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Immunogenicity Study of Chimeric Secretory IgA: TB Multi-Epitopes Protein as Vaccine Candidate in Development of Mucosal Vaccine Against Tuberculosis

Keh Min Xuan[1], **Nur Ain Mohd Asri**[1], **Mohd Nor Norazmi**[1,2], **Maryam Azlan**[1], **Frank Camacho**[3,4], **Rapeah Suppian**[1]

[1]School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, 16150, Kelantan Darul Naim, Malaysia

[2]Malaysia Genome and Vaccine Institute, National Institutes of Biotechnology Malaysia, 43000 Kajang, Selangor Darul Ehsan, Malaysia.

[3]Recombinant Biopharmaceuticals Laboratory, Pharmacology Department, School of Biological Sciences, Universidad de Concepcion, Concepcion, Chile

[4]Center for Biotechnology and Biomedicine Spa, Concepcion, Chile

kmxuan@yahoo.com

INTRODUCTION & AIM

Tuberculosis (TB), caused by Mycobacterium tuberculosis (M.tb), remains one of the leading infectious diseases worldwide. Considering Mycobacterium tuberculosis (Mtb) primarily infects the lungs, it may be more effective to match the route of infection to the route of vaccination when developing TB vaccines, besides stimulate both systemic and mucosal immunity.

The chimeric protein consists of two IgA molecules assembled with one joining chain (JC) to form dimeric IgA, which is stabilized by the secretory component (SC). It also incorporates three tuberculosis-specific epitopes: Antigen 85B (Ag85B) associated with active TB, alpha-crystallin (Acr) linked to latent infection, and resuscitation-promoting factor E (RpfE) involved in TB reactivation.

Aim: To determine the immunogenicity response through intranasal immunization of mice with the chimeric protein

Objectives:

1. To determine humoral immune response by ELISA
2. To evaluate cell-mediated immune response by flow cytometry
3. To evaluate cytokines production level (IFN-γ, TNF-α, IL-2)

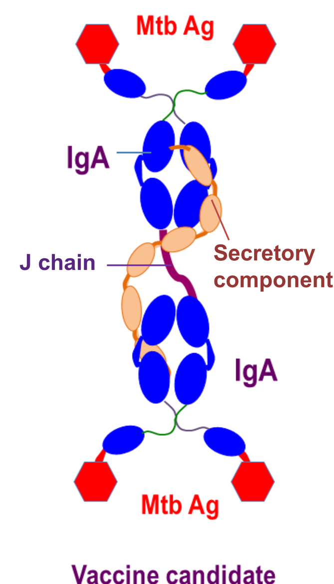

Figure 1 :Schematic illustration of current platform (Patent number: PI2021000909)

