## 2013 CFID Undergraduate Summer Research Award Summary Report

# A *Listeria monocytogenes*-based vaccine for Respiratory Syncytial Virus

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## Introduction / Background

Respiratory Syncytial Virus (RSV) is a major cause of severe lower respiratory tract infections in infants and children, leading to hospitalization of an estimated >3 million children under the age of 5 annually [1]. Despite such staggering figures, there is currently no licensed RSV vaccine. In the 1960s, a formalin-inactivated RSV vaccine candidate disastrously led to exacerbated disease [2]. Efforts since then have explored a range of strategies including subunit vaccines, viral vector vaccines, DNA vaccines and live-attenuated vaccines as possible alternatives [3]. Even though vaccine efforts have focused largely on the fusion (F) glycoprotein of RSV for its neutralizing antibody epitopes and conservation across RSV strains [4], vaccine development has also explored the possibility of T cell-based responses for protection and facilitation of RSV clearance, particularly the RSV M and M2 proteins for the CD8 epitopes they possess [5]. Importantly, it has been shown in mice that adoptive transfer of $CD8^+$ T cells offers protection from subsequent RSV infection by limiting viral replication *in vivo* [6], and that vaccine-elicited $CD8^+$ T cells can protect against RSV infection and pathogenesis [7]. In humans, studies have demonstrated an RSV-specific $CD8^+$ T cell response in infants after primary RSV infection [8], and that the presence of $CD8^+$ T cells correlates with convalescence [9]. Such a basis for a T cell-based response against RSV prompted us to consider *Listeria monocytogenes* (*Lm*) as a possible vaccine vehicle against RSV, given one of its strengths being the induction of a powerful Th1-type T cell response.

*Lm* is a bacterium that has proven to be highly promising as a live attenuated vaccine vector. We have previously demonstrated *Lm*-based vaccination to be one of the few approaches that successfully induces a strong immune response involving IFN-γ producing $CD4^+$ T cells as well as antigen-specific $CD8^+$ cytotoxic T cells early in life [10]. In addition, *Lm* is able to confer lifelong immunity following a single dose in neonatal mice, and has an established safety track record in humans [11]. Studies by other groups using *Lm* have also shown that recombinant *Lm* strains expressing antigens such as the human immunodeficiency virus Gag protein or *Francisella tularensis* IglC protein, can successfully induce antigen-specific vaccine responses when given to neonatal mice and protect from subsequent challenge [12–14]. Thus, *Lm* as a neonatal vaccine delivery vehicle appears a likely promising vaccine candidate against RSV.

We hypothesized that the F, N, M and M2 proteins of RSV can be expressed in a live-attenuated strain of *Lm*; and when subsequently introduced in neonatal mice, will induce a broad, efficient and lasting immune response sufficient for protection against RSV infection.

## Objectives

To create *Lm*-RSV constructs that a) express chosen RSV antigens, b) maintain its viability for infection, and c) induce the desired immune response (i.e. Th1-type response).

## Methods

*Generation of Lm-RSV constructs*

Our collaborators provided a Bacterial Artificial Chromosome of the RSV A2 strain, from which the relevant segments of chosen RSV genes were amplified using PCR. These were amino acid residues 1-440 of the F protein, residues 1-257 of the M protein, residues 60-177 of the M2 protein and residues 1-340 of the N protein. Primers were designed to give the cloned segments a *XhoI* restriction site at the amino terminal end and a *SpeI* restriction site at the carboxyl terminal end (with the exception of the M antigen sequence, due to the presence of an internal *SpeI* restriction site; a partial blunt-ended cloning strategy was used for the M protein), to correspond to the orientation of the *Lm* plasmid insertion site. The *Lm* antigen expression vector used in this study was pADV211, which expresses the cloned antigenic sequence as a FLAG-tagged fusion protein with truncated ActA of *Lm*. Following cloning, the antigen expression vector was sequenced and transformed into our attenuated *Lm* vaccine strain (Δ*daldatactA*). In this strain, the antigen expression vector is stably maintained as a plasmid through complementation of alanine synthesis.

