In silico approaches for *Mycoplasma pneumoniae* multi-epitope vaccine construction

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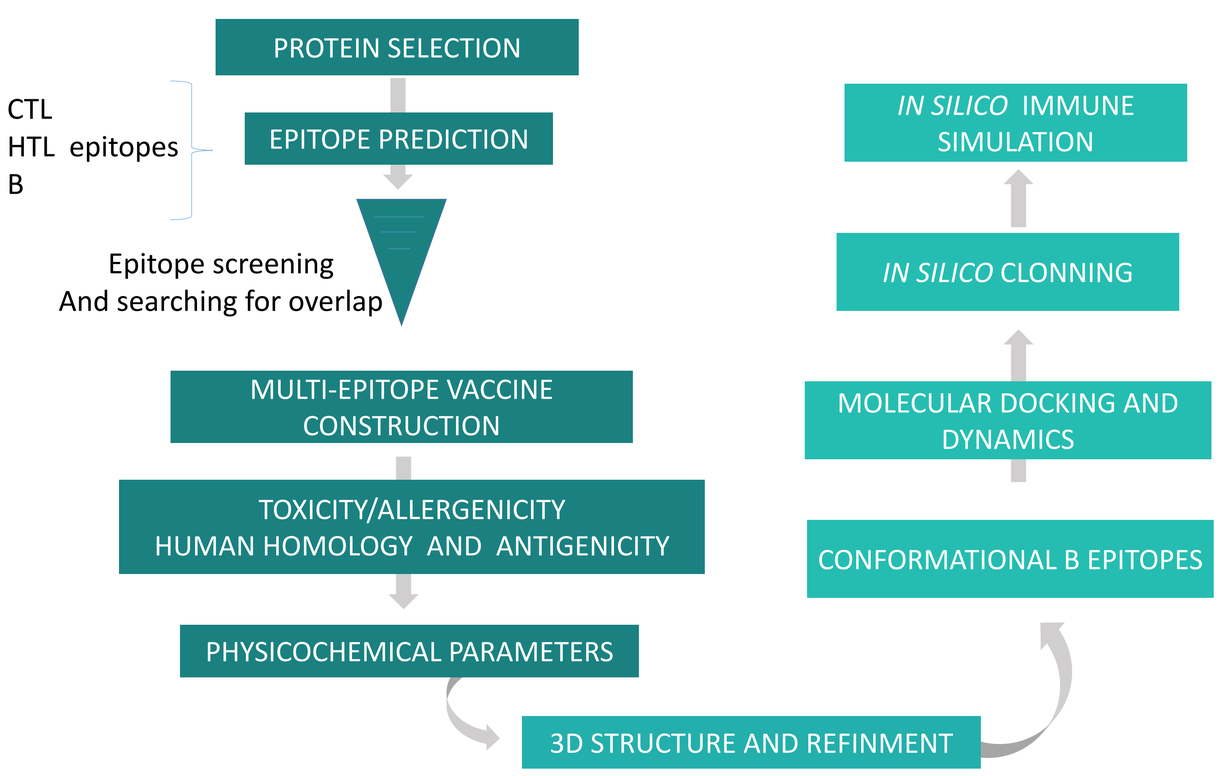
INTRODUCTION

Pneumonia is a serious health problem that causes the death of three million people annually. *Mycoplasma pneumoniae* is one of the main pathogens associated to this disease, with a significant increase in recent years and with high rate of resistance to antimicrobials. Vaccines are fundamental in preventing infections and considerably avoiding the need for hospital services and financial resources. Thus, the purpose of this study is to determine using immunoinformatics tools, a multi-epitope vaccine against *M. pneumoniae*. Multi-epitope vaccines are made of a chimeric protein, composed of carefully selected epitopes that belong to proteins determined by reverse vaccinology approaches and have proven to be a good strategy to minimize time and resources in vaccine development. Among the benefits of seeking these new vaccines are the possibility of targeting the immune response to conserved regions of the pathogen of interest, responses of high immunological capacity and less likely to induce adverse reactions in relation to attenuated or inactivated vaccines.

METHODOLOGY



RESULTS AND DISCUSSION

Eight proteins determined by reverse vaccinology and 3 others by the literature were selected for the study. More than 3.000 MHC I epitopes and another 3.000 MHC II were predicted by the two tools used. 718 B cell epitopes were found. After overlapping and filtering searches, we reached a total of 16 class I MHC epitopes and 13 highly reliable MHC II epitopes, which were predicted by the two platforms, overlap with B cell epitopes and high immunogenic capacity, belonging to 5 proteins. These epitopes were used to build the vaccine together with the adjuvant Heat-labile enterotoxin from *Escherichia coli.*

