ID No.	K3012
Project Title	Development of M2e-based intranasal universal influenza vaccine utilizing PilVax platform
Principal Investigator	Catherine Tsai (Research Fellow, Univ. of Auckland)
Project Members IMSUT Host Researcher Members	 Kohtaro Fujihashi (Project Prof., IMSUT) Hideki Asanuma (Chief, NIID)

Report

We have developed PilVax into a vaccine against influenza A virus infections. We showed that PilVax was able to present either one or two copies (in tandem) of the highly conserved epitope of influenza matrix 2 protein (M2e) efficiently on the surface of the recombinant *L. lactis* strains. The recombinant *L. lactis* was generated in Auckland, and the pilus expression was confirmed by western blot and flow cytometry using antibody specific to the pilus backbone protein Spy0128 (Figure 1).

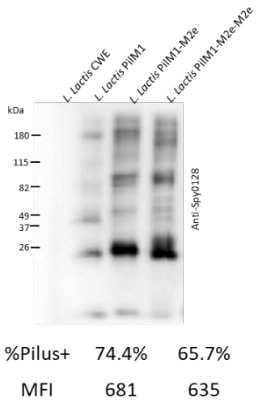

Figure 1. Western blot of *L. lactis* cell wall extract using anti-Spy0128 showed high molecular weight laddering pattern, a typical representation of the covalently-jointed pilus proteins. The pilus expression level was also quantified by flow cytometry using anti-Spy0128. CWE: cell wall extract; MFI: mean fluorescence intensity. Pilus+: pilus-positive population.

the custom on February 25, 2022 and delivered to Dr. Fujihashi's laboratory on February 28, 2022 (please see attached document).

Due to pandemic COVID-19 Dr. Tsai could not visit the IMSUT. Therefore in 2020 Drs. Asanuma and Fujihashi conducted a pilot study using the vaccine antigens obtained from Dr. Tsai. In this experiment, groups of 3 mice were nasally immunized with 1 x 10^5 CFU of either M2e-PilVax, M2e-M2e (tandem), or PilM1 (without M2e peptide) in the presence or absence of cholera toxin (CT, 1 μg) as nasal adjuvant. A positive control consisting of 1μg of inactivated whole influenza vaccine was also included. The mice were immunized

intranasally three times at weekly intervals. Eleven days after the last nasal immunization, all groups of mice were nasally challenged with influenza virus (Cal7: A/H1N1pdm09, $5LD_{50}$, 50µl). Mice immunized with adjuvant CT showed significant protection against lethal challenge with Cal7 (Fig.1). However, this protection appeared to be non-specific, as the PilM1 + CT group, where no M2e antigen was present, also exhibited better clinical presentation than the negative control group (Figure 2).

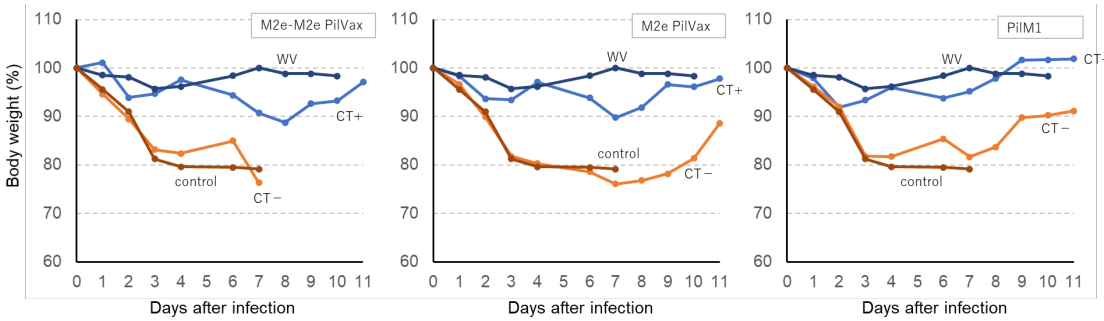

Figure 2. Percent body weight changes of mice after Cal7 challenge. WV: inactivated whole influenza vaccine; CT+: with CT adjuvant; CT-: without CT adjuvant.

