Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?

There Is Only One True Winner

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The scientific community now possesses information obtained directly from human beings that makes it possible to understand why breakthrough-enhanced dengue virus (DENV) infections occurred in children receiving Sanofi Pasteur's Dengvaxia tetravalent live attenuated vaccine and to predict the possibility of breakthrough-enhanced DENV infections following immunization with two other tetravalent live attenuated vaccines now in phase III testing. Based upon recent research, Dengvaxia, lacking DENV nonstructural protein antigens, did not protect seronegatives because it failed to raise a competent T-cell response and/or antibodies to NS1. It is also possible that chimeric structure does not present the correct virion conformation permitting the development of protective neutralizing antibodies. A premonitory signal shared by the Sanofi Pasteur and the Takeda vaccines was the failure of fully immunized subhuman primates to prevent low-level viremia and/or anamnestic antibody responses to live DENV challenge. The vaccine developed by the National Institute of Allergy and Infectious Diseases (National Institutes of Health [NIH]) has met virtually all of the goals needed to demonstrate preclinical efficacy and safety for humans. Each monovalent vaccine was comprehensively studied for reactogenicity and immunogenicity in human volunteers. Protective immunity in subjects receiving tetravalent candidate vaccines was evidenced by the fact that when vaccinated subjects were given further doses of vaccine or different strains of DENV the result was "solid immunity," a nonviremic and nonanamnestic immune response.

GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

Editors: Shane Crotty and Rafi Ahmed
Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a030700
Cite this article as *Cold Spring Harb Perspect Biol* 2018;10:a030700

Three live attenuated tetravalent dengue vaccines (LATVs) either have completed or currently entered phase III clinical efficacy testing. Each of these vaccines is structurally quite different (Table 1). All three initiated preclinical development during an era when the accepted surrogate for protective immunity for dengue infection/disease was the circulation of dengue virus (DENV)-type-specific neutralizing antibodies. All dengue vaccines must succeed at a complex challenge: they must provide lifetime protection against infection/disease with any of four different viruses to avoid breakthrough infections that may devolve into vaccine antibody-enhanced disease (ADE). The tetravalent vaccine that has completed phase III clinical testing fails this challenge. This failure has contributed important insights into the mechanisms of immune protection and of ADE of dengue infections/disease. The revised understanding of dengue protective immunity has generated questions about the outcome of vaccine efficacy trials of the two dengue vaccines currently in phase III.

DENGVAXIA ENHANCEMENT—WORRY, WORRY

In 2015, Dengvaxia, a live attenuated dengue vaccine consisting of a mixture of four yellow fever–dengue virus chimeras developed by Sanofi Pasteur, completed phase IIb, III efficacy trials in >35,000 children, ages 2–16 years in 10 dengue-endemic countries in Asia and the Americas (Hadinegoro et al. 2015). This vaccine has been recommended for use in dengue-endemic countries by the World Health Organization's (WHO's) Scientific Advisory Group of Experts (SAGE) on Immunization and is currently licensed in 14 dengue-endemic countries (www.streetinsider.com/dr/news.php?id=12511124&gfv=1). Statements from the manufacturer, SAGE background documents, and a WHO position paper failed to explicitly identify as "adverse events" children—who were vaccinated when aged 5 years or younger during year 3 of a phase III efficacy trial—hospitalized at a significantly higher rate, 20/2099 (0.99%) than controls, 2/1005 (0.2%), a relative risk of 4.95, $p = 0.03$ (Anonymous 2016; WHO 2016; Secretariat 2016). This is a clear instance of vaccine-enhanced disease. Instead, the WHO identified vaccinated children who were hospitalized during breakthrough dengue infections as having experienced a "safety signal" (Anonymous 2016). Despite published reports of the hospitalization for dengue illnesses of 295 vaccinated children of all ages, no effort was made by the manufacturer nor were recommendations made by international review panels to further identify the mechanism explaining this "safety signal" (Secretariat 2016). Further, the occurrence of adverse events was predicted to be only temporary. SAGE noted reassuringly that,

> In those children first vaccinated at ages 2–5 years in Asia, a statistically significant increased risk of hospitalized dengue was seen in vaccine recipients in the third year after the first dose, though this dissipated in years 4 and 5. The biologic mechanism behind this increased risk is currently not understood but may be related to naïve serostatus and/or age. A significant increase in hospitalizations was not seen in those older than 5 years. No other safety signal has been identified (Secretariat 2016).