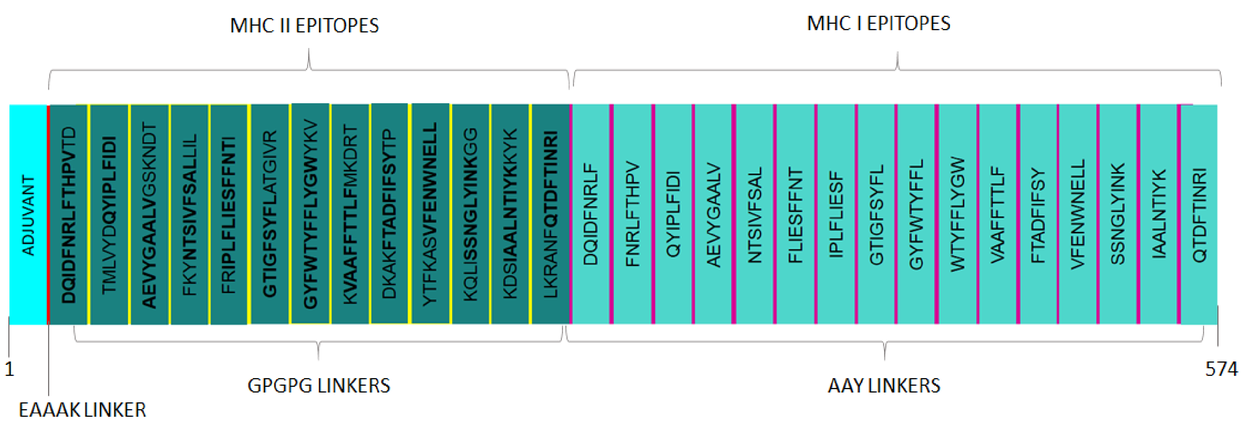


Figure 1: Multi-epitope vaccine construct with the peptide linkers and epitopes totaling 574 amino acid residues.

The vaccine was considered non-allergenic and non-toxic. Scored 0.64 by VaxiJen, demonstrated good antigenic propensity and 57 epitopes capable of inducing IFN-γ production were predicted. In addition, there was no significant similarity with the human proteome. The physicochemical characteristics of the vaccine demonstrated stability and the range of quality of the modeling reached 87.3% after structural refinement.

The expression of our vaccine in the K12 strain of *E. coli* and codon adaptation were performed satisfactorily. Regarding the simulation of the immune response, there was a significant increase in the population of memory B cells as well as a strong production of immunoglobulins and T helper cells with great differentiation into Th1 that are extremely important cells to fight intracellular microorganisms such as *M. pneumoniae.* The molecular docking between vaccine and the toll like 2 receptor showed an appropriate binding energy of -50.76, and dynamic simulation is being carried out to better understand the interaction of this complex.

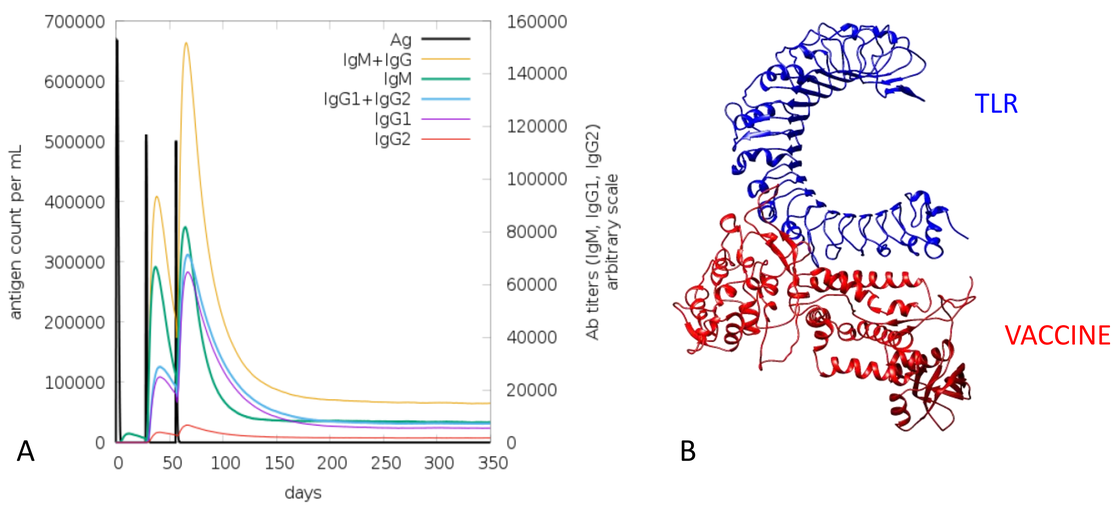


Figure 2: (A) Immuno simulation results regarding immunoglobulins production. **(B)** Representation of the molecular docking between the multi-epitope vaccine, in red, and the Toll like 2 receptor, in blue.

CONCLUSIONS

Through immunoinformatics approaches, it was possible to build a safe, highly immunogenic, stable and good population coverage multi-epitope vaccine model. In addition, the epitopes were carefully selected to reach a wide range of *M. pneumoniae* strains efficiently. Structural tests are still being carried out however, so far, a promising candidate vaccine for *M. pneumoniae* has been designed in this work, but, to ensure immunological efficiency and memory development, validation experiments need to be carried out.

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