THE EFFECT OF PRE-EXISTING IMMUNITY AGAINST JAPANESE ENCEPHALITIS VIRUS ON THE SEVERITY OF DENGUE DISEASE

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Dengue is an endemic disease for more than one hundred countries in the world, which causes a huge economic burden for most of the developing countries in South, Southeast, and East Asia. Therefore, there is an urgent need for a vaccine, drug or vector mosquito control method to fight against this disease. One vaccine has been registered, which was found to have low efficacy and safety issues. Both DENV and Japanese encephalitis virus (JEV) belong to the Flaviviridae family. JEV has approximately 50% homology with DENVs. DENV and JEV geographically coexist in South and East Asia. Therefore, there may be a higher chance of producing cross-immune responses for both JEV and DENV. Universal Japanese encephalitis vaccination is also implemented in South and East Asia and therefore it is more important to determine possible effects of pre-existing immune responses against the JEV on dengue disease severity.

According to the literature, pre-existing immune responses against JEV may give protection against dengue or it may enhance the dengue disease severity. Most of these studies have used commercial assays to determine pre-existing immune responses against JEV. Unfortunately, these commercial assays have low specificity and sensitivity. Therefore, in this study JEV specific assay was wished to develop to determine pre-existing immune responses against JEV. Bioinformatics analysis was done to identify JEV specific 20mer peptides from DENV and other flaviviruses, and subsequently 36 JEV specific peptides were identified. Twenty two out of 36 peptides were successful in commercial synthesis. Antibody and T cell responses to these 22 peptides were screened by carrying out ELISA and ELISpot assays.

Initially, the immunogenicity and specificity of the 22 JEV specific peptides were assessed on ELISA in individuals who were non-immune to JEV and DENV (JEV^-DENV^-, n=30), those who were only immune to the JEV and not DENV (JEV^+DENV^-, n=30), those who were only immune to DENV (JEV^-DENV^+, n=30) and in those who were immune to both viruses (JEV^+DENV^+, n=30). Seven out of 22 peptides were found to be highly immunogenic and specific and these 7 peptides were used as a pool to further evaluate JEV-specific responses. All 30/30 JEV^+DENV^- and 30/30 JEV^+DENV^+ individuals, and only 3/30 (10%) JEV^-DENV^+ individuals responded to this pool. This pool of 7 peptides cross immune responses were screened in patients following primary and secondary dengue infection during the convalescent period and found that the JEV-specific peptides were unlikely to cross react with DENV IgG antibodies.

To identify those commercially synthesized peptides T cell immune responses against JEV, Cultured and *ex vivo* ELISpot assays were done to individuals who have had varied DENV and JEV positivity. Using IFNγ cultured ELISpot assays, JEV-specific T cell responses were investigated in JEV^-DENV^- (n=21), JEV^-DENV^+ (n=22), and JEV^+DENV^- (n=23). The responses to these peptides were further assessed by carrying out *ex vivo* IFNγ assays and flowcytometry.

According to the results, none of the $JEV^{-}DENV^{-}$ individuals responded to any of the JEV specific peptides. A high frequency of responses was seen to 6/20 peptides by individuals who were JEV^{+} but $DENV^{-}$, where over 75% of the individuals responded to at least one peptide. P34 was the most immunogenic peptide, recognized by 20/23 (86.9%) individuals who were $JEV^{+}DENV^{-}$, followed by peptide 3 and peptide 7 recognized by 19/23 (82.6%). *Ex vivo* responses to these peptides were less frequent, with only 40% of individuals responding to peptide 34 and 16-28% to other peptides, probably as 5/6 peptides were recognized by CD4+ T cells.

Since both JEV and DENV co-circulate in the same regions and since JEV and DENV vaccines are likely to be co-administered in the same geographical regions in future, these JEV-specific T cell epitopes would be useful to study JEV specific T cell responses, in order to further understand how DENV and JEV specific cellular immune responses influence each other.

According to the results of this study, those who have had past severe dengue (Severe dengue, n=175) were significantly more likely ($p<0.0001$) to have JEV-specific antibodies than those with past non-severe dengue (Non-severe dengue, n=175) (OR 5.3, 95% CI 3.3 to 8.3). However, this should be further investigated by using a large sample number representing wider geographical regions in dengue endemic areas, before generalizing the conclusion to the general population. Furthermore, my data show that this assay is highly sensitive and specific for detection of JEV-specific antibody responses, it would be an important tool to determine how JEV seropositivity modulate dengue immunity and disease severity when undertaking dengue vaccine trials in the South and East Asia in the future.

KEYWORDS

Dengue virus, JEV, ELISA, ELISpot