Table 1. Dengue fever vaccines in clinical testing, listing the number of genes, structural and nonstructural, which are included in the final construct

	Genes (*N*)		
Type of vaccine	Structural	Nonstructural	Stage of development
Live attenuated virus (molecular mutant)	DENV1–4 (two each)	DENV1,3,4 (eight each)	Phase III tetravalent (Butantan, U.S. National Institutes of Health)
Yellow fever chimera	DENV1–4 (two each)	Yellow fever (eight each)	Completed phase III (Sanofi Pasteur)
Dengue 2 chimera	DENV1–4 (two each)	DENV2 (eight each)	Phase III tetravalent (Takeda)

DENV, Dengue virus.

Indeed, this is not the case. During year 6 of the phase IIb Thailand trial, 2.8% of children vaccinated at ages 2–5 years and 1.4% of children vaccinated at age 9 or above were hospitalized for breakthrough dengue infections (Secretariat 2016). These are astonishing and persisting instances of severe DENV disease in vaccinated children.

Because of its failure to recognize dengue vaccine–enhanced disease, SAGE called for no delay in licensing of Dengvaxia and did not issue a requirement for a targeted search for "hidden" cases of ADE-related dengue disease occurring in older age groups. This responsibility was clearly identified in the "Technical Consultation and the WHO Guidelines for the Clinical Evaluation of Dengue Vaccines in Endemic Areas," where it was stated that "a sub-immunogenic vaccine, or a vaccine whose efficacy wanes over time, could leave a recipient with an 'immune profile' which not only fails to protect, but increases the risk for experiencing severe dengue through complex immunopathological mechanisms following subsequent natural infection" (World Health Organization 2008; Bentsi-Enchill et al. 2013). Despite the claim that hospitalization of children 9 years and older was not vaccine enhanced, using published age-specific dengue antibody prevalence and dengue infection data from all 10 vaccine trial sites to model infection outcome of children who were vaccinated at age 9 or above, all of the observed hospitalizations could be attributed to children who were seronegative when vaccinated (Halstead and Russell 2016). This modeling has been extended to a larger group of patients with similar conclusions (Aguiar et al. 2016). It should be noted that antibodies raised to Dengvaxia appear to be more efficient at producing enhanced disease than are antibodies from a past DENV infection. During year 3, using published serological status and DENV infection rates of children in Asian vaccination sites, the 20 hospitalizations in vaccinated 2- to 5-year-olds were estimated to occur in a group of 176 seronegative children experiencing their first DENV infection, for a hospitalization rate of 11.4% (Coudeville et al. 2016; Halstead and Russell 2016). In the placebo population, it was estimated that there were 60 DENV monotypic-immunes of whom two were hospitalized who experienced secondary DENV infections, or 3.3%, a rate similar to hospitalization rates reported for secondary DENV infections in many prospective cohort studies (Halstead 2003; Halstead and Russell 2016). Thus, the hospitalization rate of children sensitized by vaccine is >3.5 times the rate of those sensitized by a single prior DENV infection. This increased risk may persist or possibly increase with time, as was the case when sequential infections occurred at an interval of 20 years in Cuba (Guzman et al. 2002a; Halstead and Russell 2016).

