Universitat Autònoma de Barcelona

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IN SILICO DESIGN OF A DENGUE VIRUS VACCINE

1 Background information

Dengue is an infectious disease that causes 390 million new cases of infection every year and 25000 deaths. Its infectious agent is the dengue virus (DENV), a flavivirus which codes for 10 proteins (3 structural and 7 non-structural) in an 11 kb RNA genome [1]. DENV has four different serotypes and is transmitted through *Aedes*' species bite. Despite DENV epitopes being widely studied, *Dengvaxia*® is the only available vaccine and it isn't effective enough. There are other vaccines in clinical trials, but none of them are peptide-based nor try to combinate class I & II MHC and B-cell epitopes information **(Figure 1)**.

OBJECTIVES: the purpose of this project is the identification of epitopes that could lead to the development of a peptide vaccine for DENV.

METHODOLOGY: Predictions for structural and non-structural proteins from the 4 DENV serotypes will be performed with the objective to identify its ability to bind to class I & II MHC molecules, as well as to B-cells. Those peptides predicted to be part of aggregation spots or transmembrane helixes will be dismissed. In the end, the best candidate peptides will be selected, chemically synthetized, conjugated to liposomes as carrier molecule and its success to induce immune response will be tested.

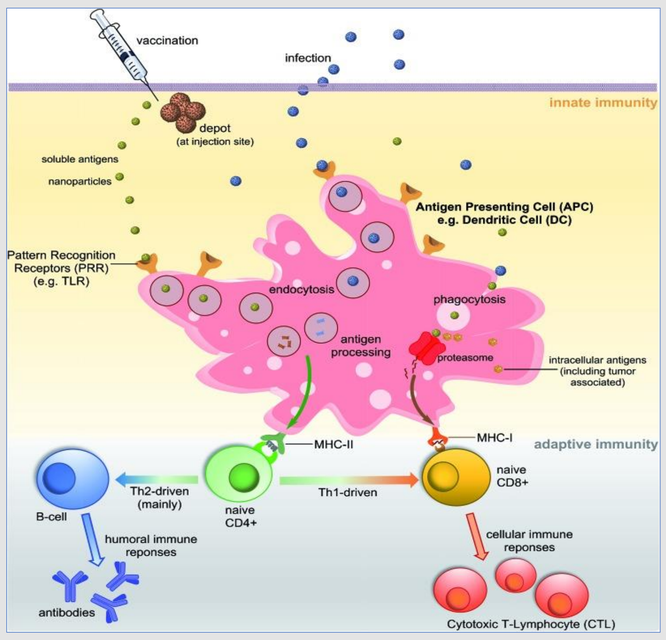

Figure 1. Schematic representation of the adaptative immune response. The major histocompatibility complex (MHC) plays a major role in the adaptive immune response. Class I MHC molecules present antigens to naïve TCD8⁺ cells and class II MHC to TCD4⁺ cells, which can induce T-dependent B-cell activation. B-cells are also capable to recognize epitopes by their own and produce T-independent humoral response [2]. For this reason, induction of these mechanisms is used in vaccine design. Image from Skwarczynski, M., & Toth, I. (2016). Peptide-based synthetic vaccines. *Chemical Science*, *7*(2), 842–854.

2 Identification of epitopes for class I & class II MHC molecules and B-cells

FUNCTION	TOOL'S NAME	INPUT	OUTPUT	AVAILABLE AT
DENV DATA RETRIEVAL	UniProt	None	File containing DENV proteins in FASTA format	www.uniprot.org
	CLUSTALW	File containing DENV proteins in FASTA format	5 files containing DENV serotype-specific and common proteins in FASTA format	www.ebi.ac.uk
MHC MOLECULES PREDICTION	IEDB MHC I binding	Proteins' file	File containing 8, 9, 10 & 11 aa-long peptides predicted to be recognized by MHC I molecules which are codified by available human alleles	www.iedb.org
	IEDB MHC II binding	Proteins' file	File containing peptides predicted to be recognized by MHC II molecules which are codified by human alleles	www.iedb.org
	SYFPEITHI	Proteins' file	8, 9, 10, 11 & 15 aa-long peptides predicted to be recognized by MHC I & MHC II molecules which are codified by available human alleles	www.syfpeithi.de
	NetMHCPan	Proteins' file	8, 9, 10 & 11 aa-long peptides predicted to be recognized by MHC I & MHC II molecules which are codified by representative human alleles	www.cbs.dtu.dk
B-CELL EPITOPES PREDICTION	BepiPred	Proteins' file	Aminoacidic sequence of the input proteins' showing a specific threshold score for each position as well as if the residue is buried or exposed and if it is part of a coil or helix	www.cbs.dtu.dk
RESULT'S FILTERING	AGGRESCAN	File containing the candidate peptides and file, in FASTA format, containing the sequences of the proteins from where such peptides were retrieved	File containing candidate peptides which are not part of transmembrane helixes or aggregation spots	bioinf.uab.es
	TMHMM			www.cbs.dtu.dk

CANDIDATE SELECTION: Peptides capable of inducing class I & II MHC molecules response, prioritizing those that can induce the response of as much of these molecules as possible, because more people will be likely to posses the gene that codifies it. Predictions obtained by *IEDB* and *SYFPEITHI* will also be given preference from pan-specific methods. Those peptides predicted by *BepiPred* with an elevated threshold and being showed as exposed will be selected as B-cell epitopes. Selected peptides will be sent to synthetize.

