SUPPLEMENT ARTICLE

Dengue Human Infection Model Performance Parameters

Timothy P. Endy

Infectious Disease Division, Department of Medicine, State University of New York, Upstate Medical University, Syracuse

Dengue is a global health problem and of concern to travelers and deploying military personnel with development and licensure of an effective tetravalent dengue vaccine a public health priority. The dengue viruses (DENVs) are mosquito-borne flaviviruses transmitted by infected *Aedes* mosquitoes. Illness manifests across a clinical spectrum with severe disease characterized by intravascular volume depletion and hemorrhage. DENV illness results from a complex interaction of viral properties and host immune responses. Dengue vaccine development efforts are challenged by immunologic complexity, lack of an adequate animal model of disease, absence of an immune correlate of protection, and only partially informative immunogenicity assays. A dengue human infection model (DHIM) will be an essential tool in developing potential dengue vaccines or antivirals. The potential performance parameters needed for a DHIM to support vaccine or antiviral candidates are discussed.

Keywords. dengue; vaccine; human; infection; model.

VIRAL HUMAN CHALLENGE MODELS

Influenza Virus Human Challenge Model

The first attempt at a human challenge model using laboratory-derived viruses was in 1933 for influenza virus [1]. In this study influenza virus was passed in infected ferrets and 1 cc of filtrate of ferret turbinate suspension was inoculated into each nostril of 2 student volunteers. They were then isolated on a hospital ward and observed for influenza infection. Neither developed illness, and they were later found to have antibody against the virus. A subsequent study in 1936 used aerosolized influenza-infected mouse lung tissue to infect 72 volunteers [2]. Volunteers inhaled atomized infected lung suspensions. Hyperemia of the nasal mucosa and edema of the turbinates lasted from 4 to 10 days in 40% of volunteers; nasal exudate occurred in 20% beginning on the second or third day after inoculation and lasted from 3 to 10 days. General symptoms occurred in a third of the volunteers, with 14 cases developing clinical influenza with fever to 39°C lasting 2 days. Detailed descriptions were made on the clinical course of those with influenza, including laboratory abnormalities, and no deaths were reported.

Recently, human challenge models for influenza virus have been developed to study the epidemiology of this infection and to test the efficacy of drugs and vaccines [3, 4]. The current influenza challenge model enrolls healthy susceptible adults who are influenza antibody negative and infected intranasally with a well-characterized pool of wild-type influenza virus. Volunteers become infected and develop a mild illness with recovery of virus from the nasopharynx. This human challenge model has been used to evaluate antiviral agents, including neuraminidase inhibitors, and influenza vaccines. The performance parameters for this challenge model were the development of viral excretion from the respiratory tract (nasal wash and throat swab) and the development of an influenza-like illness, defined as ≥24 hours of either fever >37.9°C with 1 respiratory symptom or ≥2 symptoms of which 1 is a respiratory symptom. A challenge influenza virus A/WI produced according to good manufacturing practices was given to 9 volunteers via a nasal solution containing 5.5 $\log_{10}$ median tissue culture infective dose per milliliter of virus into each nostril [3]. Oseltamivir was given

Correspondence: Timothy P. Endy, MD, MPH, State University of New York, Upstate Medical University, 725 Irving Ave, Ste 304, Syracuse, NY 13210 (endyt@upstate.edu).

The Journal of Infectious Diseases 2014;209(S2):S56–60

DOI: 10.1093/infdis/jiu112

to all volunteers from day 4 for a 5-day course to minimize post-experiment transmission. Seven of the 9 volunteers were infected (78%), with 5 reporting symptoms, of whom 4 had symptoms consistent with an influenza-like illness. One volunteer had fever, 4 were culture positive from nasal wash, 7 were polymerase chain reaction positive on nasal wash, and 7 seroconverted. There were no fatalities or severe adverse events, and the authors concluded that the influenza human challenge model was safe and a model in which to rapidly develop and down-select influenza vaccines and antivirals. The human performance parameters for the challenge model were the reproducible development of viral excretion and the development of mild clinical symptoms consistent with an influenza-like illness.