*Testing for expression of RSV antigen*

The transformed *Lm* (i.e. *Lm*-RSV vaccine construct) was cultured in Brain Heart Infusion (BHI) broth, with the supernatant proteins subsequently precipitated. Expression and secretion of the fusion antigen was confirmed using SDS-PAGE and western blot protocols. The western blot was probed using an anti-FLAG antibody to confirm both the presence and appropriate size of the fusion antigen.

*Testing for intracellular viability of Lm-RSV vaccine constructs*

Our *Lm*-RSV vaccine constructs were tested for their intracellular viability using a cellular infection assay, i.e. J774.1A, a macrophage cell-line. This assay ensures that antigen expression by *Lm* does not hinder its capability to infect and replicate in host cells, a critical requirement for vaccine immunogenicity. Cells were infected with 1 ml of infectious media ($2x10^6$ CFU/ml) at multiplicity of infection (MOI) 1, and lysed with $dH_2O$ at 5 different timepoints (-2H, 0H, 2H, 4H, 6H) for dilution and plating in order to determine the CFU count; time points were measured from the addition of gentamicin, a treatment that serves to remove extracellular bacteria. Used as controls were the 10403S and XFL7 strains; 10403S is the wild-type strain of Lm, and XFL7 is a strain with the *prfA* gene (a virulence factor of *Lm* that acts as the chief regulator of *Lm* virulence genes) inactivated and thus unable to replicate.

*Passaging of Lm-RSV vaccine constructs*

In order to attempt increasing the virulence of our strains, vaccine constructs were passaged twice through mice. For this study, neonatal male BALB/c mice were injected intraperitoneally with $10^8$ CFU of the *Lm*-RSV vaccine construct in 100 µl of saline; at 24 hours, mice were sacrificed, and their spleens isolated, homogenized and plated, for the determination of spleen CFU count 24 hours post-injection. A new batch of infection aliquots were made each time the strain was passaged, with a repeat of the J774.1A infection assay to ensure no loss of the strain's ability to infect and replicate.

## Results

Full report was received by CFID but results are suppressed here due to confidentiality agreements and pending submission of manuscript for publication.

## Conclusions and future directions

Given the urgency for an RSV vaccine and the potential for *Lm* to work as a neonatal vaccine vehicle, we attempted to generate *Lm*-RSV constructs that were capable of expressing chosen RSV antigens, maintain its viability in the cell and induce an immune response that would facilitate clearance of and protect against RSV infection. This was carried out using the F, M, M2 and N antigens of RSV.

We here report the generation of an *Lm*-M2 construct that expresses and secretes the M2 antigen of RSV and is capable of intracellular replication. Among the 4 chosen antigens, findings from a number of previous studies suggest that M2 represents the most promising vaccine candidate for the induction of antigen-specific T cell responses sufficient for dealing with RSV infection and conferring subsequent protection. Thus, even though the original objectives were not completely met, this study has succeeded in generating an *Lm*-RSV construct that is worthy of further testing, based on the results thus far. The *Lm*-N construct that was made did not appear to express the RSV antigen; nor did it seem capable of infecting the cells used in this study.

Cloning of the F and M antigens into *Lm* is still a work in progress. Finally, vaccine aliquots for the passaged *Lm*-M2 constructs were made, and will next undergo immunogenicity testing as well as challenge studies in mice.

In addition to what was written in the report, my summer project also consisted of an 8-week research education program by the Child and Family Research Institute (during which I gave an oral and poster presentation of my work, the latter of which I'm very thankful to have won), being involved in the setting up of an international collaboration for this project, and writing and submitting a review article, amongst other things – it was a most enriching experience. I am presently still continuing work on this project during my academic year.

## Works Cited


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