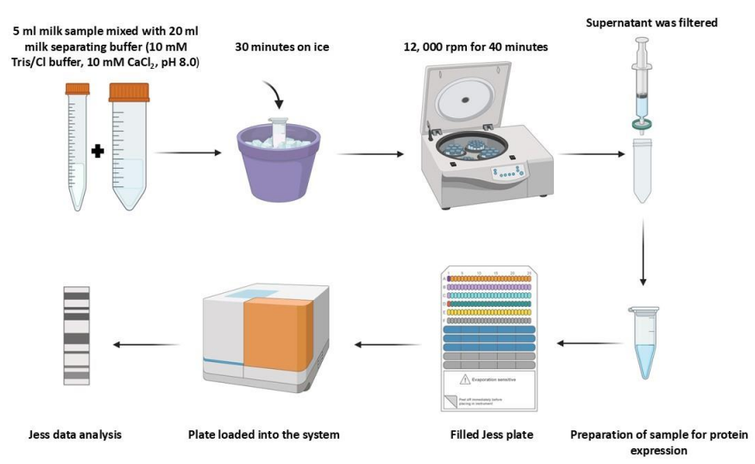

Figure 3. Sample processing for determination of protein expression in milk.

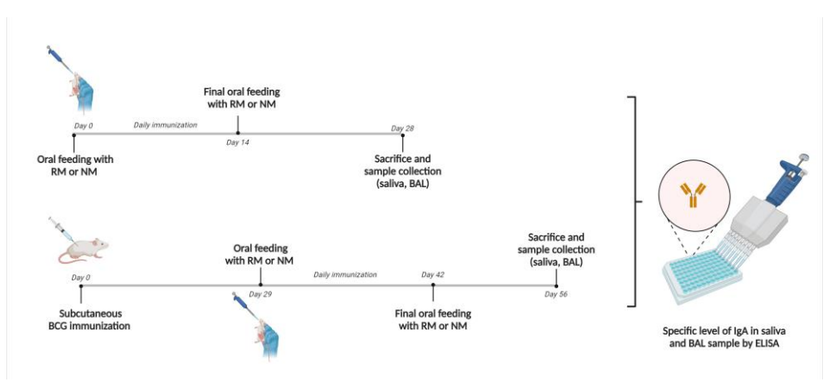

Figure 4. Immunization schedule. Five groups of Balb/C mice (n=5) were categorized as follows: recombinant milk (RM), normal milk (NM), BCG prime with RM boost (BCG-RM), BCG prime with NM boost (BCG-NM), and BCG alone (BCG). No of animal ethics approval: USM/IACUC/2023/(140)(1263)

RESULTS

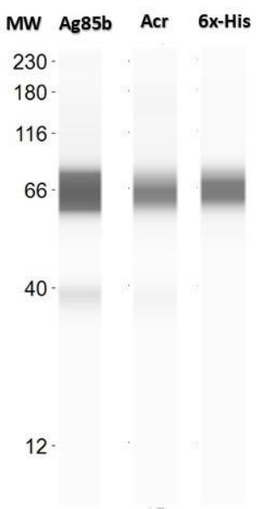

Figure 5. Jess analysis of recombinant protein expression in milk. Ag85B and Acr serve as vaccine epitopes, and 6x-His acts as the tag for the protein construct. Molecular weight (MW) markers are indicated on the left (in kDa). Clear bands around 66 kDa suggest successful expression of the tagged constructs.

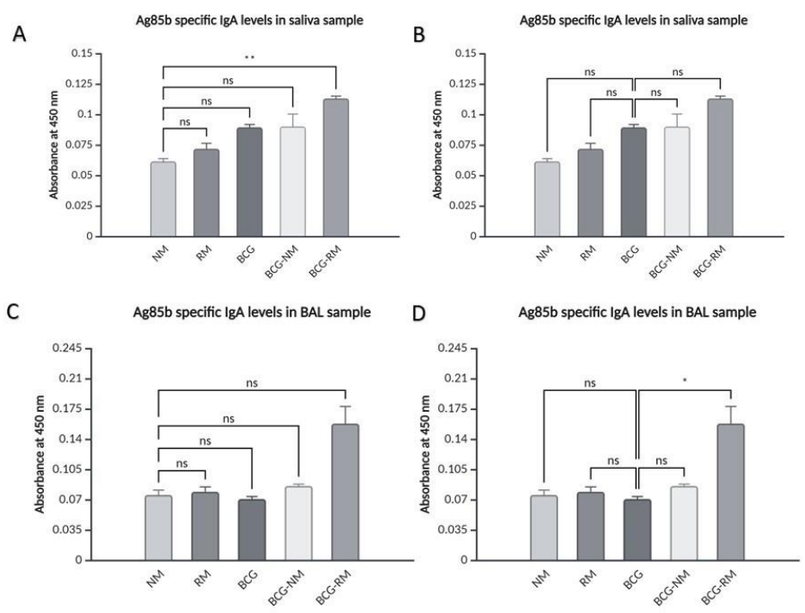

Figure 6. Comparison of Ag85B-specific IgA Antibody Levels in Saliva and BAL Samples Across Vaccination Groups

IgA levels were assessed in both saliva (A, B) and bronchoalveolar lavage (BAL) (C, D) samples using an ELISA assay to measure Ag85B-specific IgA. Five groups were compared: normal milk (NM), recombinant milk (RM), BCG, BCG+NM, and BCG+RM. Data are expressed as the mean ± SEM. N = 5 per group.

- **(A)** Comparison of Ag85B-specific IgA levels in saliva samples, using NM as the control group. Statistical significance was observed between NM and BCG-RM groups ($p < 0.01$).
- (B) Comparison of Ag85B-specific IgA levels in saliva samples, using BCG as the control group. No statistically significant differences were observed between the groups.
- (C) Comparison of Ag85B-specific IgA levels in BAL samples, using NM as the control group. No significant differences were observed between the groups.
- (D) Comparison of Ag85B-specific IgA levels in BAL samples, using BCG as the control group. A significant difference was observed between BCG and BCG-RM groups ($p < 0.05$).

Statistical analysis was performed using the Kruskal-Wallis test. The significance levels are indicated as follows: *p < 0.05, **p < 0.01; ns = not significant

CONCLUSION

Our study demonstrates that recombinant vaccine-containing milk from the mammary gland of non-transgenic goat enhances mucosal immunity with oral immunization. The significant increase in IgA levels in mice, particularly in BCG-primed groups, highlights the potential of this vaccine as a booster candidate with BCG.

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