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Dengue Human Infection Models Supporting Drug Development

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Dengue is a arboviral infection that represents a major global health burden. There is an unmet need for effective dengue therapeutics to reduce symptoms, duration of illness and incidence of severe complications. Here, we consider the merits of a dengue human infection model (DHIM) for drug development. A DHIM could allow experimentally controlled studies of candidate therapeutics in preselected susceptible volunteers, potentially using smaller sample sizes than trials that recruited patients with dengue in an endemic country. In addition, the DHIM would assist the conduct of intensive pharmacokinetic and basic research investigations and aid in determining optimal drug dosage. Furthermore, a DHIM could help establish proof of concept that chemoprophylaxis against dengue is feasible. The key challenge in developing the DHIM for drug development is to ensure the model reliably replicates the typical clinical and laboratory features of naturally acquired, symptomatic dengue.

Keywords. dengue; human infection model; clinical trial; drug development.

In most cases, fever and symptoms resolve uneventfully after 3–7 days with no long-term sequelae [1]. In a subset of cases, a transient vascular leakage syndrome develops after 3–4 days of illness, which, when severe, can lead to life-threatening hypovolemic shock (called dengue shock syndrome) and/or hemorrhage [1, 5]. The vascular leak syndrome may be associated with complement activation and a coagulopathy that can contribute to the risk of hemorrhage [7, 8]. Signs of vascular leakage include an increasing hematocrit, microalbuminuria, or the accumulation of fluids at serosal surfaces (eg, pleural effusions) [5].

Supportive care and the careful titration of minimum volumes of parenteral crystalloid fluids to maintain stable cardiac output during the 1–3-day period of vascular permeability are critical elements in dengue case management [5]. Vasopressors are commonly used in cases with prolonged shock. Clinically significant hemorrhage requiring use of blood products is more common in adults than in children [9].

Improvements in the management of severe dengue cases have seen the case mortality rate decline over the last decade in many but not all endemic settings [10–12]. At present there is no validated way of identifying which patients will progress to more severe disease, meaning that health facilities in endemic areas are often...
overwhelmed with patients with dengue who require ongoing observation as outpatients or are admitted for inpatient observation.

THE CASES FOR DENGUE THERAPEUTIC AND PROPHYLACTIC AGENTS

Antivirals
A drug that can shorten the duration of illness and reduce the risk of disease progression would be a significant advance for both patients and for overstretched health systems in dengue-endemic countries. An obvious approach is to target virus replication with a small molecule antiviral drug that given early in the course of illness has the potential to shorten the duration of infection. Moreover, because high early DENV viremia levels are a risk factor for the development of more severe disease, an antiviral drug might also reduce the incidence of severe complications [13, 14]. In addition, a drug that reduces the magnitude and duration of viremia has the potential to reduce onward DENV transmission by reducing the length of time an individual patient is infectious to mosquitoes [15]. Alternatives to small molecule antiviral drugs include therapeutic monoclonal antibodies; however, high costs and requirement for parenteral administration are currently barriers to their use.

Disease Modulators
Both host and virus factors are important in influencing the outcome of DENV infection. A range of host factors, such as age, sex, genotype, and flavivirus infection history, influence the disease outcome [10, 16, 17]. Because the manifestations of severe dengue are typically observed in secondary DENV infections and when innate and adaptive immune responses have driven steep declines in viremia levels, it is widely held that the immune system plays an important role in disease pathogenesis [18]. This represents the rationale for use of an immunomodulatory drug that can favorably modulate the poorly defined immunopathogenic mechanisms postulated to underlie severe dengue. It is possible that the dichotomous approach of targeting either the virus or the immune response is an oversimplified strategy to the development of dengue therapeutics. Other physiological systems are involved in the control of endothelial integrity, but their role in dengue remains poorly understood [19].

Prophylaxis
A vaccine would be the most effective prophylactic public health intervention for control of dengue, but the development of an efficacious tetravalent dengue vaccine is proving to be a challenge [20, 21]. An alternative or adjunctive approach is the use of drug chemoprophylaxis in the community. Chemoprophylaxis using oseltamivir has been advocated for controlling the spread of influenza within households and was commonly used in some settings during the 2009 H1N1 influenza pandemic, creating a precedent for such an approach for an acute viral infection [22, 23]. The safety profile of a prophylactic dengue drug would have to be exemplary, and there are major questions of whether this approach could be cost-effective and sustainable, given the temporally and spatially heterogenous patterns of DENV transmission in endemic regions [24, 25]. Moreover, in most dengue-endemic regions a significant fraction of the population is already immune and will not benefit from chemoprophylaxis. Consequently, in endemic areas the number needed to treat, and the duration they need to be treated for, may be large for the prevention of a single symptomatic case and very large for the prevention of severe cases. There is a marginally stronger argument for chemoprophylaxis in populations with no immunity to DENV. Examples of this include dengue-naive travelers or military personnel who are transiently present in areas of high DENV transmission. Similarly, chemoprophylaxis could be indicated for communities experiencing “virgin soil” dengue outbreaks because of importation by viremic travelers or infected mosquitoes.

