No.	K23-3044	
研究課題名	Development of a mucosal vaccine against Strep A infections	
研究代表者	Loh Jacelyn (University of Auckland・Senior Research Fellow)	
研究組織	受入教員	石井 健 (東京大学医科学研究所・教授)
	分担者	Jacelyn Mei San Loh (University of Auckland, Department of Molecular Medicine and Pathology - Infection and Immunity・Senior Research Fellow)
	分担者	Catherine Jia-Yun Tsai (University of Auckland, Department of Molecular Medicine and Pathology - Infection and Immunity・Research Fellow)
	分担者	Kohtaro Fujihashi (Chiba University Hospital, Department of Human Mucosal Vaccinology・Project Professor)
	分担者	Yoshikazu Yuki (Chiba University Hospital, Department of Human Mucosal Vaccinology and HanaVax・Project Professor)

(Form 2)

Principal Investigator
Jacelyn Loh

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Project Completion Report

Report

Streptococcus pyogenes (Strep A) is a major human pathogen. It is responsible for a range of diseases, such as pharyngitis, skin and soft tissue infections, toxic shock syndrome, and acute rheumatic fever, with at least 18.1 million cases of severe disease and over 500,000 deaths each year. Although several Strep A vaccine candidates have been reported, none have passed phase II clinical trials.

We have developed a recombinant protein vaccine against this pathogen called TeeVax, that targets the T-antigen of the Strep A pilus. In our IMSUT international joint research project we successfully formulated TeeVax with cCHP nanogel and conducted an immunogenicity study in mice in the laboratory of Professor Fujihashi. TeeVax-cChP and control formulations were delivered by the intranasal (IN) or sublingual (SL) routes, and immunogenicity was assessed.

Results from this study showed that the TeeVax-cChP formulation delivered via the IN route produced superior serum IgG/IgA and mucosal IgA compared to non-adjuvanted or cholera toxin B (CTB)-adjuvanted vaccines (Fig. 1).