METHOD

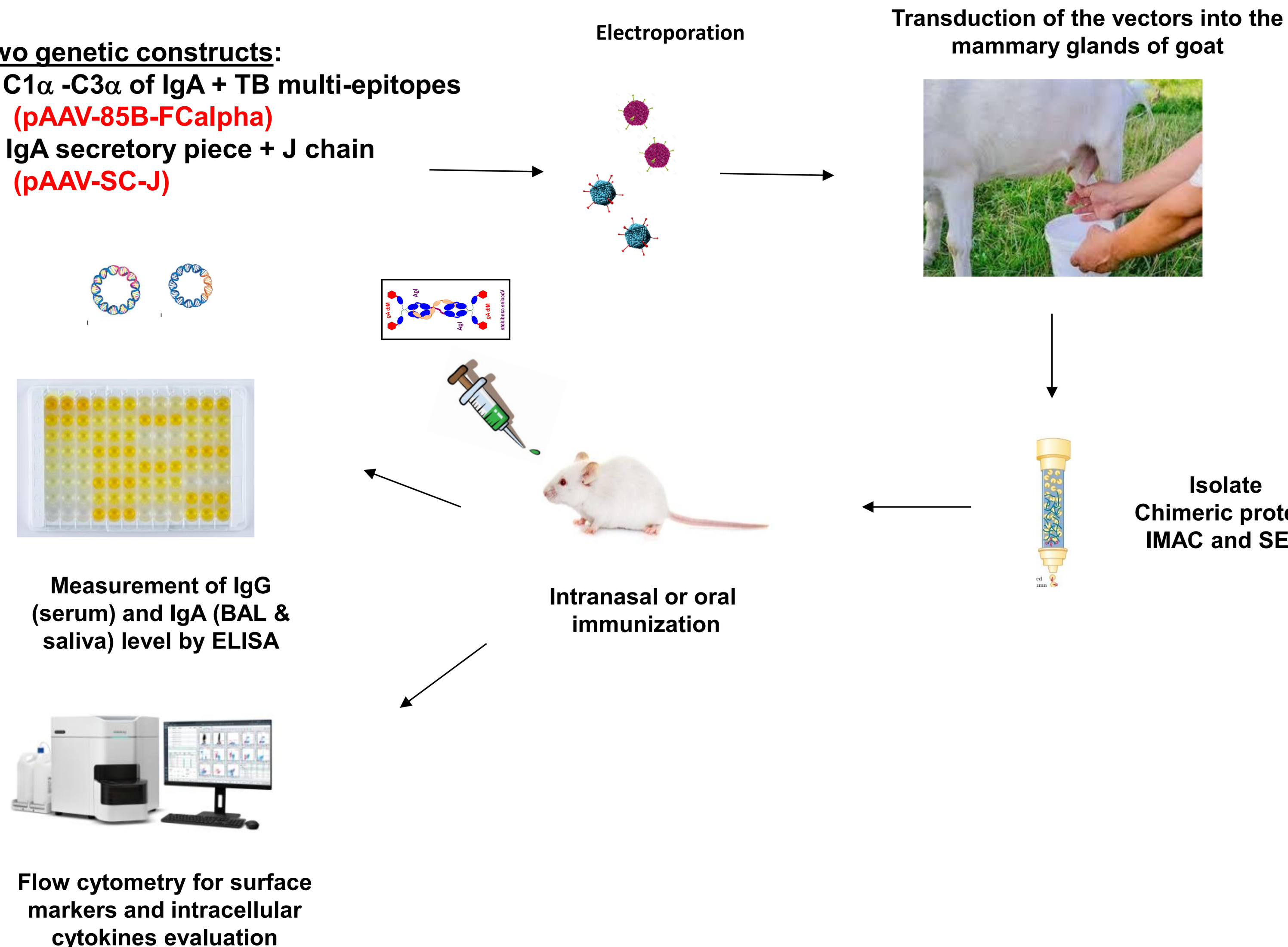

Figure 2: Project overview.

Groups	Day 0 (BCG Prime)	Week 4 (1st Immunization)	Week 5 (2nd immunization)	Week 6 (3rd immunization)	Week 8
PBS only (n=5)		10 µL of PBS	10 µL of PBS	10 µL of PBS	Sacrifice
BCG-PBS (n=5)	BCG: 100 µL of 2x10^6 CFU (Subcutaneous route)	10 µL of PBS	10 µL of PBS	10 µL of PBS	Sacrifice
mIgAm protein (n=5)	-	10 µL of 10 µg/ml of mIgAm protein	10 µL of 10 µg/ml of mIgAm protein	10 µL of 10 µg/ml of mIgAm protein	Sacrifice
BCG-prime + mIgAm protein-boost (n=5)	BCG: 100 µL of 2x10^6 CFU (Subcutaneous route)	10 µL of 10 µg/ml of mIgAm protein	10 µL of 10 µg/ml of mIgAm protein	10 µL of 10 µg/ml of mIgAm protein	Sacrifice

Figure 3: Immunization schedule. PBS is given a total of 10 µl (5 µl per nostril) by intranasal route. Protein with the concentration of 10 µg/ml is given a total of 10 µl (5 µl per nostril) by intranasal route

RESULTS & DISCUSSION

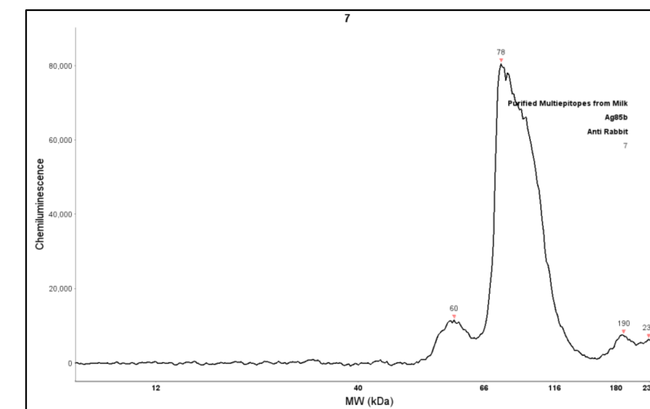

Figure 4. Expression of chimeric protein against Anti-Ag85B using automated Western blot System Jess^{TM}. The protein was loaded at concentration of 0.74mg/ml. A peak corresponding to multi-epitopes purified protein mAgmFc-alpha was detected at molecular weight of 78 kDa. This shows the expression of Ag85B epitope in the chimeric protein.

