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Short communication

C1q binding to dengue virus decreases levels of infection and inflammatory molecules transcription in THP-1 cells

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ABSTRACT

Dengue virus infection elicits a spectrum of clinical presentations ranging from asymptomatic to severe disease. The mechanisms leading to severe dengue are not known, however it has been reported that the complement system is hyper-activated in severe dengue. Screening of complement proteins demonstrated that C1q, a pattern recognition molecule, can bind directly to dengue virus envelope protein and to whole dengue virus serotype 2. Incubation of dengue virus serotype 2 with C1q prior to infection of THP-1 cells led to decreased virus infectivity and modulation of mRNA expression of immunoregulatory molecules suggesting reduced inflammatory responses.

Dengue virus (DENV) is a significant human pathogen, causing 250–500 million new infections per year and 2.5 billion of people live in endemic areas, making it an important public health problem (Bhatt et al., 2013). Dengue disease presents in a range of manifestations from a relatively benign, self-limiting febrile illness (dengue fever, DF) to life-threatening vascular leakage (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS) (World Health Organization, 2012). Mechanisms which might lead to severe clinical outcomes in DENV infections have been described, such as Antibody Dependent Enhancement (ADE) (Halstead and O'Rourke, 1977), Original Antigenic Sin in T cells (Mongkolsapaya et al., 2003) and Cytokine Storm (Dong et al., 2007).

The complement system, an arm of the innate immune, consists of three main pathways: the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway. It comprehends several proteins found in the blood, which normally circulate as inactive precursors that, when stimulated, initiate an amplifying cascade of further cleavages, resulting in release of chemokines, opsonization and activation of the cell-killing membrane attack complex (Ricklin et al., 2011). The expression of complement-related genes has also been found altered in DHF as compared to DF (Nascimento et al., 2009a; Ubol et al., 2008). In addition, it has been previously observed that DHF patients have hyper-active complement activation (Bokish et al., 1973a,b; Nascimento et al., 2009b). These data support that the complement system is activated to a greater extent in DHF patients than in subjects developing DF. However the mechanisms leading to hyper-activation are not completely understood. In addition, direct interactions of DENV with complement factors

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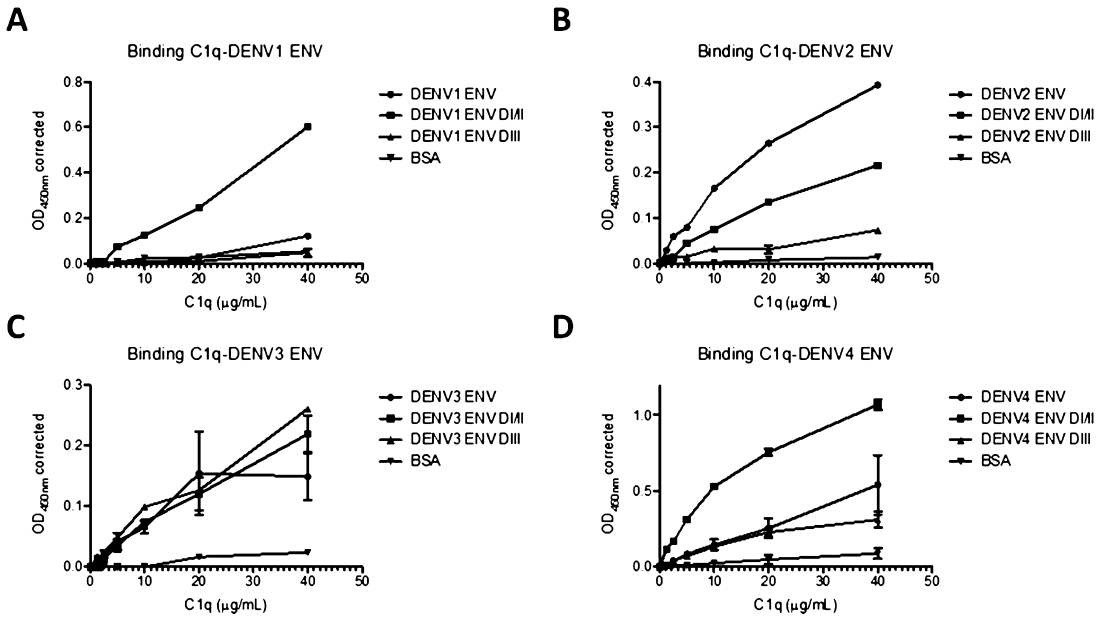