Taking into account that the vaccination schedule used in this study is at a much lower dosage than the original PilVax publication (1×10^5 CFU, 3 doses in total, compared to 1×10^8 CFU, 12 doses in total), we concluded that low dose of PilVax vaccination requires nasal adjuvant to elicit protective immunity. Subsequently, another immunization and challenge study was planned using the same vaccine schedule established in the original PilVax study (Wagachchi & Tsai, *et. al.* 2018). This time, mice were immunized with 1×10^8 CFU of either M2e-PilVax, M2e-M2e (tandem), PilM1 (without M2e peptide), or WT *L. lactis* without adjuvant, on 3 consecutive days, 2 weeks apart, for a total of 12 doses. Nine days after the last immunization, mice were infected with lethal dose of influenza virus (Cal7: A/H1N1pdm09, $5LD_{50}$, 50µl). Disappointingly, no protection was seen in PilVax vaccination (Figure 3).

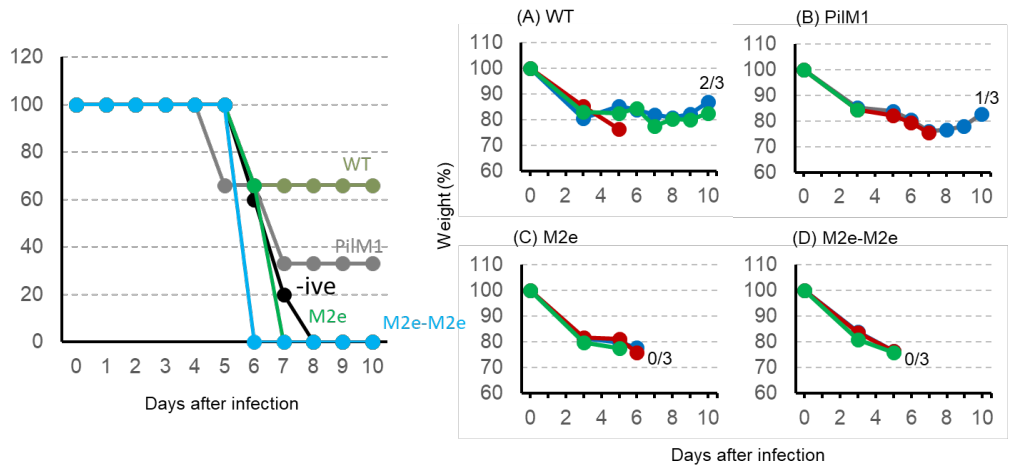

Figure 3. Survival rate and percent weight loss after Cal7 challenge.

The saliva samples collected before each boost (on Days 13, 27, 41) and before challenge (Day 52) will be examined for anti-M2e IgA titres by ELISA to verify the immunization efficiency.

Based on the recent finding that the GAS pilus protein is a potent immune stimulator, and activate the innate immune receptor TLR2, the team will now include a study to investigate the suitability of pilus protein as a protein adjuvant. Dr. Tsai has designed four different constructs to produce Spy0128- or Spy0125-conjugated M2e peptide. These recombinant proteins are being produced in Auckland, and will go through protein integrity, immunogenicity, and TLR2 bioassays in Auckland, before being shipped to Tokyo for animal immunization and challenge studies. Fusing to the TLR2 ligand Spy0125 or Spy0128 is expected to improve immunogenicity and efficacy of the M2e vaccine candidate.

Dr. Tsai presented at the IMSUT FY2021 Research Presentation Meeting on 4 March, 2022 via Zoom, and is planning to arrive in Tokyo at the end of June to physically work with Dr. Fujihashi

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Thus far, we had three Zoom meetings and 48 (with Dr. Tsai) and 16 (with Dr. Asanuma) e-mail discussions. Furthermore, Dr. Asanuma has visited IMSUT and performed preparation of experiments and performed preliminary experiments.