Rather than undertaking comprehensive studies to determine the etiology of hospitalized breakthrough cases among vaccinees, the manufacturer and WHO scientists issued demurrals, declaring (1) breakthrough dengue hospitalization were not instances of vaccine-enhanced disease as there were no clinical differences between disease course in vaccinees or placebos, (2) there was no direct evidence that hospitalized disease occurred in vaccinated seronegatives who had been identified in the "serological cohort" (i.e., the 8%–19% of children bled prior to receiving vaccine), (3) hospitalized cases are the result of a "cluster" phenomenon, an imbalance of cases resulting from the inclusion of a large number of 2- to 5-year-old seronegatives, and, remarkably, (4) the vaccine sensitization of seronegatives that led to hospitalization during breakthrough dengue infections is not of public health importance because these children, in the absence of vaccine, are fated to experience sequential dengue infections (Sabchareon et al. 2013; Capeding et al. 2014; Villar et al. 2014; Anonymous 2016; Arredondo-Garcia et al. 2016; Guy and Jackson 2016; Secretariat 2016; Wilder-Smith et al. 2016).

Responses to the demurrals:

1. *No clinical difference in disease in vaccinees and placebos.* Seronegative children of all ages responded to one or more doses of Dengvaxia by regularly developing DENV1- to 4-neutralizing antibodies. These antibodies were poorly protective (Sabchareon et al. 2012; Capeding et al. 2014; Villar et al. 2014).

Indeed, antibodies raised by Dengvaxia declined rapidly to low levels in adults, predominantly seronegative, whose antibodies 5 years after vaccination failed to protect mice against DENV2 challenge (Velumani et al. 2016). The Dengvaxia-induced antibody profile resembles very closely the dengue-neutralizing antibodies observed in infants born to dengue-immune mothers (Halstead et al. 2002). When titers reach infection-enhancing levels, they should amplify wild-type DENV infections to exactly the same clinical syndrome observed in infants circulating passively acquired enhancing antibodies or children experiencing a secondary dengue infection. No data were provided by the manufacturer to defend an argument that dengue vaccine–enhanced disease should be more severe or why such infections should differ clinically from naturally acquired antibody-enhanced dengue disease.

2. *No excess disease in vaccinated seronegatives who had been identified in pre-bled serocohorts*. In the Sanofi phase III trials, only a small fraction of hospitalized cases were bled prior to vaccination (Wilder-Smith et al. 2016). During years 3–6, the hospitalization-only surveillance phase, sufficient numbers were not available to calculate efficacy separately for seronegatives and seropositives. The only efficacy data for seronegatives and seropositives are for year 2 when larger numbers of milder DENV illnesses were identified by an intensive surveillance system (Table 2) (Hadinegoro et al. 2015). According to Wilder-Smith et al. (2016), the manufacturer offered "new data" describing lower incidence rates of "hospitalizations and/or severe disease" in vaccinated versus placebo cohorts among children whose serological status was defined by tests on blood obtained prior to vaccination. It should be noted that these new data, unavailable to the public, comprise a denominator that differs from published hospitalization data. Data available to the public do not provide assurance that >/= 9-year-old vaccinated seronegative children have not or will not experience severe vaccine-enhanced disease.

3. *Hospitalizations are the result of a "cluster" phenomenon*. The large number of hospitalizations among 2- to 5-year-olds created an epidemiological aberration described as "temporal clustering," the inclusion in the clinical trial of a large number of vaccinated seronegatives (Guy and Jackson 2016; Secretariat 2016; Wilder-Smith et al. 2016). Dengue is transmitted seasonally and, therefore, "clustering" occurs routinely manifested as large dengue outbreaks following the introduction of serotypes that differ from those of an earlier period. This is particularly true for island populations. For example, following the "clustered" transmission of

Table 2. Year 2 chimeric-yellow fever-dengue (CYD) vaccine protective efficacy in an intention-to-treat (post-dose 1) analysis in persons who prior to vaccination were seronegative or seropositive for dengue virus (DENV), Japanese encephalitis (JE) antibodies (Southeast Asia), or DENV antibodies (Americas)

