Unveiling a Drift Resistant Cryptotope within Marburgvirus Nucleoprotein Recognized by Llama Single-domain Antibodies

Marburg virus (MARV) is a highly lethal hemorrhagic fever virus that is increasingly re-emerging in Africa, has been imported to both Europe and the US, and is also a Tier 1 bioterror threat. As a negative sense RNA virus, MARV has error prone replication which can yield progeny capable of evading countermeasures. To evaluate this vulnerability, we sought to determine the epitopes of llama single-domain antibodies (sdAbs or VHH) specific for nucleoprotein (NP), each capable of forming MARV monoclonal affinity reagent sandwich assays. Here, we show that all sdAb bound the C-terminal region of NP, which was produced recombinantly to derive X-ray crystal structures of the three best performing antibody-antigen complexes. The common epitope is a trio of alpha helices that form a novel asymmetric basin-like depression that accommodates each sdAb paratope *via* substantial complementarity-determining region (CDR) restructuring. Shared core contacts were complemented by unique accessory contacts on the sides and overlooks of the basin yielding very different approach routes for each sdAb to bind the antigen. The C-terminal region of MARV NP was unable to be crystallized alone and required engagement with sdAb to form crystals suggesting the antibodies acted as crystallization chaperones. While gross structural homology is apparent between the two most conserved helices of MARV and *Ebolavirus*, the positions and morphologies of the resulting basins were markedly different. Naturally occurring amino acid variations occurring in bat and human *Marburgvirus* strains all mapped to surfaces distant from the predicted sdAb contacts suggesting a vital role for the NP interface in virus replication. As an essential internal structural component potentially interfacing with a partner protein it is likely the C-terminal epitope remains hidden or “cryptic” until virion disruption occurs. Conservation of this epitope over 50 years of *Marburgvirus* evolution should make these sdAb useful foundations for diagnostics and therapeutics resistant to drift.

UT Health San Antonio

Alex Taylor
John Garza
Laura Sherwood
John Hart
Andrew Hayhurst