2013 CFID Undergraduate Summer Research Award Proposal

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

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Background / Rationale

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]. From these studies, it has become clear that of the three surface glycoproteins that RSV expresses, the fusion (F) protein is the most promising vaccine antigen. The F protein is conserved across RSV strains [4], and is capable of generating the kind of immune response necessary for the clearance of, and protection against, RSV infection. An optimal vaccine against RSV would have to induce a predominantly T_H1 type immune response, i.e. IFN-γ producing $CD4^+$ T cells, RSV-specific $CD8^+$ cytotoxic T cells, and high titers of neutralizing antibodies [5, 6, 7]. A major challenge in designing such an optimal RSV vaccine will be to generate such a strong and balanced immune response in neonates – a target population which has proven to be poorly responsive to many vaccination strategies [3].

Listeria monocytogenes (Lm) is a bacterium that has proven to be highly promising as a live attenuated vaccine vector. A fundamental aspect of this proposal is that the Kollmann lab has shown Lm-based vaccination to be one of the few approaches that successfully induces IFN-γ producing $CD4^+$ T cells, as well as antigen-specific $CD8^+$ cytotoxic T cells early in life. Thus, Lm as a neonatal vaccine delivery vehicle appears to fulfill the necessary requirements for a successful neonatal RSV vaccine. Especially noteworthy is the finding that Lm is able to confer lifelong immunity following a single dose in neonatal mice [12]. Furthermore, Lm has an established safety track record in humans [8, 9]; and as a delivery vehicle, Lm has been able to stimulate protective immunity against several pathogens [10]. In short, Lm is essentially the broadest and most efficient neonatal vaccine platform to date [11].

Hence, given that Lm is able to induce the kind of immune response known to be essential for the clearance of and protection from RSV infection, as well as being safe in neonates, an Lm vaccine vector expressing the F-glycoprotein of RSV is highly promising as a vaccine candidate.

Hypothesis

The F glycoprotein of RSV can be expressed in a live-attenuated strain of *Listeria monocytogenes*, and when subsequently introduced in neonatal mice, will induce a broad, efficient and lasting immune response sufficient for protection against RSV infection.

Objectives

Given the available time-span to conduct research over the summer, my goal for this project will be to generate a recombinant strain of live-attenuated Lm that expresses the RSV F- glycoprotein.

More significantly, this summer research project will lay the groundwork for an important line of inquiry that will ensue following its completion. The F-expressing Lm strain will undergo immunogenicity testing, and subsequent challenge studies. Success in this endeavor will generate new frontiers in the quest for an RSV vaccine – and in the grander scheme of things, the fight against major infectious diseases.

Methodology

Clone RSV antigen into Lm vector. The nucleotide sequence that will be cloned is that which encodes the F gene of RSV – this corresponds to nucleotides 6420-8322 on the RSV Bacterial Artificial Chromosome (RSV-BAC) that will be provided by collaborators [13]. The relevant segment of the F gene will be amplified from the BAC using PCR. The F gene sequence contains many known T cell epitopes and antibody-binding sites, including the site to which Palivizumab binds (Palivizumab is an antibody used therapeutically against RSV, inhibiting its entry into cells) [14]. The specific sequence will be cloned into our Lm antigen expression vectors (pADV134, which expresses antigens as a FLAG-tagged fusion protein with truncated listeriolysin – this enhances antigen secretion and immunogenicity). The antigen expression vector will be 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.

Assessment of F antigen expression by Lm. The transformed Lm will be grown in broth culture, and subsequently supernatant proteins will be precipitated. Expression and secretion of the fusion antigen will be confirmed using SDS-PAGE and western blot protocols. The western blot will be probed using an anti-FLAG antibody and Palivizumab to confirm both the presence and appropriate size of the fusion antigen.

Testing for intracellular viability. Once the successful expression of the F glycoprotein by our Lm vaccine strain has been established, a cellular infection assay will be carried out in J774A, a macrophage-like 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.

Preparation F-expressing Lm vaccine aliquots for future steps in the project. Single-use aliquots of the RSV-specific Lm vaccine strain will be prepared and frozen. Also, the dose (cfu/µL) will be confirmed using titration in order to ensure precise vaccine dosage.

Future follow-up

The vaccine prepared during this project will be assessed for immunogenicity in mice, as a potential prophylactic vaccine. After which, challenge studies – also in mice – will be conducted.

Literature Cited

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