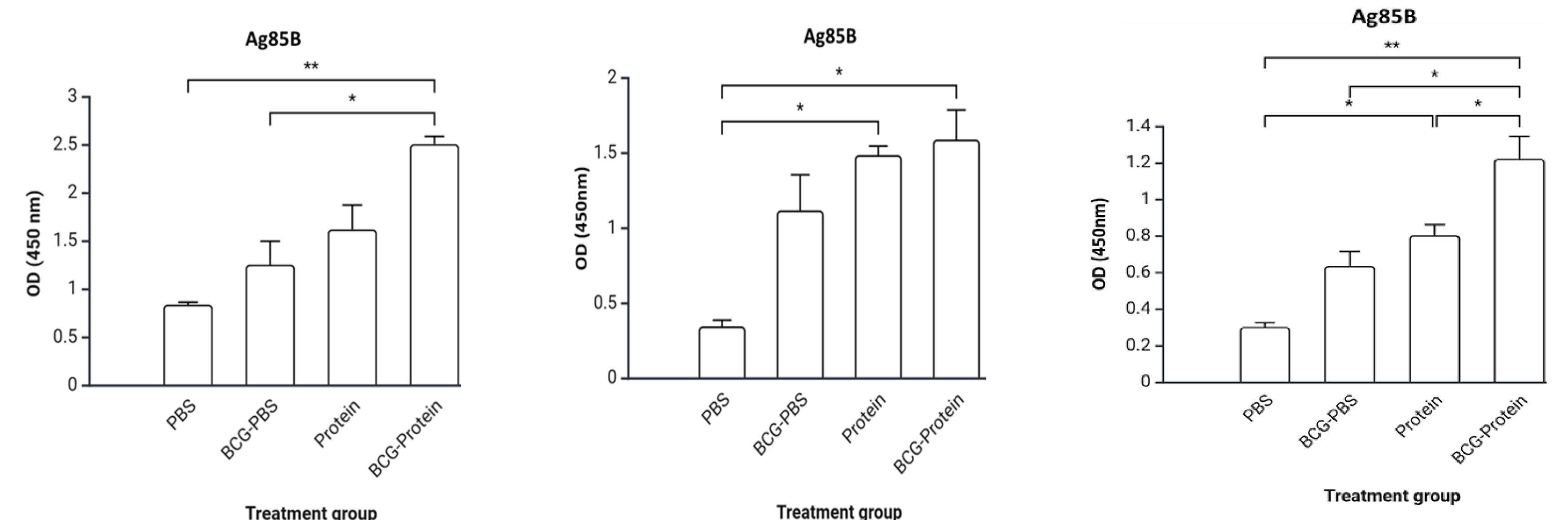

Figure 5: IgG in serum, IgA in saliva, and IgA in Bronchoalveolar lavage (BAL) samples were assayed against Ag85B antigen by ELISA. BALB/c mice (n=5 per group) were immunized with either phosphate-buffered saline (PBS), BCG-PBS, protein or BCG-protein. Data are expressed as triplicate means ± standard error of the mean. Significant differences between groups are indicated with asterisks measured by one-way ANOVA and Tukey's multiple comparison tests. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

