



Evaluating the Neutralizing Antibody Responses to a Dengue Vaccine in Seropositive & Seronegative Children from Cebu, Philippines

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Abstract

Dengue virus, a tropical mosquito-borne *Flavivirus*, presents global health concerns that require an effective vaccine. Dengvaxia, the only commercially available tetravalent vaccine against dengue, has had a complicated past and has called into question its power to produce neutralizing antibodies. In a unique approach, 10 paired samples were taken from a cohort of children in Cebu, Philippines both before vaccination and a year after vaccination with a single dose. By mixing the human sera samples with pure virus and VERO-81 cells, staining with primary and secondary antibodies, and the use of TrueBlue, the antibody response can be visualized. The results produced seemingly random responses, with naive, primary, and multitypic baseline serotypes producing positive and negative vaccine responses. A larger sample and more reproducible protocols must be used to make more conclusive statements about Dengvaxia's power to produce neutralizing antibodies.

Background

Dengue Virus

The global implications of dengue virus, a mosquito-borne tropical *Flavivirus* (related to West Nile & Zika) are immense. With 390 million annual infections (a fourth of which manifest clinically) and 40,000+ deaths a year, an effective vaccine is desperately needed¹.

Four Serotypes

There are four genetically distinct serotypes of dengue virus. A phenomenon known as antibody-dependent enhancement (ADE) complicates the development of a vaccine,

as prior infection of one serotype can cause more severe symptoms if re-infected with a different serotype. An effective vaccine, therefore, must produce antibodies for all 4 serotypes to be effective.

Dengvaxia

Despite initial excitement, Dengvaxia was concluded to "Exacerbate cases of dengue in children never previously infected" due to improper considerations of ADE. The WHO now recommends the vaccine with the caveat that "pre-vaccination screening" be a requirement to ensure only previously infected individuals receive the vaccine².

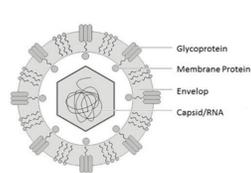


Figure 2. Basic biology of dengue virus



Figure 1. *Aedes mosquito, the vector for dengue virus*



Figure 3. Dengvaxia, the commercially available vaccine

Purpose

The purpose of this experiment is to investigate the neutralizing antibody response to a single dose of Dengvaxia before and after vaccination of 10 paired samples from children in Cebu, Philippines. Analyzing these paired samples will aid in plugging the holes of the Phase III clinical trials which did not properly evaluate the serostatus of its subjects prior to vaccination. By using paired samples, the different antibody responses between seropositive and seronegative children can be evaluated. In addition, the use of a set of positive control samples to serve as internal quality controls (IQCs) will aid in assessing the variability of the neutralization assay used to evaluate these samples' responses to the vaccine.

Methods

Virus Titration

Before the antibody response of human samples could be evaluated, the proper concentration of virus to ensure replicable & readable results was determined. This involved comparing traditionally lab replicated dengue virus and virus that was developed with an overexpression of the furin protein to mirror human systems. A dilution of virus was calculated for each of the four serotypes of dengue.

Neutralization Assays

Using the optimal virus concentration of each serotype, neutralization assays were completed on 10 paired samples with the following cycle:

Day 1: Seeded plates of VERO-81 cells with a concentration of 2×10^5 cells/mL in 5% FBS growth media; incubated for 24 hours at 37° C.

Day 2: Sera was diluted to create sigmoidal dose response curve with a factor of three. Virus was thawed and reacted with human sera to produce antibody response. Next, the solution was transferred to seeded plates to measure response further within protocol; incubated for 40-48 hours depending on serotype at 37° C.

Day 4: Fully incubated 96-well plates were neutralized with PFA and set in 1x Perm buffer

Day 9: Neutralized plates were reacted and incubated with a primary antibody, secondary antibody, and then TrueBlue reagent to visualize antibody response of human sera; plates were scanned into BioSpot software.

Days 10-13: The number of foci, or clusters of infected cells, were counted and checked for quality control with ImmunoSpot DC software. The fewer number of foci, the stronger the response in the samples.

Data Analysis

With the data collected, each sample's response to each serotype of dengue virus was normalized and transformed to measure the EC50, or dilution of 50% maximal response. Determinations were made as to prior infection and relative effectiveness of a single Dengvaxia dose.

