Modelling the AIRR response to DENV1: a human challenge study

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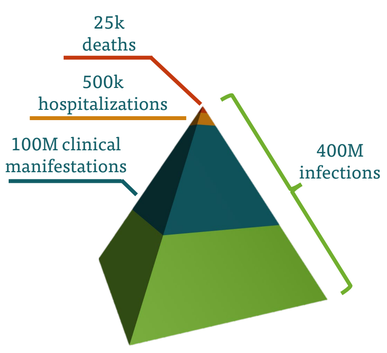
IMPACT

Dengue fever is caused by four serotypes of the **dengue virus** (DENV)[1], belonging to the genus *Flavivirus* in the family of *Flaviviridae*. The virus is transmitted through the bite of an infected female **mosquito** belonging to the genus *Aedes*[2].

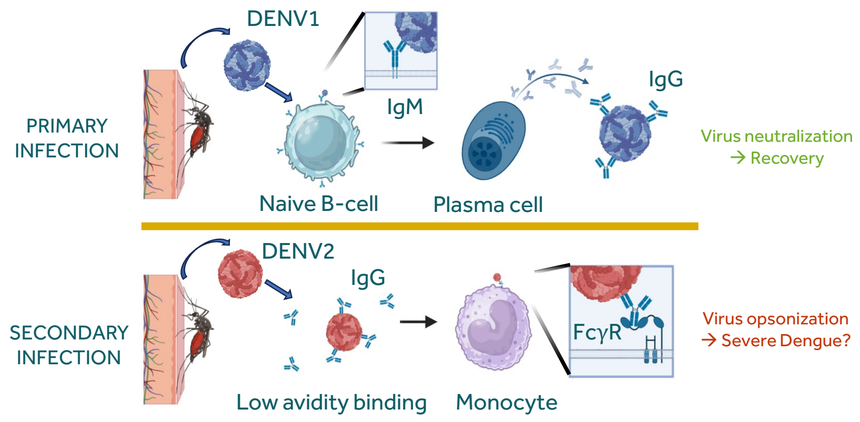
Most infections are asymptomatic, but some can lead to severe conditions like **dengue hemorrhagic fever** and **dengue shock syndrome**. So far, no reliable predictive biomarkers for severe disease have been identified[3].



Annual dengue burden of disease[4]

SEVERE DENGUE, AN IMMUNOPATHOLOGY?

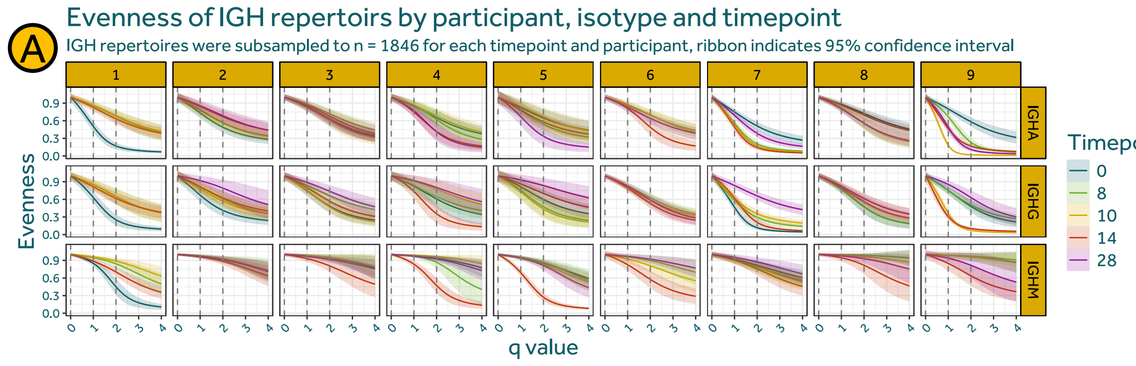
Severe cases are often linked to **secondary heterotypic infections**, likely due to **antigenic imprinting** and **antibody-dependent enhancement (ADE)**[5], whereby weakly-neutralizing cross-reactive antibodies increase viral replication by inducing opsonization and promoting infection of FcγR expressing cells[6].