	Seronegatives			Seropositives		
	Cases/person years			Cases/person years		
Clinical study	Vaccine	Control	Vaccine efficacy (%) CI	Vaccine	Control	Vaccine efficacy (%) CI
Southeast Asia	23/424	18/216	34.9% (−18.0–64.1) $p = 0.16$	18/901	34/444	73.9% (54.3–86.3) $p = <0.001$
Americas	9/258	9/149	42.2% (−42.3–76.6) $p = 0.24$	8/1073	23/512	83.4% (63.2–92.5) $p = <0.001$
Combined	32/682	27/365	36.6% (−4.2–61.4) $p = 0.08$	26/1974	57/956	77.9% (65.1–86.0) $p = <0.001$

Data combined with that from supplemental Figure S6 in Hadinegoro et al. (2015).

DENV1 in 1977 in Cuba, a "clustered" DENV2 outbreak occurred in 1981. The infections in 1981 occurred within a few months and affected a wide age range, 2–60+ years, producing many thousands of hospitalizations during secondary infections of children, inversely related to age. But in the same outbreak virtually all seronegative dengue-infected children experienced inapparent infections or mild disease (Guzman et al. 1990). "Clustering" did not result in a large fraction of severe cases that occurred in seronegative children. The dengue disease syndromes that occur in children who were seronegative at the time of vaccination should be compared to dengue disease syndrome that occurred in seronegative dengue-infected children in the same population. Vaccine-enhanced disease is identified when rates of severe disease in the former group, normalized for age, differs significantly from disease syndrome distribution in the latter.

4. *All children are fated to experience secondary dengue infections*. Wilder-Smith et al. (2016) have written that vaccinating seronegatives of any age may be epidemiologically desirable because after such individuals experience a first wild-type dengue infection (and survive) they will be immune to further dengue infections. That is true. But being hospitalized with that first dengue infection is the problem. Dengvaxia may enhance first DENV infections more efficiently than a wild-type DENV infection enhances a second DENV infection (Halstead and Russell 2016). Also, the resultant disease may be more severe because the earlier in life an individual is sensitized and infected, the more severe the resultant dengue disease (Guzman et al. 2002a). Finally, vaccine sensitization imposes a lifetime of risk (Guzman et al. 2002b).

DISCUSSION

WHO's SAGE completely missed the insight that vaccine efficacy calculations for potentially enhanceable viruses differ from calculations for viruses not subject to ADE. In the Dengvaxia clinical trials, hospitalized dengue disease in vaccinees and placebos occurred in two different populations. In placebos, hospitalizations accompany second heterotypic dengue infections. In vaccinees, hospitalizations are occurring in seronegatives who have been converted to "monotypic-immune equivalents" and are hospitalized during first DENV infections. Because of these denominator differences, the efficacy calculations used to justify release of Dengvaxia for public distribution are incorrect (Halstead 2016c). The rates cannot be compared.

How and why did members of the dengue scientific community and international health institutions fail to identify breakthrough dengue disease in vaccinated subjects as serious adverse events? It is difficult to understand the contorted explanations offered (Halstead 2016a,b). Vaccine-enhanced DENV infections had clearly been anticipated in clinical trial preparatory documents. Further, there are clear precedents for viral vaccine-enhanced disease, for example, breakthrough wild-type virus disease following administration of killed measles and respiratory syncytial virus vaccines to humans (Fulginiti et al. 1967; Kim et al. 1969; Hombach 2009; Bentsi-Enchill et al. 2013). Vaccine-enhanced disease occurs after administration of scores of veterinary viral vaccines (Halstead et al. 2010; Ubol and Halstead 2010). There is no plausible biological reason why the vaccine-enhanced dengue disease observed in 2- to 5-year-olds should not accompany DENV infections of vaccinated seronegatives of any age. It has already been noted that children 9 years and older are at lower risk to develop clinically serious vascular permeability than are children ages 2–5 years (Halstead and Russell 2016). But their risk is not zero! The recommendations by SAGE and the manufacturer to administer Dengvaxia to individuals 9–45 years of age who reside in communities experiencing high dengue endemicity will inevitably place large numbers of seronegatives at risk to enhanced disease essentially for life. To conclude that vaccine-enhanced disease cannot occur in individuals 9 years and older without providing supportive evidence is ethically and scientifically wrong. It is the responsibility of the manufacturer to study the immu-

nologic history of all hospitalized vaccinated children ages 2–16 years. All hospitalizations in vaccinees must be placed in the correct prevaccination group, seronegatives or seropositives. It should be possible to make this determination retrospectively by testing early convalescent sera from hospitalized children (Halstead 2016c; Halstead and Russell 2016).