3 Liposome preparation

Five different types of liposomes will be synthetized, one for each set of predicted peptides. The liposome containing peptides from the conserved regions between serotypes will not include B-cell peptides. An universal TCD4⁺ response inductor, the PADRE peptide [3], will also be included alongside with the MHC-I and MHC-II peptides inside the liposome, and B-cell peptides will be conjugated to the liposome's membrane **(Figure 2)**.

Evaluation of peptide incorporation to the liposome's membrane will be performed by confocal microscopy and evaluation of peptid encapsulation will be performed by protein quantification after centrifugation.

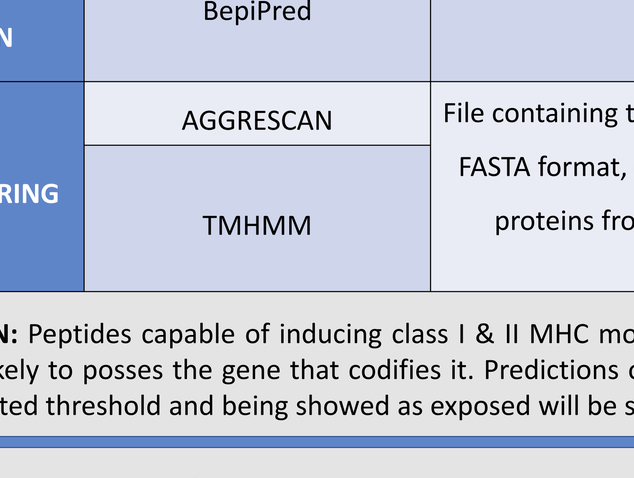

Figure 2. Liposome design. Created with BioRender.com

4 Results validation

To test the ability of the liposomes cocktail to induce immune response, an assay with mice will be performed to validate the results **(Figure 3)**.

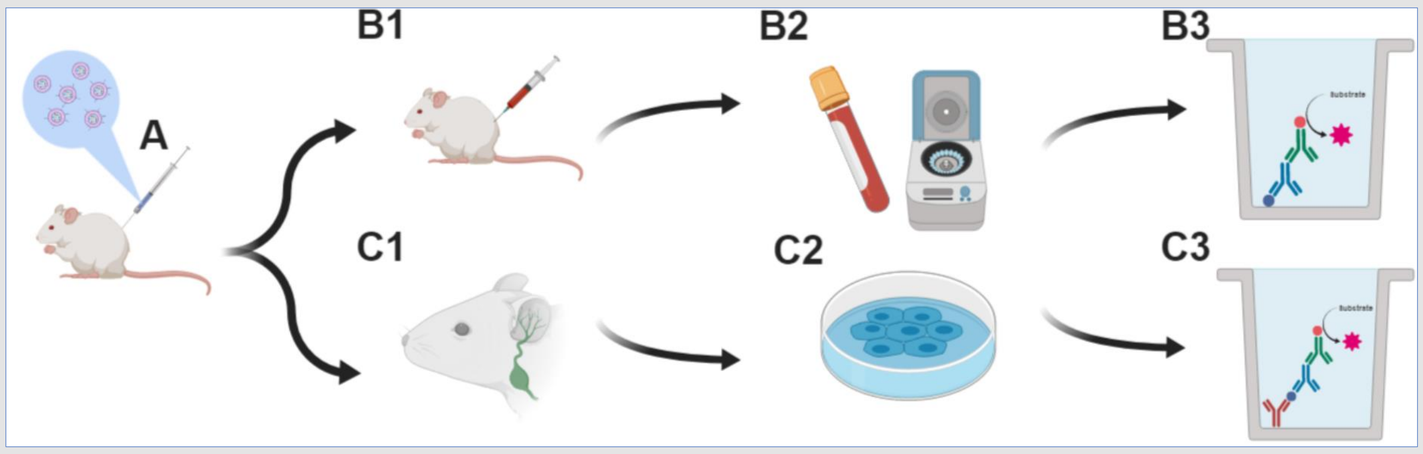

Figure 3. Results' validation process. Administration of liposomes cocktail to mice **(A).** On one side mice blood extraction **(B1)**, centrifugation to obtain serum **(B2)**, and specific antibody detection by ELISA using the peptides added to the liposomes **(B3)**. On the other side extraction of mice lymph nodes **(C1)**, T-cell and B-cell culture **(C2)** and α, β and γ interferon detection by sandwich ELISA **(C3)**. The protocol for this assay will be presented to an ethics committee and modification will be effectuated, if needed. Created with BioRender.com.

5 Expected results

It would be expected for peptide predictions to identify an epitope codified by a large amount of human alleles capable of inducing both MHC-I and MHC-II response. If such epitope isn't found, it would be expected that due to the presence of PADRE, a TCD4⁺ universal inducer, those peptides capable of inducing a MHC-I response alone would be enough. Referring to B-cell predictions, it would be expected to find serotype-exclusive peptides with the ability to induce humoral response.

Ultimately, it would be expected to obtain a vaccine design that could be able to confer immunity against all DENV serotypes that could be presented to preclinical and clinical trials. Additionally, as the vaccine should be able to induce both T-dependent and T-independent humoral response, it could be used as a preventive and therapeutic vaccine.

References:
[1] WHO (2009). *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control.* World Health Organization.
[2] Kindt, T. J., Goldsby, R. A., & Osborne, B. A. (Eds.). (2007). *Immunology* (6th ed). New York: W.H. Freeman.
[3] Alexander, J., Sidney, J., Southwood, S., Ruppert, J., Oseroff, C., Maewal, A., … Sette, A. (1994). Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides. Immunity, 1(9), 751–761.