Measles Virus Human Challenge Model

The first recorded use of a live-attenuated viral vaccine as a challenge virus to test the immunogenicity of a vaccine against the same virus was in the testing of the killed measles vaccine [5, 6]. In these series of experiments, the efficacy of a killed alum-adsorbed measles virus vaccine developed by Eli Lilly and Company was evaluated by challenging vaccine recipients with an attenuated live measles virus vaccine developed by Lederle Laboratories. The performance parameters of this challenge model were to induce fever and clinical symptoms and signs of measles infection. In this reported series, 680 children were challenged with live attenuated vaccine virus as follows: 1 month after 3 doses of killed vaccine (n = 25), 5–13 months after 3 doses (n = 50), 1 month after 2 doses (n = 117), and after 1 dose of killed vaccine (n = 488). Clinical illness occurred after challenge in 15% of children on or after the fifth postchallenge day. Very few had rash and none developed classic measles infection. No control subjects were used to establish the challenge model with attenuated vaccine alone and disease severity. The authors concluded that the killed vaccine produced solid immunity based on the attenuated measles virus challenge. Of note, the killed vaccine was eventually licensed and then pulled off the market due to the occurrence of atypical measles infection upon infection with wild-type measles virus and its association with previous immunization to killed measles vaccine [7]. This experience underscores the importance of using controls to validate the clinical severity or lack of symptoms of the challenge virus strain.

Albert Sabin Studies on a Dengue Human Challenge Model

Human dengue challenge experiments were performed by Dr Albert Sabin in the 1940s during World War II [8]. Human volunteers were experimentally infected with wild-type dengue virus (DENV) obtained from infected individuals and then rechallenged with the same DENV or a heterologous strain at different time periods after initial infection. The performance parameters for these experiments were to induce symptomatic DENV infection, primarily fever, rash, and leukopenia. Sabin's findings demonstrated the following: (1) human volunteers could be successfully inoculated with passaged DENV to induce clinical dengue fever; (2) human volunteers were protected from reinfection to the same strain of DENV up to 18 months following initial infection (the longest time period tested) and resulted in complete homotypic protective immunity; (3) following initial infection, humans developed heterologous protective immunity to challenge from heterologous DENV strains for up to 2 months after initial infection; and (4) rechallenge with heterologous DENV resulted in an attenuated illness that lasted up to 9 months after initial infection. For this discussion, the key concept that arose from Sabin's findings, which have implication for a modern-day dengue human infection model, is that human volunteers could be safely experimentally infected with DENV to test if homotypic immunity to a specific DENV serotype provides solid protective immunity to that specific serotype, a key tenet for testing a potential DENV vaccine.

Recent Dengue Human Experimental Infection and Challenge Studies

The Walter Reed Army Institute of Research performed the first modern-day series of 3 DENV human experimental infection and challenge studies since the Sabin studies of the 1940s [9] (see Lyons et al in this supplement). In the published work of Sun and colleagues (study 3) [9], 10 subjects previously vaccinated with a live attenuated tetravalent dengue vaccine (TDV) and 4 DENV-naive control subjects were challenged by subcutaneous inoculation of previous monovalent live-attenuated DENV vaccine candidates that were too reactogenic as a vaccine candidate. All 5 TDV recipients were protected against DENV-1 challenge; of recipients challenged with DENV-3, 2 were protected. All DENV-3 challenge subjects who developed viremia also developed elevated liver enzyme levels, and 2 had values that were >10 times the upper limit of normal. All 4 control subjects developed dengue fever from challenge. The DENV-1 and DENV-3 strains were chosen because these caused fever in previous experimental human dengue infection studies 1 and 2. The development of fever was one important component of the performance challenge model. When given at 10^4 plaque-forming units subcutaneously, both strains consistently elicited uncomplicated dengue fever in 4 and 5 adult subjects, respectively. Both DENV-1 experimental infection controls developed fever of 39.3°C and had dengue symptoms that lasted 2–6 days. The incubation periods in the control subjects were long, at 17 and 21 days. Some subjects developed elevations in liver function tests, rash, and leukopenia. No subjects had any evidence for classic dengue hemorrhagic fever (DHF), although some had asymptomatic effusions.

CLINICAL DENGUE VIRUS INFECTION

Many studies have documented the symptoms of both primary and secondary dengue infection. For the sake of this discussion,

the World Health Organization's description of dengue fever is characterized by a high-grade fever, severe headache, pain behind the eyes, nausea, vomiting, rash, and a low total white blood cell count [10]. Thrombocytopenia and bleeding are features of severe dengue, although this may also occur in milder disease. Severe dengue is characterized by increased vascular permeability and plasma leakage leading to shock, clinically known as DHF. As a discussion on performance parameters for a dengue human infection model, the spectrum of clinical illness ranges from subclinical with limited viremia, subclinical with viremia and fever and few symptoms, clinical dengue fever, and severe dengue with hemorrhagic manifestations. For risk to human subjects, with risk being defined as mortality or prolonged morbidity, we know that severe dengue infection with hemorrhagic manifestations is treatable with supportive care, but mortality can range from 1% to 5% in certain populations. Dengue fever is not normally associated with mortality but can result in severe morbidity with several days of hospitalization. Unlike influenza and other viral infections, there is no antiviral drug that modifies disease severity for DENV infection.

