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Analysis of Sero-Specific Immune Responses using Epitope Grafted Dengue ED3 Mutants

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1 Introduction

Dengue fever is a re-emerging tropical disease and its severe form is caused by cross-reactivity between its four serotypes. The third domain of the viral envelope protein (ED3) contains the two major putative epitopes(E1 and E2) and is a highly suitable model protein for examining the molecular determinants of a virus' sero-specificity. Here, we aim at characterizing the sero-specificity and cross-reactivity of the immune response against DEN3 and DEN4 ED3 using six epitope grafted ED3 variants where the surface-exposed epitope residues from DEN3 ED3 were switched to those of DEN4 ED3 and vice versa. Further, we report the structure of a DEN4 ED3 variant, where E2 was grafted from DEN3 (referred as DEN4_E2^{DEN}, hereafter).

2 Experiments

Synthetic genes encoding the ED3 sequences were cloned into a pET15b vector (Novagen) as reported previously [1]. The proteins were over-expressed in E. coli, purified by reverse phase HPLC, and lyophilized. 3-4 week old Swiss-Albino mice were immunized by weekly injection, and after the final injection, the sera were collected from the supernatant of blood sampled from heart. Interaction strengths were monitored by ELISA using purified ED3. Finally, X-ray diffraction data were recorded from single crystals at the Photon Factory, and DEN4_E2^{DEN3}'s structure was determined by molecular replacement.

3 Results and Discussion

We prepared six ED3 variants, in which the surface-exposed epitope residues from DEN3 ED3 were switched to those of DEN4 ED3 and vice versa. We raised sera against DEN3 ED3 and DEN4 ED3 and analyzed their reactivity against the grafted ED3 variants. Both anti-sera exhibited high sero-specificity with little cross reactivity to their counter serotype. Reactivity against epitope-grafted mutants indicated that E2 in DEN3 ED3 and E1 in DEN4 ED3 play a comparatively major role in determining sero-specificity. Although the reactivity recovered by replacing the epitopes in the counter serotype's ED3 by those against which the serum was raised was weak in absolute terms, the relative reactivity pattern was approximately in line with our specificity-transfer hypothesis (Fig 1). Finally, we solved the crystal structure of a DEN4 ED3 variant, where E2 was grafted from DEN3 ED3. The structure of DEN4_E2^{DEN3} was essentially identical to that of the wild type DEN4 ED3 (RMSD: 0.33 Å) despite E2's residues being substituted from DEN3. Detailed examination of the electrostatic surfaces (Fig.2) nevertheless provided some insights into the sero-specificity and cross-reactivity of DEN ED3s that were unnoticed from sequence information only [2].

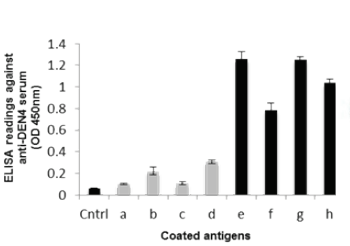

Fig 1: ELISA of anti-DEN4 sera. DEN4 are shown with back and DEN3 are in gray bars. The variant's identities are: a) DEN3 ED3, b) DEN3 _E1^{DEN4}, c) DEN3_E2^{DEN4}, d) DEN3_E1E2^{DEN4}, e) DEN4 ED3, f) DEN4_E1^{DEN3}, g) DEN4_E2^{DEN3} and h) DEN4 _E1E2^{DEN3}, where super-scripts indicate the epitope's serotype.

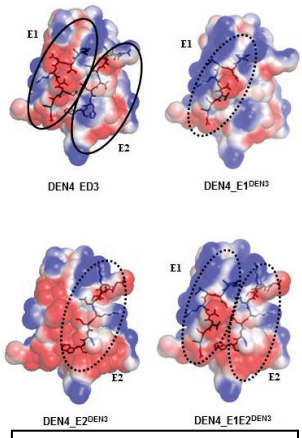

Fig 2: Electrostatic potential surface of DEN4 and its epitope-grafted mutants. The epitope regions are shown in wire models and encircled.

References

[1] Elahi M et al Biochim Biophys Acta. Proteins and Proteomics ,1844(3), 585-92 (2014)

[2] Kulkarni M et al, Biochim Biophys Acta. Proteins and Proteomics in press (2015). The coordinates of DEN4_E2^{DEN3} are deposited in the Protein Data Bank (PDB: 4x42).

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