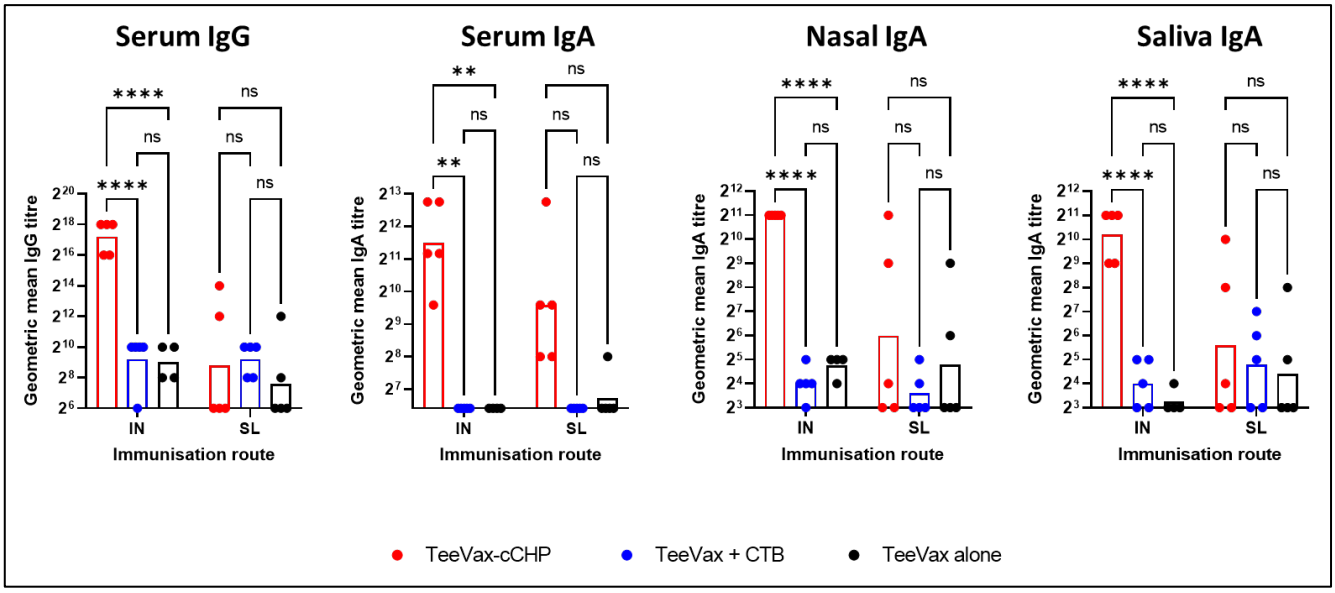

Fig. 1. Immunogenicity of TeeVax vaccination. Balb/c mice (n=5) were immunised with TeeVax-cCHP, TeeVax+CTB, or TeeVax alone at weekly intervals using either IN or SL delivery. One week after the third immunization, biological samples were collected and analysed by ELISA against TeeVax antigens. Symbols indicate the log2 titre from an individual animal, bar indicates the geometric mean titre. Statistical analysis by 2way ANOVA (**** $P < 0.0001$).

The next step was to test the efficacy of the TeeVax-cCHP nanogel in a challenge model. Although we originally planned to test this vaccine only in our colonisation model, we extended our studies with some co-funding from the Maurice Wilkins Centre (New Zealand) to test also in our invasive disease model.

TeeVax-cCHP was administered intranasally into mice using a 3-dose weekly schedule, then challenged 2 weeks later with Strep A intranasally (colonisation model) or subcutaneously (invasive disease model). Bacterial load or survival was measured, respectively (Figure 2). Results from these studies showed that TeeVax-cCHP significantly reduced bacterial burden in the colonisation model, but did not significantly enhance protection from invasive disease (Figure

2). However, it was observed in the invasive disease model that male mice succumbed to infection very rapidly compared to female mice (Figure 3). When stratifying the survival rates by sex, a trend was observed that immunization with TeeVax-cCHP improved survival compared to TeeVax alone in female mice (Figure 3). A larger sample size will be required in the future to confirm this sexual dichotomy in protection.