GOALS OF A DENGUE HUMAN INFECTION MODEL

The ultimate goal of a dengue human infection model (DHIM) is to predict the clinical efficacy of a vaccine or antiviral drug to protect against dengue disease following natural infection with any of the 4 DENV serotypes. The challenge is to know what factors are important to develop a highly predictive human infection model. Certainly on one end of the spectrum, using wild-type DENV, such as Sabin used in his studies to produce actual symptomatic infection, would be highly predictive, as the vaccine or drug would be protecting against actual wild-type infection. On the opposite end of the spectrum, using a highly attenuated DENV strain that produces little or no viremia and no symptoms would probably not be predictive at all of wild-type DENV. Between the two ends of the spectrum lies a risk/benefit matrix in which to develop an infection model (discussed in the next section). Ultimately, a challenge virus and infection model will be developed that balances risks and benefits, is safe for the volunteer and acceptable for institutional review boards, and meets the goals of a DHIM. These goals, in order of importance, are to (1) predict clinical efficacy of a vaccine or antiviral to protect against all 4 serotypes and their circulating genotypes of wild-type DENV, (2) validate host biomarkers of protection in which to cross-reference and develop second- or third-generation vaccines and antivirals, (3) validate host biomarkers that indicate the type of infecting DENV at the time of infection or in convalescence as diagnostics for clinical care or in epidemiology studies, and (4) study the host response during DENV infection to understand the pathogenesis of the virus–host response that leads to severe DENV infection.

PROPOSED PERFORMANCE PARAMETERS OF DHIM

With the goals of a DHIM in mind, Figure 1 displays a risk-benefit matrix in which to determine performance parameters of an infection model. Across the top of the matrix is the host response to DENV infection, ranging from subclinical viremia with no symptoms to classic dengue fever and DHF. Along the y-axis is the potential predictive ability of a challenge model to predict how well the vaccine or drug will work against wild-type DENV, in descending order from low prediction to high prediction, based on the assumption that the closer the challenge virus is to wild-type virus, the higher predictive ability the model will be. Along the x-axis is risk, with the assumption that the highest risk will be from clinical dengue fever and DHF and the lowest risk from subclinical infection. As indicated by this figure, the ideal balance would be a challenge virus that produces viremia and clinical symptoms that are more severe than subclinical infection but less than classic dengue fever. To be more specific, the performance parameters of a DHIM would have the following features: (1) incubation period of 3–5 days (practical consideration for human clinical trials); (2) measurable and sustainable viremia of 3–5 days' duration at a viral load of at least 2 logs (reflective of natural infection); (3) fever defined as ≥38°C for 24 hours' duration but no more than 72 hours; and (4) 2 of the following symptoms or clinical/laboratory findings: headache, myalgia, rash, elevation in liver function tests no more than 2 times normal, leukopenia not <2000 cells/μL, and thrombocytopenia not <100 000 platelets/μL. Table 1 is the toxicity grading scale as recommended by the Food and Drug Administration during clinical trials [11]. For the DHIM, using the toxicity grading scale, it is suggested that the injection site reaction should be no more than a grade 2; fever should be no more

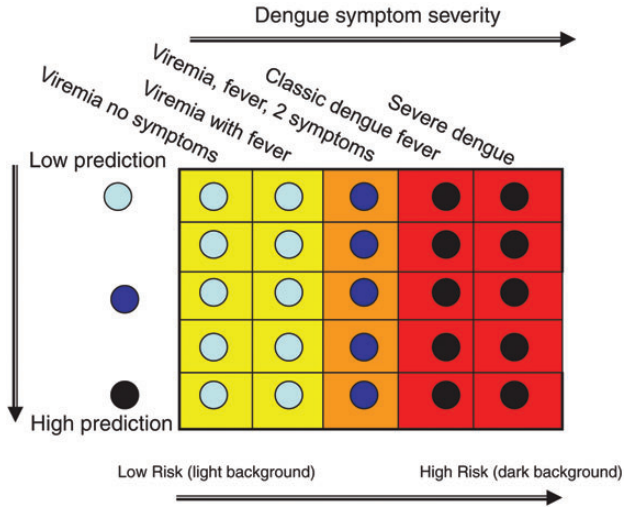

Figure 1. Risk-benefit matrix for a human dengue challenge model.

