DIFFERENTIATION OF FLAVIVIRUS INFECTIONS USING A MULTI ANTIGEN ELISA BASED ON RECOMBINANT ENVELOPE PROTEINS WITH MUTATIONS IN THE CONSERVED FUSION LOOP DOMAIN

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Introduction and Objective:

The high cross-reactivity of antibodies derived from flavivirus infections is a challenging fact in serodiagnosis of important human pathogens such as Dengue, Zika, Yellow fever, Japanese encephalitis, and Tick-borne encephalitis viruses (DENV, ZIKV, YFV, WNV, JEV, and TBEV, respectively). Moreover, areas of co-circulating flaviviruses are increasing and clinical symptoms are similar in many cases which raises the need of a reliable serological differentiation of these infections. The envelope (E) protein is a major target of the humoral immune response and its highly conserved fusion loop (FL) domain binds the majority of cross-reacting antibodies. Therefore, we inserted mutations in the FL domains of DENV, ZIKV, WNV and TBEV E proteins.

Methods:

Recombinant quadruple E protein mutants (Equad) of DENV (Serotype 1-4), ZIKV, WNV and TBEV were stably expressed in Drosophila S2 cells and purified from culture supernatants with affinity and size exclusion chromatography. Antibody responses were measured in IgM- and IgG- based ELISAs with DENV, ZIKV, WNV, TBEV infected, YFV vaccinated and flavivirus negative human serum samples.

Results:

All serum samples from infected individuals were detected as positive for at least one flavivirus; hence sensitivity and specificity are 100 %. Many DENV-positive sera displayed residual cross-reactivity, mostly to ZIKV Equad. Sera from DENV infected patients bound strongly the DENV antigen and less to the others, including ZIKV, whereas ZIKV positive serum samples behaved the opposite way. By simultaneous measurement of the same serum sample with antigens from several viruses a specific differentiation of all tested flaviviruses could be achieved.

Conclusion:

Here, we present a multi antigen based ELISA system for the simultaneous testing of human serum samples for DENV, ZIKV, WNV and TBEV IgM- and IgG antibodies which allows their discrimination with high specificity and sensitivity.