Results

Virus Titration

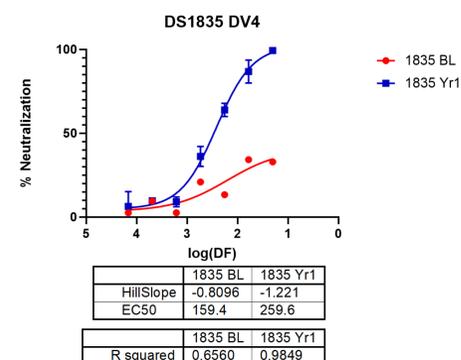
The goal was to find a dilution factor for each serotype that yielded approximately 60 foci per well; that is, to find a stock titer to complete further neutralization assays:

$$\frac{FFU}{mL} = (foci\ count) \times (dilution\ factor) \times \frac{1000}{15}$$

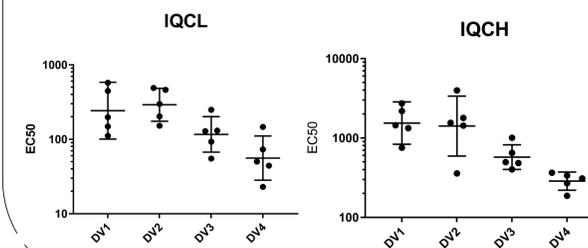
V-F DV1	V-F DV2	V-F DV3	V-F DV4
$6.71 \times 10^7 \frac{FFU}{mL}$	$6.53 \times 10^6 \frac{FFU}{mL}$	$6.06 \times 10^6 \frac{FFU}{mL}$	$3.20 \times 10^5 \frac{FFU}{mL}$

Cebu Sample Analysis

After quantifying the number of foci present in a plate, the information from the baseline and year 1 samples can be used to construct a sigmoidal dose response curve such as this example from DS1835's response to DV4:



Classification	Sample ID	Virus Serotype Antibodies Present at BL	Conclusions
Naive	1835		Vaccine induced titers for all four serotypes with a positive vaccine response against DV1, DV2, & DV3.
	137		Positive vaccine response against DV2; negative vaccine response for other 3 serotypes
	177	N/A	Negative vaccine response to all four serotypes; failed quality control
	214		Negative vaccine response to all four serotypes; failed quality control
	2307		Positive vaccine response to DV1, DV2, & DV4; negative response to DV3
Primary	922	DV1	Negative vaccine response to all four serotypes
	925	DV1	Boosted response against DV1; Positive vaccine response against DV2 & DV3
Multitypic	1909		Negative vaccine response to all four serotypes
	784	DV1; DV2	Boosted response against DV1 & DV2; positive vaccine response against DV3 & DV4
	1988	DV1; DV2; DV4	Reduced titers for DV1, DV2, and DV3; Negative vaccine response to all four serotypes



Conclusion

- Virus titrations are inherently variable, and values came within standards used in the de Silva laboratory
- Approximately 3/4 of the samples passed quality control; more experience with technique and a more standardized & reproducible protocol could decrease the amount of variability
- The response to the vaccine was seemingly random; seronegative, seropositive with one infection, and seropositive with multiple infections all produced positive and negative vaccine responses
- The use of IQCs displayed the variability of biological assays and that proper experimental technique was used in a majority of plates run
- Dengvaxia's rough past continues as the vaccine produced varying responses in naive samples and, in some cases decreased the neutralizing antibody response
- A larger sample is required to make more conclusive statements about Dengvaxia's power to produce neutralizing antibodies; the failure of Phase III trials to evaluate the baseline serostatus before vaccination has led to conflicting recommendations from the biomedical establishment that must be clarified



Figure 4. Cebu City, the Philippines; tropical regions like Cebu bear an immense healthcare costs due to vector-borne illnesses like dengue

References

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- Figure 2: DIAGNOSTICS. (2022). *Dengue virus antigens*. Creative Diagnostics. Retrieved April 19, 2022, from <https://www.creative-diagnostics.com/tag-dengue-virus-antigens-14.htm>
- Figure 3: Sanofi Pasteur, Inc. (2022). *Dengvaxia*. MPR. Retrieved April 19, 2022, from <https://www.empr.com/drug/dengvaxia/>
- Figure 4: P199. (2009). *Cebu City, Philippines* [Online image]. Wikicommons.