Fig. 1. C1q binds to full recombinant envelope proteins of all dengue virus (DENV1-4) serotypes (A–D, respectively) but not to bovine serum albumin (BSA; negative control). Within each serotype, binding of C1q occurs preferentially to the portion of ENV containing DI/II (A, B and D), except for serotype 3 (C). ELISA plates were coated with DENV ENV full length, ENV DI/II and ENV DIII (aa 1–413, aa 1–296 and aa 297–413, respectively; Table S1) fragments and BSA, and incubated with C1q concentrations ranging from 1 up to 40 µg/ml. Results obtained are representative of 5 independent experiments. Statistical analysis was done using GraphPad program and Student's paired, 2-tails *t*-test. Values were considered significant when $p < 0.05$.

have been poorly investigated. It has been observed that at least one complement factor, mannose-binding lectin, binds directly to DENV (Avirutnan et al., 2011; Fuchs et al., 2011). Such could be a mechanism of defense similar to other pathogens, i.e., to be covered by molecules of the immune system to avoid detection and elimination from the host (Hilleman, 2004). C1q activates the classical complement pathway, either by directly binding specific structures on the surface of pathogens and apoptotic cells or by binding immunoglobulins, immunocomplexes and pentraxins (Ricklin et al., 2011). It was recently reported that C1q binds to DENV non-structural protein 1 (NS1) (Silva et al., 2013). Also, C1q is able to inhibit ADE in vitro and in the mouse model in an IgG subclass-specific manner (Mehlhop et al., 2008).

DENV envelope protein (ENV) is a structural protein involved in attachment and fusion of the virus to host cells. ENV proteins have 3 domains, DI, DII and DIII. DI connects DII and DIII through flexible hinges that participate in the conformational changes that drive DENV fusion process, DII interacts with the membranes of the target cell during fusion and DIII is an immunoglobulin-like domain that is thought to mediate interactions between the virus and structures on the host cell involved in virus attachment (Modis et al., 2004). To determine which complement proteins bind DENV, we performed binding assays using purified complement proteins and ENV proteins from all 4 serotypes of DENV. Complement factors that have been found in altered levels in patient sera during severe DENV infections (C1q, C3, C4, C5, factor B, factor D and factor H; Quidel, San Diego CA, USA) (Bokish et al., 1973a; Nascimento et al., 2009b) or in cellular infection in vitro (CD46; Sino Biological, Beijing, China) (Nascimento, unpublished data) were selected for this study. The complement factors were tested at several concentrations, including their physiological concentrations (as determined in Nascimento et al., 2009b). Recombinant ENV proteins were obtained commercially (Prospec, Ness-Ziona, Israel; MyBioSource, San Diego CA, USA). We observed that full ENV proteins from all DENV serotypes bound to C1q (Fig. 1A–D). No significant interactions were detected with the remaining complement factors tested (data not shown). The binding to C1q occurred preferentially

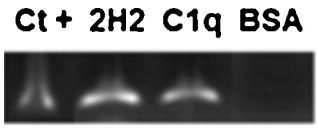

Fig. 2. Dengue viruses (DENV2) bind to C1q. DENV2 bind to 2H2 antibody and to C1q, but not to BSA. Ct+, positive control used was an extract of Vero Cells infected with DENV2. Amplicon size, 189 bp. To capture the viruses, wells were coated with 2H2 antibody, C1q and BSA and incubated with DENV2. Viral RNA was extracted by Trizol and DENV2 presence was confirmed by PCR using specific primer sequences for this virus. Results obtained are representative of 3 independent experiments.

in the DI/II of ENV proteins within each serotype except DENV3 (Fig. 1A–D). For the latter, C1q appears to bind similarly to both DI/II and DIII (Fig. 1C). The functionality of all recombinant proteins used was previously validated by assessing their binding to antibodies derived from plasma samples of a Dengue cohort study using both Luminex® and flow cytometry (Soares de Melo, 2012, in preparation).

To determine if C1q could bind whole, intact virus we employed a modified ELISA-capture protocol pioneered to detect HCV association with CD59 which has been shown to be much more sensitive than the conventional colorimetric ELISA system (Amet et al., 2012). ELISA plates were coated with a commercial monoclonal mouse antibody (IgG2a) against DENV (2H2, Millipore, Billerica MA, USA) with C1q or with BSA (negative binding control) and then incubated with DENV2 strain 16681 (BEI Resources, Manassas VA, USA). Virus were previously expanded and tittered as described elsewhere (De Melo et al., 2011). Plates were washed to remove any unbound DENV and RNA was extracted from each well. The specific virus cDNA synthesis was performed and followed by PCR with specific primers to DENV2 (Table S2). As expected, DENV bound to the wells containing 2H2 antibody and C1q, but not BSA (Fig. 2).

A structural protein of human astrovirus type 1, the coat protein (CP), has been demonstrated to bind C1q (Bonaparte et al., 2008), inhibiting activation of classical complement pathway in vitro (Hair et al., 2010). However, in similar conditions, ENV protein from all