Development of a recombinant antigen and multiplex PCR for differentiation of tick-borne and mosquito-borne flaviviruses

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Background: Crimean Congo haemorrhagic fever (CCHF) virus is widely distributed in Africa. Though cases of suspected CCHF infections are routinely investigated, less than 10% are confirmed. A proportion are usually *Rickettsia* spp. infections but a large number remain undiagnosed. This warrants investigation of other tick-borne pathogens such as flaviviruses. Although the presence of mosquito-borne flaviviruses in southern Africa is known, that of tickborne flaviviruses was suggested by serological evidence in cattle but not confirmed. *Ixodes* ticks are the known principal vectors of tick-borne flaviviruses and are endemic in southern Africa. The domain III of the *flavivirus* envelope protein was reported to differentiate between the highly cross-reactive tick- and mosquito-borne flaviviruses. Our aim was to prepare a noncross reactive representative tick-borne recombinant antigen that will differentiate between antibodies against the tick- and mosquito-borne members, and to develop a nested multiplex PCR that can be used for differentiation. Langat virus, a tick-borne flavivirus, was selected as representative for preparation of a recombinant antigen because it can be handled in a BSL 2 laboratory.

Methods: The domain III region of the envelope protein (EDIII) of Langat virus was amplified by PCR and cloned into a pQE-80L expression vector. The recombinant protein was expressed in a bacterial expression system, purified in Nickel-charged columns and characterized using Western blot. The protein was evaluated in an ELISA for cross-reactivity with mosquito-borne flaviviruses. For the nested multiplex PCR, primers that amplify conserved regions of flaviviruses and CCHF were identified.

Results: The assay was nested to increase sensitivity and detected the viral nucleic acid of 10 known flaviviruses and CCHF virus. A 13kDa EDIII recombinant protein was expressed and purified. The antigen was able to differentiate between antibodies directed against tickand mosquito-borne flaviviruses and also showed clear cut-offs when tested against known positive antibodies directed against louping ill, tick-borne encephalitis and West Nile viruses.

Conclusion: The recombinant antigen is an important tool for the differentiation of flaviviral groups and will be used to screen human and cattle sera for antibody against tick-borne flavivirus. Similarly the multiplex PCR will be a useful tool for screening acute serum samples and ticks.

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A novel cell encapsulation mode for delivery of therapeutic antibodies against West Nile Virus infections that maintains steady plasma antibody levels throughout therapy

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Background: West Nile virus (WNV) is currently endemic in various parts of all five continents in the world. Being a member of the Japanese encephalitis virus subgenus, WNV can cause potentially fatal neuro-invasive diseases such as encephalitis and meningitis. Unfortunately, to date, no vaccine or antiviral therapy has yet been approved. One antiviral strategy in development involves the passive administration of neutralizing antibodies. As with most immuno-therapies, plasma antibody levels diminish between treatments. This is especially detrimental in flavivirus immuno-therapies as sub-neutralizing concentrations of antibodies can instead enhance infection. Here, we report a proof-of-concept for a novel mode of delivery using encapsulation of hybridoma cells producing therapeutic antibodies which enables the maintenance of a steady level of antibody in the plasma, thus preventing any possible antibody-dependent enhancement (ADE).

Methods: Recombinant domain III of the envelope glycoprotein (rE-DIII) of WNV was cloned, expressed in bacteria, and purified. It was then inoculated into balb/c female mice and splenocytes harvested to generate hybridomas. Limiting dilution was subsequently performed and producers of antibody specific to WNV were selected. Following which, the selected hybridomas were encapsulated in polymers of sodium cellulose sulfate. Viability of the encapsulated hybridoma clones, their ability to continuously secrete antibodies, and most importantly, whether antibodies can be released from the capsules were then characterized.

Results: Pilot batches of hybridomas remained viable and divided beyond 100 days postencapsulation. Moreover, the encapsulated hybridoma cells and their progeny were able to continuously secrete WNV-specific antibodies, and the secreted antibodies could be released from the capsules.

Conclusion: Our preliminary results showed that encapsulation of cells producing therapeutic antibodies can indeed be a potential solution to improving treatment outcomes for immunotherapies. This is especially essential for immunotherapies against flavivirus infections so as to minimize any potential ADE side-effect. However, the application of this technology for the treatment of infectious diseases is still at its infancy and we are currently in the midst of further optimizing and characterizing this technology.

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