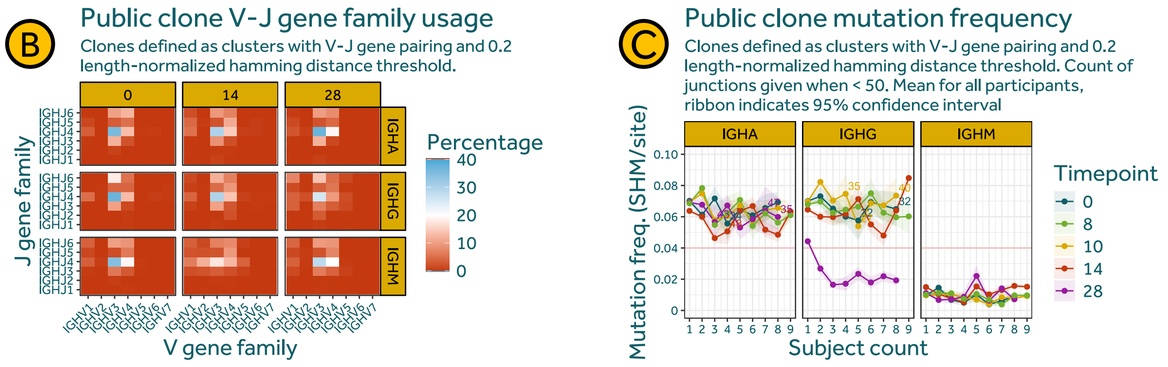
This phenomenon has also **hindered vaccine development**, as vaccination of seronegative individuals mimics a primary dengue infection, potentially **worsening disease severity** upon secondary infection.

RESULTS

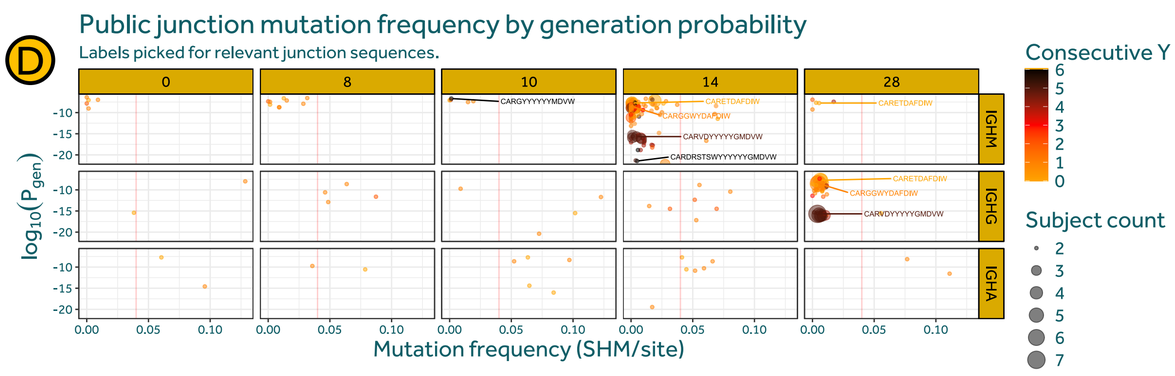
Clonal expansion is evident across all isotypes in the majority of samples (A). Although variations exist among participants and isotypes, a trend emerges indicating that evenness is lowest on days 10 and 14.



DENV1 infection causes diversification of V-J gene pairing on day 14 in IGHM sequences and increases J6-V1 pairing for IGHG on day 28 (B).

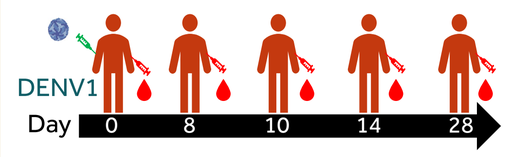


Public IGHG clones have significantly lower mutation frequencies on day 28 (<0.04 SHM/site) compared to other timepoints (C). Public IGHM junctions of day 14 correspond with IGHG of day 28 (D) and contain tyrosine-repeats, likely from J6-V1 pairing as previously described[8].

