No.	K25-3119	
Project Title	A new vaccine strategy to combat RSV infection	
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(Form 2)

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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

Summary of Research

The global burden of mortality and morbidity caused by infectious diseases remains alarmingly high. The recent COVID-19 pandemic reminded us of the ongoing threat of severe infections caused by existing and emerging pathogens. Vaccination is an effective preventative measure for life threatening and debilitating infections, and has helped eliminate a number of important infectious diseases in the history. However, many widespread infections still await an optimal vaccine for effective disease control. Respiratory syncytial virus (RSV) is one of such disease targets that deserves substantial vaccine efforts. The lower respiratory tract infections caused by RSV take a high toll on young children and older people, claiming 101,400 children's lives and over 40,000 geriatrics deaths in a single year [1, 2]. The development of a RSV vaccine could save countless lives but has been a long time challenge.

Mucosal vaccination presents an alternative strategy to tackle the unmet vaccine needs. Most human pathogens use the mucosal tissues as their entry portal. Therefore, inducing adaptive immunity at the mucosa is a logical approach to hinder the intruding pathogens from establishing infections. The mucosal vaccination route also offers additional advantages in convenience that may enhance accessibility and compliance. This is especially beneficial for vaccine roll-out in lower-income countries, or for global vaccination attempts during a pandemic.

We have developed PilVax, a novel mucosal vaccine platform using the pilus (fimbriae) structure of Group A Streptococcus (GAS) as peptide carrier, and the food-grade bacterium *Lactococcus lactis* as a naturally adjuvanting vehicle. We have demonstrated that the stabilised and amplified antigens integrated in the PilVax construct can elicit desirable humoral and cellular immune responses when given by the convenient nasal delivery route [3]. **The aim of this project is to develop a mucosal RSV vaccine based on the PilVax platform**.

Progress to Date

We have constructed six RSV mucosal vaccine candidates, each containing a validated epitope from RSV. The surface attachment (G) or fusion (F) glycoprotein of are crucial for RSV infectivity and pathogenesis, and are targeted by vaccine candidates and monoclonal antibody therapies. However, F protein goes through conformational change which alters its functional epitopes, while G protein is type specific meaning some epitopes vary between RSV-A and RSV-B. The six epitopes used in this project were carefully selected to circumvent these caveats: $F_{255-275}$ and $F_{422-438}$ are neutralising epitopes found on both the pre- and post-fusion forms of F protein [4]; $G_{144-159}$, $G_{164-176}$, $G_{171-187}$, and $G_{190-204}$ are linear B cell epitopes that are shared between the G proteins of both A and B subtypes, and have been shown to confer protection in mouse and cotton rat challenge studies [5]. Importantly, $G_{164-176}$ and $G_{171-187}$ are located in the central conserved domain (CCD) of the G protein, which contains a sequence motif similar to one in the chemokine CX3C that is the culprit of vaccine-enhanced disease (VED). Antibodies against this CCD region can block the action of CX3C, thus reduce the risk of VED while preventing RSV.

Preliminary immunisation studies have been conducted in Balb/c mice. Antigen-specific antibodies were detected in post-immunisation serum, nasal wash, and bronchoalveolar lavage samples, especially high titres of serum IgG and nasal IgA. A repeat immunisation cohort is on the way, and Th1/Th2/Th17 cytokine response will also be evaluated in re-stimulated lymphocytes isolated from the cervical lymph nodes, mesenteric lymph nodes, and spleens.

Research Plan for FY2026

The preliminary immunisation data are encouraging. We are currently preparing for assays looking into antibody functionality, e.g. neutralising assays and plaque assays. Technique and protocol transfer has been done during a visit to Tokyo/Chiba in March 2026, and a second trip is planned for June 2026 with samples being shipped to Chiba University. Meanwhile, we have obtained biosafety approval to establish the assays with RSV-A and RSV-B virus strains in Auckland.

Our previous studies have demonstrated the increased immunogenicity of individual peptide delivered by the PilVax platform [3, 6]. In this study we will trial new designs that allow for the delivery of multiple antigens. We expect the presence of multiple antigens will maximise the efficacy and expand the versatility of this subunit mucosal vaccine platform. We have established cloning strategies to allow for the insertion of multiple peptides in the pilus structure [3]. These methods will be used to insert at least one G epitope and at least one F epitope into Spy0128, either at the same loop region or in separate loop regions. Pilus expression in the resulting recombinant L. lactis strains will be analysed by western blot and flow cytometry, using our established protocol [7]. The presence of incorporated epitopes will also be detected by polyclonal antibodies raised in rabbits vaccinated with the synthetic RSV peptides mixed with cholera toxin B (CTB) adjuvant.

In a pilot study we have successfully generated PilVax strains each carrying one of the six selected RSV epitopes. We have previously showed that co-administration of L. lactis strains expressing different antigens can simultaneously induce antibodies against all antigens [8]. Therefore, as a second strategy to deliver a combination of F and G epitopes, these RSV PilVax strains will be given as a mixture.

These multi-valent vaccines will be assessed for vaccine-induced humoral and cellular immune responses in mice. Based on the results from the immunogenicity studies, lead vaccines will be selected for efficacy evaluation in a mouse challenge model. Female Balb/c mice (n=10) will be challenged with RSV-A and RSV-B strains two weeks post immunisation [9]. Three to four days later the mice will be sacrificed, and their nasal turbinates and lungs will be removed for preparing tissue homogenates. Viral titres in the turbinates and lungs will be determined by RT-qPCR and $TCID_{50}$ assay to evaluate the protective effect of the vaccine in the upper and lower respiratory tract, respectively.

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