Optimizing conjugated meningitis vaccine with flagellin adjuvants

Project Aims-
Bacterial meningitis is a common infection with worldwide prevalence, caused by the gram-negative bacteria *Niesseria meningitidis*. It is a commensal organism with an obligate human host that colonizes the upper respiratory tract. However, in some circumstances, the central nervous system can become infected, causing shock, swelling and inflammation which can be fatal, especially in infants and young children. There are six different subtypes of *N. meningitides*, groups A, B, C, W-135, X and Y which are the strains primarily responsible for causing disease. While *N. meningitides* is prevalent throughout the world, each region has different endemic subtypes.

There are several different meningitis vaccines which are currently licensed, which protect against some of the six subtypes. The targets for most of these vaccines are subtype-specific polysaccharides on the outer bacterial membrane, usually conjugated to a protein component. While the primary mechanism of protective immunity from these vaccines is eliciting a bactericidal antibody titer against the membrane polysaccharide,[4] conjugation with a peptide or protein allows for the creation of lasting adaptive immunity via a T cell dependent mechanism. Polysaccharides alone cannot be displayed on an MHC molecule which is the necessary first step in a memory response. Despite the wide success of many vaccination campaigns, meningitis epidemics continue to occur to this day, primarily in what is known as the meningitis belt of sub-Saharan Africa[2]. However, no multivalent vaccine exists that can protect against all meningitis subtypes, and in particular subtype B is endemic in several regions and has been especially difficult to vaccinate against. Additionally, there needs to be increased efficacy and cost-effectiveness for subtypes which are endemic in sub-Saharan Africa in order to create high enough immunity in the population to successfully decrease incidence of disease.

Towards this end, we propose attempting to conjugate the meningococcal polysaccharide with flagellin as a carrier protein in order to produce a more effective vaccine[3]. This would serve the dual purpose of conjugating the polysaccharide to a protein component and thus exposing it to the adaptive immune system and enhancing antibody immunity, as well as serving as an intrinsic adjuvant. Current meningitis vaccines are conjugated to a protein such as tetanus or diphtheria toxoid, which are sufficient to stimulate the immune system and produce immunologic memory. However, flagellin is an agonist of the innate immune system, specifically toll like receptor (TLR) 5, and we believe that conjugation with this protein could prove to increase overall immunogenicity of the meningococcal polysaccharide.

**Specific Aim 1: Develop a system to conjugate meningococcal polysaccharides to flagellin adjuvant across different subtypes.** In order to test the hypothesis that conjugation with flagellin could be a beneficial adjuvant for next-generation meningitis vaccines, the first step is to develop a system of conjugation. Conjugation involves a set of chemical reactions to activate the purified polysaccharide and protein for covalent bonding, and the initial aim will be to see whether this conjugation is possible, and develop a system of production. In order to test efficacy of this system across all subtypes, distinct polysaccharides from each subtype would ideally be cross-linked to the flagellin, using
polysaccharides which have previously been identified as antibody targets in other vaccines, meaning that at least six flagellin-polysaccharide conjugates must be created, one from each main meningococcal subtype.

**Specific Aim 2: Test activation of toll-like receptors in vitro.** Once conjugate vaccine targets have been created, the next step is to test the immunogenicity of these constructs by seeing whether or not they activate toll-like receptors in vitro. One of the main features of the pathogenesis of meningococcal disease is caused by inflammation mainly associated with the activation of TLR4 because of its high density of lipopolysaccharides on the bacterial cell wall. However, by conjugating the vaccine with flagellin this should activate TLR5, which is another innate immune mechanism designed to protect against bacteria. Using a bacterial antigen to produce immunity against a bacterial pathogen would likely be a productive way to elicit a robust immune response against challenge without causing over-stimulation of the TLR4 pathway which can amplify disease progression. By using an in vitro system with an immune competent cell line, activity of TLR pathways 4 and 5 can be assessed after introduction of this conjugate protein. Ideally, TLR5 pathways would be activated, indicating that the flagellin is acting in its capacity as an adjuvant.

**Specific Aim 3: Assess immunogenicity and production of specific antibody titers using an animal model.** Although *N. meningitidis* is an obligate human pathogen, there do exist several animal models which can be used to assess immunogenicity of specific vaccine constructs. In particular, using an inbred mouse line that has a well categorized immune system can be used to study the effects of the vaccine. With this tool, we propose testing the production of antibodies against the meningococcal polysaccharide, and particularly whether or not these antibodies are in fact bactericidal and therefore capable of limiting infection. A vaccination schedule will be developed involving an initial vaccination and then a booster shot some weeks later, and then assessing serum antibody levels after several more weeks. If tests show what are thought to be protective antibody levels, there is the possibility of doing a challenge experiment post immunization. Specifically, it would be prudent to not only test whether or not the flagellin-conjugated vaccine produces antibodies in general, but ideally we would compare this antibody titer to that of an already approved conjugate vaccine to see whether it produces a more robust immune response. One particular advantage that could be seen from conjugating the meningococcal polysaccharide with flagellin could be a decreased dose needed to produce immunity, which would help in overall cost-effectiveness. Therefore, in the animal model, dose-escalation studies would also be performed to compare antibody titers across different vaccine dosages.