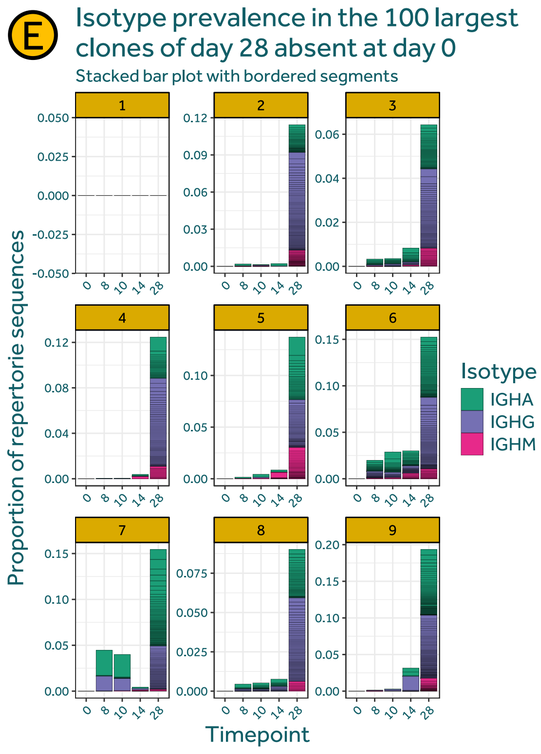


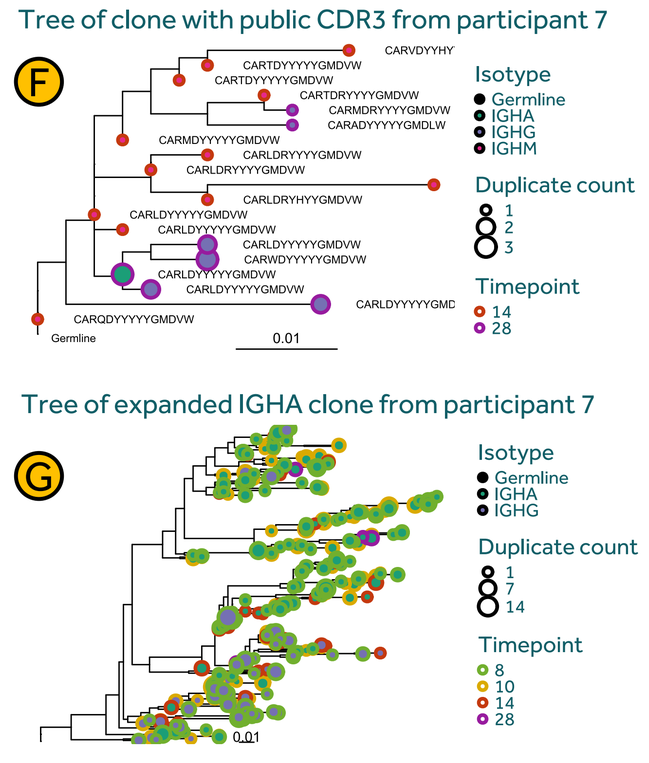
EXPERIMENTAL SETUP

To investigate the **B-cell** adaptive immune **receptor repertoire** (AIRR) response to DENV, we utilized biobanked samples from a human challenge study[7] involving nine healthy volunteers infected with an **attenuated DENV1 strain (45AZ5)**. **Whole-blood** samples (2.5 ml) were collected in PAXgene Blood RNA Tubes at fixed timepoints. Libraries were prepped with 0.66 µg of total RNA using the **NEBNext Immune Sequencing kit** and sequenced on an **Element-AVITI** system. Sequences were assembled through **nf-core/airrflow V4.1.0.** Data was analyzed and plotted in R, supported by **Immcantation** and **ggplot2** libraries.



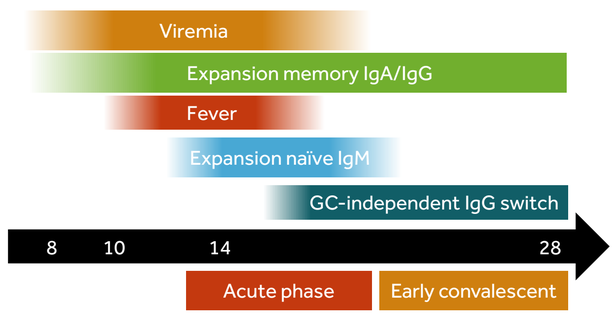
While no public IGHA CDR3s were detected (D), IGHA forms a significant part of post-infection expanded clones (E). Clonal trees with public CDR3s (F) correlate with collection timepoints and start at day 14, whereas clones rich in IGHA (G) show no correlation, are more evolved, and begin earlier.





CONCLUSIONS

The results support a model of immune response to primary DENV1 infections involving two cell groups: Days 8-10 show **reactivation** of cross-reactive **IgA/IgG memory B-cells**[7], while the acute and early convalescent phase (days 14-28) see expansion of **naïve B-cells** and **germinal center-independent** isotype switching to **IgG plasma cells**[9].



FUTURE PROSPECTS

Work in progress:

- Characterize public clones
- DENV targets of IgA clones

Open questions:

- Impact low SHM clones
- Origin IgA clones

DIGITAL

SOURCES & MORE