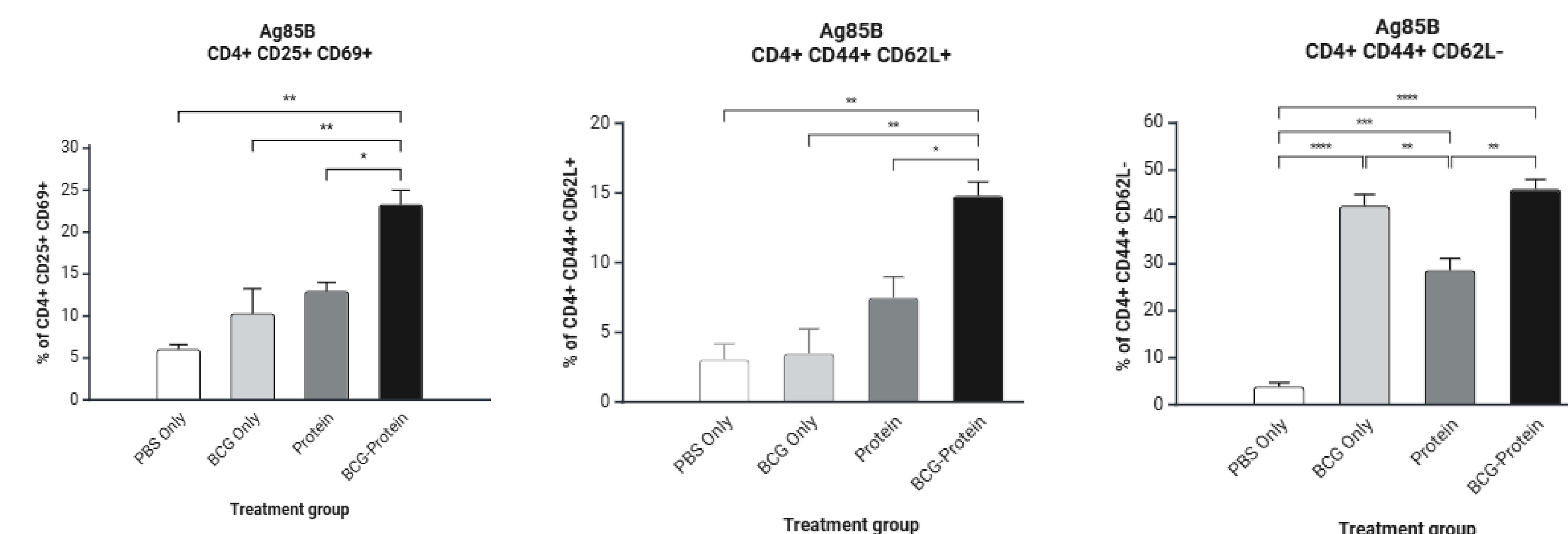

Figure 6. Expression of T helper cell activation, central memory T cell, and effector memory T cell markers from CD4+ T cells after 72 hours of stimulation with Ag85B. Results are shown in mean ± standard error of the mean (n=5) according to One-Way ANOVA test with Tukey test. P<0.05 was considered statistically significant. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

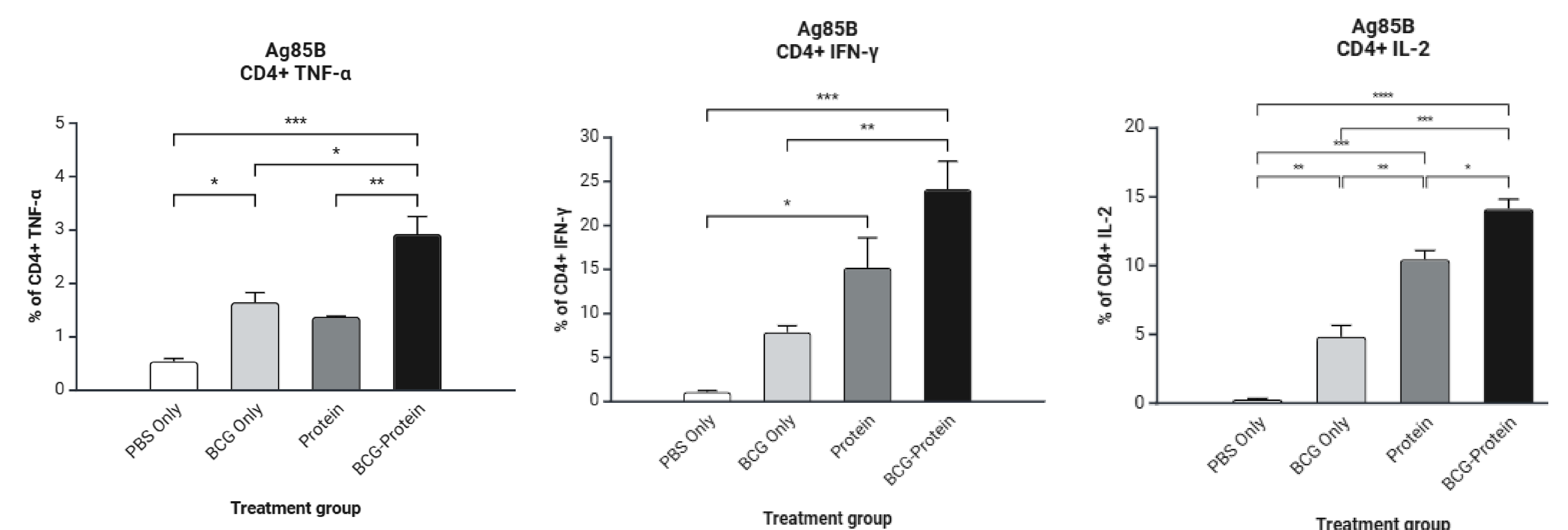

Figure 7: Percentages of CD4+ T cells producing TNF-α, IFN-γ and IL-2 cytokines after 72 hours of stimulation with Ag85B according to One-Way ANOVA test with Tukey test. Results are shown in mean ± standard error of the mean (n=5). P<0.05 was considered statistically significant.

CONCLUSION

The purified protein elicited both cell-mediated and humoral immune responses in Balb/c mice. Notably, these immune responses were significantly enhanced when the vaccine candidate was administered using a BCG prime-boost regimen.

FUTURE WORK / REFERENCES

- Prove that intranasal administration of purified protein is protective against virulent strain, H37Rv.
- Conduct GLP toxicity and safety studies of the mouse version in mice.
- Challenge studies in mice with clinical isolates (Harlam and Beijing).
- Conduct non-GLP studies for toxicity, safety and challenge studies in NHP.