If these experiments prove to be successful, flagellin-conjugated vaccines for *N. meningitidis* could be a possible avenue for the development of future meningitis vaccines. If a multivalent vaccine could be produced that is able to protect against all six subtypes with long lasting immunity, this could eventually be used to control and even eliminate bacterial meningitis from what are currently epidemic regions, and provide relief to a major global health burden.
Significance-
Bacterial meningitis caused by *N. meningitidis* is a public health problem across the globe, in both developed and developing countries alike. Meningitis is uniquely able to cause outbreaks even in communities with moderate vaccination rates[^4], and so in order to achieve control of this disease, widespread and cost effective vaccinations must become available for all subtypes.

Figure 1[^4] shows the distribution of the various subtypes of meningitis globally, with emphasis placed on the so-called meningitis belt in sub-Saharan Africa where epidemics remain unfortunately common. Also highlighted are regions where subtype B is prevalent, as this is the last major subtype to which a vaccine proves elusive.

Historically, there have been multiple vaccines developed and licensed for prevention of meningitis. The first was a simple purified polysaccharide, which induced antibody titers but because it did not contain a protein component was unable to create significant memory immunity and only lasted for several years[^1]. Moreover, re-vaccination with this vaccine was unsafe, as it could cause antibody-dependent adverse effects and actually increase disease upon meningococcal infection. Later, conjugated vaccines were developed, using tetanus and diphtheria toxoid as a peptide base. Now, there are two fully licensed conjugate vaccines, targeting subtypes A, C, W135, and Y, and two more recently developed quadrivalent vaccines undergoing licensure processes in the European Union[^1]. One bivalent vaccine has been developed targeting specifically subtypes C and Y. The only conjugate vaccine specifically targeting subtype A meningitis was recently licensed through
grant money established through The Meningitis Project, which is a foundation dedicated to stopping meningitis outbreaks mostly in sub-Saharan Africa\textsuperscript{[2]}, While several vaccine candidates have been made to subgroup B, which is especially prevalent and causes ongoing outbreaks in Australia, New Zealand, and the Pacific, there are currently safety and licensure concerns due to antigenic similarity between meningococcal polysaccharides and human neuronal tissue.

It is clear from the number and variety of vaccines available that there is not yet a single optimal vaccine to prevent bacterial meningitis. It has been documented that differences in vaccination recommendations can cause pockets of unvaccinated and therefore vulnerable populations. These differences include which populations and age ranges can be vaccinated effectively using different vaccines, the longevity of protection which is elicited, and the subtype vaccinated against, all of which vary by country. An additional consideration when producing a meningitis vaccine is cost. It was one of the goals of The Meningitis Project, when developing a subtype A conjugate vaccine for sub-Saharan Africa, that each dose cost less than $0.50. While this goal was achieved, and subtype A vaccination is now feasible in many countries in the meningitis belt, this still leaves populations susceptible to other subtypes, as quadrivalent vaccines are more costly. There is also the possibility of emerging new virulent strains, such as was seen with the appearance of the W-135 strain in Africa in the last decade. This makes it especially important to optimize vaccines to the strains we already know about, and hopefully develop improved delivery systems that can be used in the event of an appearance of a new strain.

All in all, vaccine coverage against meningitis needs to be improved in order to prevent future epidemics. By conjugating the vaccine with not a simple peptide delivery system, but a flagellin which can serve the secondary function as an adjuvant, it stands to reason that there could be improved immunogenicity, which could correlate to better protection against meningitis.

**Research Design and Methods**

The main focus of this research would be to develop an efficient conjugation system for flagellin and meningococcal polysaccharides. In order to do this, the well-characterized polysaccharides from among the different meningitis subtypes would be used, eliminating the need to find and categorize our own polysaccharides. Similarly, as flagellin-conjugated antigens have been researched as adjuvants in other contexts, it would be possible to use established bacterial strains to produce the flagellin and purify it for conjugation. There are many different chemical reagents and reactions that can be used for covalently attaching different chemical structures, and this work would be a matter of deciding on a set of optimal protocols given the structure of the polysaccharides and flagellin that can then be tested to see which works the best. The immunogenicity studies can likewise be carried out using established cell lines and mouse lines and standard assays for TLR activity, as well as detection and characterization of serum antibodies.
Future Directions-
If a flagellin-conjugated vaccine proves to be viable to produce and a suitably immunogenic construct, the next steps in furthering this research would be to conduct safety and efficacy studies. If the ultimate goal is to produce a vaccine that would be able to protect against all six subtypes, the different conjugates would need to be studied in combination to ascertain the optimal ratio of subtypes to provide protective immunity. Any additional safety concerns that have arisen during the initial testing would need to be addressed. Efficacy would need to be further tested as well, ensuring that immunologic memory has been established, and there is long-term persistence of antibodies. Dose escalation studies in an animal model would also be an essential feature. If, at the end of these studies, the flagellin-conjugated vaccine seems to stand up to measure with established vaccine standards for other meningitis vaccines, it could conceivably be advanced to human clinical trials.

Timeline-
Year 1-2: The first year or two would certainly be spent constructing as many subtype conjugates as possible, with the goal of establishing polysaccharides for all six subtypes with a flagellin. Much of this time would likely be spent optimizing protocols and preparing quantities or a production scheme large enough to use in later immunogenicity studies.
Year 2-3: Immunogenicity of the constructs will be assessed during this time, both in vitro and in vivo. First the viability of the constructs should be verified, followed by an assessment of immune activity.

Budget-
Reagents and materials for cloning.................................$5,000
Tissue culture supplies.....................................................$10,000
Immunogenicity assays.....................................................$5,000
Mouse line purchase and upkeep....................................$15,000
Equipment and maintenance............................................$5,000

References-