Abstract

MHC stabilization assay using RMA/S cell line and ELISA based assays were used to validate potentially good binders as predicted by bioinformatics tools. Some of the peptides were validated to bind to MHC in both assays while other peptides showed discordant results. It could be either due to the sens itivity of the assay or the quality of 51 synthesized peptides. Thus, for ELISA very small quantities of peptides were used while for cellular stabilization of MHC a larger concentration of peptides were required and if there are some contaminant inhibitors w ere present in the peptide preparation, they could skew the results as we observed in both the assays. None the less this study predicted some novel peptides of DENV proteins that could potentially be used for in vivo studies to establish their value to wards a subunit vaccines and deciphering immunological events in the immunity and immunopathology caused by DENV. Peptides for CHIKV were also predicted but their validity was not established and could be part of future studies.] Some of predicted peptides st abilized the MHC - I monomer and gave positive results. Now these monomers can be exploited for making MHC tetramer so that Specific T- cells against respective peptides can be detected in a system. Different online tools and software follows different approaches to generate different lists of immunogenic peptides such as SYFPEITHI uses the concept of anchor residue and preferred residue while IEDB ANN uses artificial neural network and SMMPMBEC uses position specific scoring matrices. There is no guarantee that a predicted epitopes will also be a good epitope in experimental conditions. High throughput studies are always better to conduct but in limited resource condition we can use an approach to increase the probability to find some epitopes but using this approach, we validated H2Kb and H2Db peptides in vitr o condition. Conservation analysis was necessary as if we select an epitope which shows sequence similarity with host then that peptide will not work in spite of the fact that how good is that epitope. The peptide will be identified as a self and no immunological response will be generated. Sequence should also not similar to other organism, as epitope will not be specific.