Purification of Vaccine Candidates Isolated from *Streptococcus mutans*

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Introduction

Endocarditis is a disease where the inner tissue around heart valves becomes inflamed due to a bacterial infection that originates in the bloodstream. It is commonly known that the bacteria from the genus *Streptococcus* can initiate infection (Moreillon & Que, 2004). No vaccine against streptococcal endocarditis is currently available (Moreillon & Que). Despite lack of success in vaccine development there is a new reason for optimism: a recent achievement in immunology called reverse vaccinology (Rappuoli, 2001). This strategy uses a combination of i*n silico, in vitro* and *in vivo* techniques and is an efficient way to uncover and to assess universal vaccine candidates. Reverse vaccinology has been defined as a method of producing a vaccine by first studying the genomic information of the organism (*in silico)* to determine which peptides are most likely to be antigenic (Rappuoli). These predicted peptides will be highly expressed (*in vitro)* and compiled to represent all the virulent species of Streptococcus. Animal testing of the vaccine candidates will confirm whether or not the selected peptides stimulated an immune response in the host (*in vivo).*

By using reverse vaccinology researchers are able to identify vaccine candidates and subsequently test them for effectiveness across all strains of *Streptococcus* (Barocchi, Censini, & Rappuoli, 2007). In collaboration with others, this particular project will focus on *Streptococcus mutans* in order to deduce a universal vaccine for streptococcal endocarditis.

Methods

The *in silico* aspect of reverse vaccinology requires utilization of bioinformatic tools to predict surface proteins for the species in question. By accessing databases that contain full proteomic sequences, the different types of surface proteins (i.e. lipoproteins, transmembrane proteins) can be predicted by looking for signatures such as signal peptides and transmembrane domains. Once a list of protein candidates from *Streptococcus mutans* is identified *in silico*, other proteomes of other streptococcal species (more specifically, the ones that cause endocarditis) will be searched. The more evolutionarily conserved a protein candidate is (across the genus of *Streptococcus*), the more attractive it will be as a vaccine candidate. Since it is the goal to produce a 'universal' vaccine, the protein should be commonly expressed by a diversity of potential streptococcal pathogens. Another factor of importance would be to eliminate those proteins too similar to those found in humans by comparing the *Streptococcus* proteomes to humans via available bioinformatic databases. This reduces the possibility of an autoimmune reaction.

After gaining the knowledge of what proteins might be present on the exterior of the cell, *in vitro* techniques can be employed. Once organismal DNA has been isolated, primers will be designed and utilized to isolate surface protein-coding genes from *S. mutans* (minimum of 36). The genes, amplified by PCR, will then be transferred to a vector. Each vector, now containing a single surface protein-coding gene, will be transformed into *E. coli* BL21 strain for further replication and will then be sequence confirmed to make sure the correct gene was accepted by the vector. The vectors used will be induced using isopropyl β-D-thiogalactoside (IPTG) to express the gene coding for the vaccine candidate. A polyhistidine-tag, encoded in the vector at either the N- or C-terminal end of candidate protein, will facilitate isolation of pure protein samples. The proteins containing the polyhistidine-tag will bind to a column containing metal

ions, allowing all other molecules to pass through. Upon eluting the sample, the target proteins will unbind from the column. Polyacrylamide gel electrophoresis will be run on the extracted proteins to determine the level of purification. The final *in vivo* steps will be conducted by collaborators who will immunize rats with the amplified proteins and assess strength of the vaccine candidates for prevention of endocarditis.

Possible Results and Implications

By observing the strength of the vaccine candidates produced using *in vivo* techniques, an anthology of proteins successful in initiating an immune response can be compiled. Testing of this collection enables further studies to be conducted with different varieties of proteins. Eventually, the vaccine will be tested on humans with the hopes of preventing streptococcal endocarditis.

References

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