Table 1. Suggested Toxicity Grading Scale for the Dengue Human Infection Model[a]

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of nonnarcotic pain reliever for >24 h or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/redness	2.5–5 cm	5.1–10 cm	>10 cm	Necrosis or exfoliative dermatitis
Induration/swelling	2.5–5 cm and does not interfere with activity	5.1–10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis
Fever, °C	38.0–38.4	38.5–38.9	39.0–40	>40
Tachycardia (beats per min)	101–115	106–1306	>130	ER visit or hospitalization for arrhythmia
Bradycardia (beats per min)	50–54	45–49	<45	ER visit or hospitalization for arrhythmia
Hypertension (systolic), mm Hg	141–150	151–155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic), mm Hg	91–95	96–100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic), mm Hg	85–89	80–84	<80	ER visit or hospitalization for hypotensive shock
Respiratory rate (breaths per min	17–20	21–25	> 25	Intubation
Nausea/vomiting	No interference with activity or 1–2 episodes per 24 h	Some interference with activity or >2 episodes per 24 h	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2–3 loose stools or <400 g per 24 h	4–5 loose stools or 400–800 g per 24 h	6 or more watery stools or >800 g per 24 h or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of nonnarcotic pain reliever >24 h or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Abbreviations: ER, emergency room; IV, intravenous.

[a] Derived from the Food and Drug Administration toxicity grading scale for clinical trials [11].

than a grade 3, vital signs a grade 1; systemic symptoms including headache, fatigue, and myalgia no more than a grade 2; all laboratory abnormalities a grade 1 except for white blood cell and platelet count, a grade 2; and urine a grade 1. For consistency of performance, 90% of controls should manifest the parameters as outlined.

CONCLUSIONS

A dengue human infection model will be an important model in which to rapidly down-select dengue vaccine candidates and potential antiviral drugs. Historically and currently, other human challenge/infection models have been used to test other viral vaccines; thus, a DHIM will be in keeping with these models. In light of the recent clinical trials of the most advanced dengue vaccine candidate in clinical development, it becomes imperative to develop this model in which to test dengue vaccines [12]. The DHIM performance parameters will need to strike a balance between risk of harm and predictive ability to determine how a vaccine or drug will perform in actual efficacy studies in the human population. This article proposes a way to look at this balance and suggests parameters in which to develop this model.

Note

Potential conflicts of interest. T. P. E. is a coinvestigator on a phase 1 vaccine trial with Inviragen, serves a data safety monitoring board for a dengue vaccine trial with Merck and Co, and receives research funds from the National Institutes of Health and the Department of Defense.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Andrewes CH, Laidlaw PP, Smith W. Influenza: observations on the recovery of virus from man and on the antibody content of human sera. Br J Exp Path **1935**; 16:566–82.
2. Smorodintseff AA, Tushinsky MD, Drobyshevskaya AI, Korovin AA, Osetroff AI. Investigation of volunteers infected with the influenza virus. Am J Med Sci **1937**; 194:159–70.
3. Killingley B, Enstone JE, Greatorex J, et al. Use of a human influenza challenge model to assess person-to-person transmission: proof-of-concept study. J Infect Dis **2012**; 205:35–43.
4. Killingley B, Enstone J, Booy R, et al. Potential role of human challenge studies for investigation of influenza transmission. Lancet Infect Dis **2011**; 11:879–86.
5. Karelitz S, Berliner BC, Orange M, Penbharkkul S, Ramos A, Muenboon P. Inactivated measles virus vaccine. Subsequent challenge with attenuated live virus vaccine. JAMA **1963**; 184:684–7.
6. Lin CY, Huang CH, Chen YH. Classification of dengue: the clinical use of World Health Organization 2009 guideline. J Formos Med Assoc **2013**; 112:61–3.
7. Alejandria MM. Dengue haemorrhagic fever or dengue shock syndrome in children. Clin Evid (Online) **2009**; 2009. pii:0917.
8. Sabin AB. Research on dengue during World War II. Am J Trop Med Hyg **1952**; 1:30–50.
9. Sun W, Eckels KH, Putnak JR, et al. Experimental dengue virus challenge of human subjects previously vaccinated with live attenuated tetravalent dengue vaccines. J Infect Dis **2013**; 207:700–8.
10. World Health Organization, Department of Child and Adolescent Health and Development. Dengue, dengue haemorrhagic fever and dengue shock syndrome in the context of the integrated management of childhood illness. Geneva, Switzerland: WHO, **2005**.
11. US Food and Drug Administration. Guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Rockville, MD: Center for Biologics Evaluation and Research, Food and Drug Administration, US Department of Health and Human Services, **2007**.
12. Sabchareon A, Wallace D, Sirivichayakul C, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. Lancet **2012**; 380:1559–67.