A question must be asked and answered: "Why did the yellow fever dengue chimeric vaccine fail to protect seronegatives?" One possibility is that packaging dengue envelope proteins on the yellow fever nucleic acid core does not present a structure that optimally raises antibodies directed at quaternary epitopes on the virion surface (Nivarthi et al. 2016). These type-specific DENV-neutralizing antibodies may be important components of protective immunity (Messer et al. 2014). However, it does appear that antibodies raised after Dengvaxia vaccination attach to quaternary structures on DENV4 (Henein et al. 2017). This dilemma needs to be resolved. The most serious immunological defect in the human immune response to the yellow fever dengue chimera results from the absence of DENV nonstructural proteins. Several lines of evidence suggest that both $CD4^+$ and $CD8^+$ T cells may be crucial to protection against homologous reinfection or heterologous dengue infection (Weiskopf and Sette 2014; Tian et al. 2016). DENV-specific human $CD4^+$ T and $CD8^+$ T cells proliferate, produce interferon γ (IFN-γ), and lyse-infected target cells, suggesting that serotype-specific T cells are activated and functional in humans with primary DENV infection. Furthermore, higher frequencies of DENV-specific IFN-γ-producing T cells are present in children who subsequently develop subclinical infection, compared with those who develop symptomatic secondary DENV infections. Studies in a murine model of DENV infection demonstrated that both $CD4^+$ and $CD8^+$ T cells contribute to protection against DENV challenge (Weiskopf and Sette 2014). Protection against heterologous DENV infection derives T cells that recognize nonstructural DENV epitopes. The four DENV serotypes exhibit distinct immunodominance patterns. DENV1, DENV2, and DENV4 all elicit $CD8^+$ T-cell responses that are predominantly focused on nonstructural proteins (mainly NS3, NS4b, and NS5). In contrast, DENV3 elicits $CD8^+$ T-cell responses that target both structural proteins (C, M, and E) and nonstructural proteins (Weiskopf et al. 2016). The substitution of yellow fever for DENV nonstructural proteins, therefore, has crippled the protective T-cell response.

Whatever the full reason why Dengvaxia does not protect seronegatives from dengue infection or disease, a clear signal of vaccine failure was delivered by subhuman primates who were challenged with wild-type DENVs or humans who were given booster doses of vaccines (Guirakhoo et al. 2004; Morrison et al. 2010). In both instances, they experienced marked anamnestic antibody responses (less so for DENV4). As demonstrated years ago and once widely understood, protective immunity to a live DENV challenge is demonstrated by absence of an anamnestic antibody response to homotypic viral challenge (Halstead and Palumbo 1973; Halstead et al. 1973). "Solid" immunity implies neutralization of the inoculum, in situ. This proposed immunological criterion of protective immunity has been validated in human clinical trials (Durbin et al. 2011b, 2016; Kirkpatrick et al. 2016).

Several additional complications confront the rational or effective large-scale use of Dengvaxia. First, Dengvaxia may provide short-term heterotypic cross-protection against severe dengue disease similar to that following a single dengue infection (Anderson et al. 2013). Much more needs to be learned about the nature and duration of protective efficacy for vaccinated seronegatives and seropositives. Second, the manufacturer offers no proven method of boosting Dengvaxia protection; indeed, while the numbers are small, the efficacy attained after two or three doses of Dengvaxia did not differ in efficacy from the first vaccine dose. This implies that a "booster" dose of Dengvaxia will not improve protective efficacy. Third, preliminary evidence suggests that the antibodies raised by Dengvaxia resemble those following administration of a monotypic DENV4 vaccine (Dorigatti et al. 2015; Henein et al. 2017). Are these

"protective"? Protection data show only 80% efficacy against DENV4 (derived from a mixture of seronegative and seropositive vaccinees).