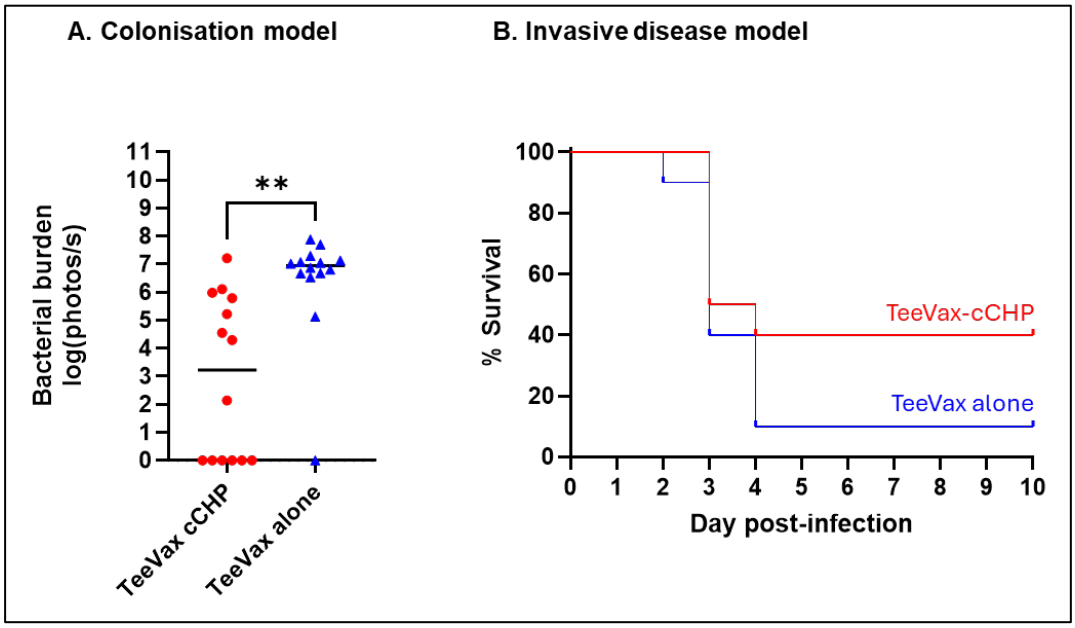

Figure 2. Protection in challenge models. A) FVB/n mice (n=14) were immunised 3 times at weekly intervals and then challenged intranasally with bioluminescently-labelled StrepA. Bacterial burden in the nasopharynx was measured by biophotonic imaging and the percentage drop in signal from the initial infection was calculated. Statistical analysis by 1-way ANOVA (** $P < 0.01$). B) Humanised plasminogen mice (n=10) were immunised 3 times at weekly intervals and then challenged subcutaneously with StrepA. Mice were euthanized at pre-determined humane endpoints, and survival analysis was performed by Log-rank test.

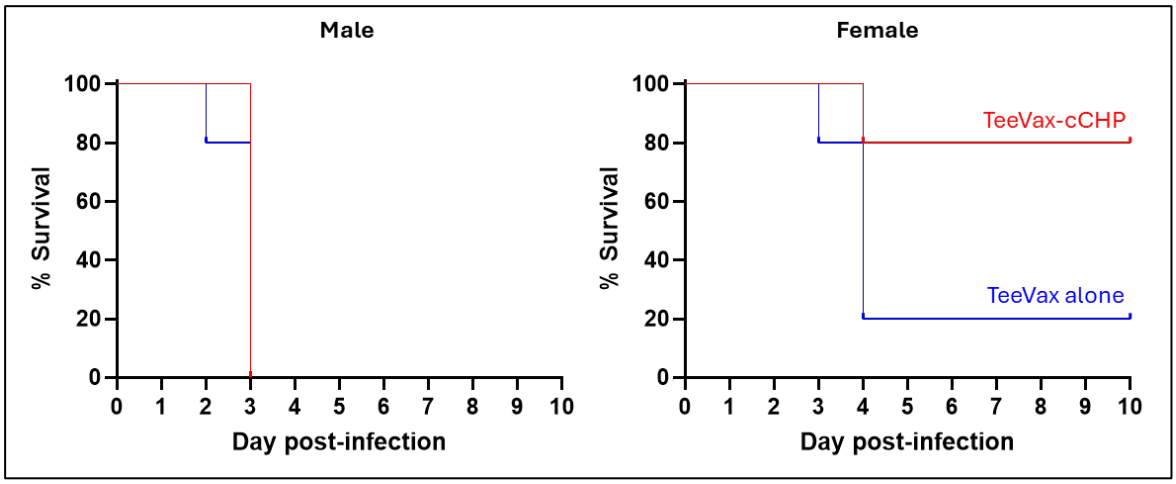

Figure 3. Sex differences in protection from invasive disease. Humanised plasminogen mice were immunised 3 times at weekly intervals and then challenged subcutaneously with StrepA. Mice were euthanized at pre-determined humane endpoints, and survival analysis was performed by Log-rank test. Data stratified by sex (n=5).

Joint publications:

Tsai CJY, Loh JMS, Fujihashi K, Kiyono H. Mucosal vaccination: onward and upward. Expert Rev Vaccines. 2023 Jan-Dec;22(1):885-899. doi: 10.1080/14760584.2023.2268724. Epub 2023 Oct 17. PMID: 37817433.

Communications:

Jacelyn Loh and Catherine Tsai conducted initial immunogenicity studies in Prof. Fujihashi's laboratory in July-Aug 2023 and met with Prof. Ishii at IMSUT during this time.

Nanogel formulation was subsequently exported to NZ to conduct animal infection studies at the University of Auckland.

Dr. Jacelyn Loh and Dr. Catherine Tsai also met with ISMUT collaborators during the FIMSA advanced training course and the 52nd Annual Meeting of the Japanese Society for Immunology held in Jan 2024 and again at the 53rd Annual Meeting of the Japanese Society for Immunology in Dec 2024.

Dr. Jacelyn Loh presented work online at the IMSUT International Joint Usage/Research Center Research Findings Presentation Meeting in 2024.

All other communications were done online.