PREVIOUS CLINICAL TRIALS OF THERAPEUTICS FOR DENGUE AND RELEVANCE TO THE DENGUE HUMAN INFECTION MODEL

For a disease with an estimated global burden prevalence of 100 million cases per year, remarkably few clinical trials of treatment approaches have been performed. Early trials tested fluid resuscitation approaches in patients with hypovolemic shock [26–28]. More-recent trials have tested the candidate antiviral agents chloroquine and balapiravir or have targeted the immune response by use of oral corticosteroids [29–31]. Although none of these trials have demonstrated therapeutic efficacy, they have provided a framework for the conduct of such trials and has led to the publication of recommendations for a standardized approach to dengue clinical trial design and conduct [15]. In addition, a trial of lovastatin in dengue is currently underway and further trials are planned [15, 32]. The virological findings in these trials, consistent with previous literature, have underscored the need for antiviral therapy to commence early in the course of illness, because illness resolves and patients are no longer infectious to Aedes aegypti mosquitoes by 5–7 days after illness onset [14, 15, 33–35].

POTENTIAL ROLES FOR A HUMAN INFECTION MODEL IN DENGUE DRUG DEVELOPMENT

In principal, a dengue human infection model (DHIM) provides opportunities for fast-tracking dengue drug development, particularly therapeutic and prophylactic antivirals. The overall strength of the DHIM is that it allows for controlled prophylactic or therapeutic studies of DENV infection in susceptible participants who have been preselected for a set of desirable...
characteristics. In contrast, prophylactic or therapeutic studies of DENV infection in an endemic country population are subject to the vagaries of ethnic diversity of the patient population, fluctuating serotype and genotype prevalence, variable viremia levels, and prior flavivirus infection histories. Collectively, these sources of variation in an endemic country population probably boost the required patient sample size required for detection of therapeutic or clinical safety signals.

**CRITICAL HURDLES FOR A DHIM**

Central to the usefulness of a DHIM is whether it mimics the main features of a symptomatic dengue case. This is a fundamental and critical hurdle in the development of a successful DHIM for use in dengue therapeutic development. Thus, the kinetics of clinical, virological, hematological, and biochemical changes that are typically present in a naturally acquired and clinically uncomplicated adult dengue case should also manifest in the adult DHIM. In the beginning, at least, we assume that the DHIM will involve participants who are flavivirus naive, and thus all experimental infections will be primary infections.

In the context of dengue drug development, the characteristics of a symptomatic, naturally acquired DENV infection that a DHIM would need to replicate include acquisition of infection and certain clinical, virological, and laboratory features.

**Acquisition of Infection**

A DHIM could use DENV-infected mosquitoes to elicit infection in human volunteers. Although this approach may be optimal in replicating the natural history of DENV infection because, for...
example, the immunoregulatory effects of mosquito saliva would be replicated, it may present additional regulatory and logistical hurdles. Alternatively, a DHIM could use subcutaneous inoculation of laboratory-cultured virus, as used in the challenge experiments by Sun and colleagues [36]. This approach bypasses issues surrounding use of mosquitoes, although it remains to be determined if there are material differences between needle-delivered and mosquito-delivered DENV infection.

Clinical Features
Typical signs and symptoms of dengue that a DHIM would need to replicate in otherwise healthy adults include fever, headache, myalgia, musculoskeletal pain, and nausea or vomiting [37]. Minor hemorrhagic features may occur between days 3 and 6 of illness (eg, petechiae) [37]. Prevention or rapid amelioration of these self-limiting symptoms will be a target of dengue therapeutic drug development. Primary infections in otherwise healthy adults are rarely associated with severe clinical complications, but it would be prudent to exclude the elderly and those with comorbid conditions from the volunteer pool, because these groups are at elevated risk of severe complications [38, 39].

Virological Features
The intrinsic incubation period of DENV infection has been estimated to be between 4–10 days [5]. Work that reanalyzed historical mosquito exposure experiments estimated that the intrinsic incubation period in primary infection was approximately 6 days [40]. The duration of viremia varies by serotype and DENV infection history. The peak viremia level is often not observed in naturally occurring infections because it precedes the time point when patients attend health care facilities, but findings suggest that the peak level occurs later in primary infections as compared to secondary [33]. Studies suggest that primary infections with DENV-1 are associated with a longer viremic period and higher viremia levels [33, 41]. An optimal DHIM would replicate the virological features of a naturally acquired DENV infection to enable confident predictions of therapeutic or prophylactic drug efficacy in the field.

Laboratory Features
A DHIM should elicit thrombocytopenia and leukopenia, both typical features in patients with primary or secondary dengue. In addition, many patients have minor elevations in transaminase and creatine kinase levels [9, 42]. Clotting abnormalities are also often observed in severe cases with elevated activated partial thromboplastin times and reduced levels of fibrinogen; however, these occur less frequently in primary infections [43].

STRENGTHS AND WEAKNESSES OF A DHIM FOR DRUG DEVELOPMENT
The strengths and weaknesses of a DHIM for performing early-phase clinical studies compared to performing such studies in naturally acquired cases in an endemic country are summarized in Table 1. In short, the great advantage of the DHIM for early-phase drug development is the ability to perform controlled prophylactic or early therapeutic studies in a setting amenable to intensive pharmacological and basic research investigations.

FOCUS AREAS IN WHICH THE DHIM COULD SUBSTANTIALLY ADVANCE CLINICAL DRUG DEVELOPMENT
A DHIM that mimics natural infection would be particularly well suited to early-phase studies of chemoprophylaxis and accompanying pharmacokinetic and pharmacodynamic investigations. For example, the immunoregulatory effects of mosquito saliva would be replicated, it may present additional regulatory and logistical hurdles. Alternatively, a DHIM could use subcutaneous inoculation of laboratory-cultured virus, as used in the challenge experiments by Sun and colleagues [36]. This approach bypasses issues surrounding use of mosquitoes, although it remains to be determined if there are material differences between needle-delivered and mosquito-delivered DENV infection.

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