Given the many questions posed, it is crucial that the efficacy and immune responses to Dengvaxia be urgently and comprehensively studied. Existing technologies should make it possible to identify retrospectively those individuals who were vaccinated as seronegatives by studies on patient convalescent sera (Halstead 2016b). Large quantities of Dengvaxia will be delivered in licensing countries. Because dengue vaccine–enhanced disease has not been recognized by international health authorities, the instructions needed to identify and avoid such vaccine-enhanced severe dengue disease have not been discussed or formulated. Regulatory authorities are in a perplexing quandary. Dengvaxia-enhanced disease has created a major ethical dilemma for the vaccine community, an enduring public health management crisis, and legal nightmare. Vaccines should not harm recipients, directly or indirectly. WHO and the manufacturer owe the customer a safe product.

TAKEDA TDV ENHANCEMENT—WHO KNOWS?

This vaccine consists of a live attenuated DENV2 strain and three chimeric viruses containing the prM and E protein genes of DENV1, 3, and 4 expressed on the backbone of the DENV2 genome (Huang et al. 2000, 2013). These viruses have a long history. Parental DENV1 and 2 from Thailand, DENV3 from the Philippines, and DENV4 from Indonesia were furnished to the Centers for Disease Control and Prevention (CDC) Vector-Borne Diseases Branch, Fort Collins, CO from the authors' laboratory. Beginning in 1980, DENV1, 2, and 4 were adapted to primary dog kidney and DENV3 to African green monkey kidney (GMK) first in Hawaii for 15 sequential passages and then transferred to Mahidol University, Bangkok where they were further passaged, tested for virulence markers, and shown to be attenuated and immunogenic at different passage levels by inoculation in susceptible adult volunteers (Bhamarapravati and Yoksan 2000; Halstead and Marchette 2003). The attenuated viruses were licensed to Sanofi-Aventis, tested in thousands of volunteers of all ages, but ultimately abandoned as a result of under-attenuation of the DENV3 strain that had been serially passaged in GMK (Sanchez et al. 2006). Of the four viruses, DENV2 16881 PDK-53 was the most successful vaccine candidate when inoculated into susceptible human volunteers producing exceptionally high rates of seroconversions in seronegatives with only minimal dengue signs or symptoms (Vaughn et al. 1996). Its attenuating mutations have been identified and an infectious complementary DNA (cDNA) clone constructed (Kinney et al. 1997; Butrapet et al. 2000).

This vaccine was selected for chimerization and preclinical testing at the CDC and then by Inviragen and more recently by Takeda, where two doses of the TDV given at a 2-month interval were shown to result in tetravalent-neutralizing antibody responses in mice and seronegative cynomolgus monkeys (Huang et al. 2003; Osorio et al. 2011a; Brewoo et al. 2012). In cynomolgus monkeys, on challenge with wild strains of DENV1–4, 90 days after receiving a second dose of vaccine, low levels of viremia were detected in animals challenged with DENV1 and viral RNA detected in animals challenged with DENV1 and 3. Almost all animals challenged with DENV1, 3, and 4 experienced marked anamnestic-neutralizing antibody responses (Osorio et al. 2011a). In phase I and II studies in adults and children, TDV was well tolerated and two doses given at a 2-month interval generally resulted in tetravalent-neutralizing antibody responses (Osorio et al. 2011b, 2014, 2016; Rupp et al. 2015). TDV is entering phase III clinical trials.

