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Review

Identifying protective dengue vaccines: Guide to mastering an empirical process☆

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ABSTRACT

A recent clinical trial of a live-attenuated tetravalent chimeric yellow fever-dengue vaccine afforded no protection against disease caused by dengue 2 (DENV-2). This outcome was unexpected as two or more doses of this vaccine had raised broad neutralizing antibody responses. Data from pre-clinical subhuman primate studies revealed that vaccination with the monotypic DENV-2 component failed to meet established criteria for solid protection to homotypic live virus challenge. Accordingly, it is suggested that preclinical testing adopt more rigorous criteria for protection and that Phase I testing be extended to require evidence of solid monotypic protective immunity for each component of a dengue vaccine by direct challenge with live-attenuated DENV. Because live-attenuated tetravalent DENV vaccines exhibit evidence of immunological interference phenomena, during Phase II, volunteers given mixtures of DENV 1–4 vaccines should be separately challenged with monotypic live-attenuated DENV. Immune responses to live-attenuated challenge viruses and vaccine strains should be studied in an attempt to develop useful *in vitro* correlates of *in vivo* protection. Finally, it will be important to learn if DENV non-structural protein 1 (NS1) contributes to pathogenesis of the vascular permeability syndrome in humans. If so, immunity to dengue 1–4 NS1 may be crucial to prevent severe disease.

Contents

1. Introduction

Because standard public health measures have proved insufficient to contain the 20–21st century dengue pandemic, major control efforts have been directed to developing effective and safe vaccines [1–5]. Five candidates are or have been in stages of human testing and one, a phase IIb clinical trial of the sanofipasteur tetravalent chimeric dengue-yellow fever vaccine (CYD 23), has proceeded to a test of efficacy in humans [6–10]. In the CYD 23 trial, from studies of a small random sample it was estimated that 90% of enrolled Thai children circulated neutralizing antibodies from previous Japanese encephalitis (JE) vaccination or wild-type DENV or JE infections. Despite this background flavivirus immunity and significant boosts in DENV 1–4 neutralizing antibodies after 2 or 3 doses of vaccine neither the initial, second nor third dose of vaccine protected against DENV-2 disease [10,11]. Although

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Table 1
Dengue illnesses observed in children given CYD vaccine or placebo following one, two or three doses or after any dose (Intention to Treat) shown by type of dengue virus recovered [10].

Doses	DENV	Dengue Vaccine		Control		Efficacy (95% C.I.)	*P* value*
		At risk**	Cases	At risk**	Cases		
>28 days after 3rd dose	1	2536	9	1251	10	55.6	0.1
	2	2510	31	1250	17	9.2	0.8
	3	2541	1	1257	2	75.3	0.5
	4	2542	0	1263	4	100	**0.02**
>28 days after 2nd dose, before 3rd dose	1	1018	1	510	4	87.5	0.083
	2	1014	12	509	3	−101	0.406
	3	1018	1	510	4	87.5	0.083
	4	1019	0	511	0	–	
After 1 and before 2nd dose	1	1290	4	645	1	−99.9	0.876
	2	1290	6	644	4	25.1	0.909
	3	1291	2	643	5	80.1	0.082
	4	1291	0	645	1	100	0.723
Intention to treat	1	5343	14	2666	18	61.2 (17.4–82.1	**0.01**
	2	5312	52	2622	27	3.5 (−59.8–40.5)	0.9
	3	5348	4	2667	11	81.9 (38.8–95.8)	**0.0026**
	4	5353	1	2679	5	90.0 (10.6–99.8)	**0.03**

Bold values signify $p < 0.05$.
* Two-tailed chi square with Yates correction.
** Person-years at risk.

numbers are small, successive doses of vaccine resulted in no obvious trend for increased protection against disease with DENV -1, -3 or -4 (Table 1). When cases from all post-immunization periods were combined (intention to treat) individual protective efficacy for DENV-1 was 61.3, for DENV-3 was 82.0 and for DENV-4 was 90.0, all judged statistically significant by the study's authors (Table 1). Additional results for this vaccine from the many other clinical trials now in progress should harden these data.

Following conventional vaccine development strategies, the low efficacy of the sanofipasteur vaccine was not recognized until formal clinical efficacy trials. Fortunately, there is much to be learned from the CYD23 clinical trial that, if acted upon, may save time and reduce the cost of identifying new protective constructs. Here, the CYD 23 trial results are analyzed to identify possible mechanisms underlying reduced protective efficacy and to make suggestions of how live virus vaccines tests in humans should be modified permitting early demonstration of vaccine efficacy, identification of correlates of vaccine protection and, if necessary, recompose vaccines or redesign administration schedules to achieve improved homotypic, heterotypic and multitypic DENV protective efficacy. The empirical nature of this process is noted.

2. Protective dengue immunity

In the design of vaccines and immunization schemes to protect against the four DENV it was recognized that natural protection against dengue infection and/or disease is observed under three circumstances: (1) monotypic, (2) heterotypic and (3) multitypic immunity. Evidence for these types of immunity and what is known about mechanisms are briefly reviewed:

(1) *Monotypic immunity* In experimental animals, CD8+ T cells contribute importantly to the containment of a primary DENV infection [12]. Solid and presumably lifelong protection against re-infection with the same DENV ensues following a primary dengue infection. This has been proved by challenge of human immunes with homotypic live DENV [13–16]. This protective immunity has been attributed to antibodies as monotypic immune serum protected against lethal encephalitis in mice by homotypic DENV and against dengue fever in humans [17–19]. Also, it has been established that passive transfer of monoclonal antibodies to DENV envelope proteins or to domain III protected against intracerebral challenge with homotypic live virus in mice [20]. It is important to note that in monotypic immune monkeys a homotypic live virus challenge was followed by absence of viremia and no anamnestic antibody response, a response labeled "solid immunity" [21,22]. Other workers have extended these observations to rhesus monkeys and humans showing an absence of viremia and stable neutralizing antibody responses following homotypic live DENV challenges of monotypic immune individuals [23,24]. The long-term stability of circulating antibodies in the face of revaccination has also been demonstrated in vaccinia-immune humans [25]. Excitingly, a quaternary structure on the intact virion has been identified as an attachment site for monotypic DENV neutralizing antibodies [26,27].

(2) *Heterotypic immunity* Sabin observed that susceptible adult American volunteers convalescent from overt DENV-1 infections were refractory to DENV-2 infection for 3 months, experiencing modified disease for up to 9 months [16]. The shorter the interval between DENV-1 infection and DENV-2 challenge, the greater the protection. Similar phenomena have been observed in other settings. For example, a live-attenuated DENV-1 mouse brain vaccine given during an on-going 1963 epidemic in Puerto Rico produced a protective efficacy of 39% against DENV-3 dengue fever (DF). [28] Modification of enhanced disease severity accompanying a second dengue infection occurs commonly as evidenced in several settings. In Iquitos, Peru in 1995, a large DENV-2 outbreak followed the prior introduction of DENV-1 in 1990. These are the conditions for the occurrence of DHF/DSS, but no cases were observed. [29] Compellingly, the 1995 infecting virus, a DENV-2 American genotype, was uniformly neutralized *in vitro* by DENV-1 immune sera from Iquitos residents. [30] Aotus monkeys immune to DENV-1 showed significant protection against viremic American genotype DENV-2 infection compared with viremias in susceptible controls. [31] A similar phenomenon was observed in Cuba where the severity of DENV-2 disease in DENV-1-immunes increased as the interval between infections increased from 4 to 20 years [32]. *In vitro*, DENV-2 neutralization by DENV-1-immune sera was greater at shorter than at longer