The developers have based hopes for successful protection against DENV infection/disease upon the achievement of broad neutralizing antibody responses following two doses of TDV. It should be noted that immunization of seronegative monkeys with Dengvaxia failed to prevent low-level viremia (Guirakhoo et al. 2004). Therefore, both Dengvaxia and TDV have failed to raise solid protective immunity in subhuman primates (Halstead 2013). In the

only TDV live virus challenge study in subhuman primates, performed at a relatively short interval, viremia or RNAemia and widespread anamnestic antibody responses were observed (Osorio et al. 2011a). This can only mean that challenge viruses initiated systemic infection. Solid sterile immunity was achieved only to the DENV2 vaccine component, which is a classic live-attenuated whole dengue virus. Complicating an interpretation of results, the challenge interval was too short to adequately test long-duration protection. In humans, several months of heterotypic protection against wild-type DENV2 infection/disease followed inoculation of an attenuated strain of DENV1 (Sabin 1952). These results suggest that it is prudent to wait for at least 6 months after completion of TDV immunization before assessing vaccine-derived protection to wild-type virus challenge. Further complicating the issue of protective immunity to dengue viruses in humans is the evidence discussed above that $CD8^+$ and $CD4^+$ T-cell immunity may contribute importantly to protection following wild-type dengue virus infection (Weiskopf et al. 2013, 2015b,c). In humans, T-cell immunity is critically directed to nonstructural protein antigens. In TDV, all nonstructural proteins are DENV2. Will DENV2 T-cell immunity be sufficient to protect vaccinated humans from infection with DENV1, 3, and 4 viruses? If so, will such immunity be durable? A final piece of the puzzle is the pathogenic role of dengue NS1 and the potential necessity of NS1 immunity to protect against dengue disease (Beatty et al. 2015; Modhiran et al. 2015). Will immunity to dengue 2 NS1 provide sufficient protection of vaccinees against disease caused by DENV1, 3, or 4?

Based on these considerations, the outlook for a successful outcome of phase III clinical studies of TDV is cloudy.

NATIONAL INSTITUTES OF HEALTH (NIH) LATV ENHANCEMENT—PROTECTION LOOKS GOOD!

Remarkably, the NIH vaccine is almost everything Dengvaxia and TDV are not. LATV is composed of three dengue viruses that have been attenuated by deleting nucleotides from the nontranslated region. The vaccine expresses all genes of dengue 1, 3, and 4 viruses. The fourth component is a chimera with a dengue 4 backbone expressing dengue 2 structural genes (Durbin et al. 2011a). The important point in the development of this vaccine has been the testing for immunogenicity and attenuation of each monovalent component in seronegative human volunteers (Durbin et al. 2011a). Successful monovalent vaccines were then combined resulting in tetravalent-neutralizing antibody responses to a single dose (Durbin et al. 2013; Kirkpatrick et al. 2015). Critically, at a 6-month interval, volunteers given a single dose of LATV showed evidence of solid protection when challenged with a heterologous strain of live dengue 2 virus (Kirkpatrick et al. 2016). Not only did challenged volunteers have no viremia or any anamnestic antibody response to a strain of DENV2 that differed from that in the vaccine but all challenged volunteers failed to express the faint total body maculopapular rash that characterizes response to vaccination in seronegatives. That this protection is likely durable is evidenced by the solid immune response observed to a booster dose of live-attenuated vaccine given at a 12-month interval (Durbin et al. 2016). These excellent results have been complemented by ample evidence that a single dose of LATV raises monospecific neutralizing antibodies conformationally similar to those raised by wild-type dengue virus infections (Smith et al. 2013). In addition, LATV raises the broadly cross-reacting dengue antibodies that are thought to be protective (Tsai et al. 2015). Moreover, the T-cell responses to LATV closely resemble those raised to infections with wild-type dengue viruses (Weiskopf et al. 2015a). Finally, LATV contains genes for three of four DENV NS1 proteins. The NIH group are planning to extend challenge to DENV other than serotype 2. LATV has entered phase III clinical testing in Brazil.

The outlook is that LATV will raise durable, solid protective immunity in seronegatives and seropositives and thus prevent ADE.

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