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Review

Trials and tribulations on the path to developing a dengue vaccine

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ABSTRACT

Dengue is a rapidly expanding global health problem. Development of a safe and efficacious tetravalent vaccine along with strategic application of vector control activities represents a promising approach to reducing the global disease burden. Although many vaccine development challenges exist, numerous candidates are in clinical development and one has been tested in three clinical endpoint studies. The results of these studies have raised numerous questions about how we measure vaccine immunogenicity and how these readouts are associated with clinical outcomes in vaccine recipients who experience natural infection. In this review the authors discuss the dengue vaccine pipeline, development challenges, the dengue vaccine-immunologic profiling intersection, and research gaps.

1. Introduction

Development of vaccines against dengue has been identified as a priority by the World Health Organization, the US Health and Human Services, the US Department of Defense, the Bill and Melinda Gates Foundation, and Ministries of Health from dengue endemic nations. Dengue is the acute febrile disease caused by the flaviviruses classified as dengue virus serotypes 1-4 (DENV-1, DENV-2, DENV-3, and DENV-4). Human infection with DENVs occurs almost exclusively through a transmission cycle between humans and mosquitoes of the genus Aedes, with *Aedes aegypti* serving as the principal vector. Trends in the growth and distribution of human and mosquito populations over the past 50 years have been highly favorable to DENV transmission. As a result, both *Aedes aegypti* and DENVs are widely distributed in tropical and subtropical areas of the world. It has been estimated that over 3 billion people are at risk for infection and that 390 million infections occur annually, of which approximately 96 million result in clinically apparent disease [1].

Dengue's unique clinical and epidemiologic characteristics contribute to its global health impact and challenge disease control efforts. As is true for many other viruses, many infections pass without recognized symptoms or signs [2]. Nevertheless, recent evidence confirms that clinically inapparent infections are likely to contribute to overall DENV transmission and thus are important to overall efforts to control dengue [3]. Among those infections that are clinically apparent, the spectrum of disease severity is very broad, extending from an uncomplicated febrile illness lasting several days to a life-threatening plasma leakage syndrome. At one end of this spectrum, school or work absences and disruption of normal activities due to mild dengue add significantly to overall morbidity due to the potentially overwhelming number of cases [4]. At the other end, although supportive care is very effective at keeping the case-fatality rate low, dengue is a significant concern to parents and clinicians in endemic areas and the high number of hospitalizations places a major strain on the public health infrastructure that is disproportionally borne by low and middle income countries (LMIC).

Infection with the DENV generates protective immunity, providing a theoretical basis for vaccination. In the case of natural infection, protective immunity develops in a step-wise fashion. However, the balance of humoral and cellular immune responses which constitutes an immuno-protective profile remains incompletely understood. Immune responses to a first (primary) DENV infection are mainly serotype-specific, and appear to provide long-lasting, perhaps life-long, resistance to re-infection with the same DENV serotype. Immune responses to the other (heterologous) DENV serotypes are detectable at low levels after a primary infection, and individuals become resistant to infection with heterologous serotypes, but this cross-reactive protective immunity lasts for only a few months [5]. Following this short period, secondary infection with another DENV serotype can occur, with important differences from primary infection.

Although the spectrum of dengue disease severity is equally wide in primary and secondary infection, the distribution of cases following secondary infection differs substantially; a smaller

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percentage of infections are inapparent while the likelihood of plasma leakage and hospitalization is markedly increased [6]. Immune responses in secondary infection display an anamnestic profile. Antibody levels reach high titer and show broad serotype-cross-reactivity, and T cell responses are predominantly directed at serotype-cross-reactive epitopes. These immune responses appear to provide protection against infection with the remaining serotypes for a year or longer [7]. Hospital-based seroepidemiology studies suggest that the risk of severe disease and hospitalization is lower during post-secondary infections; however, firm data are lacking because antibody profiles do not permit a clear distinction of secondary and subsequent infections [8]. In contrast to natural infection, when an individual receives a dengue vaccine he or she is exposed to attenuated or fragments of all four DENVs simultaneously. It is unclear what single dengue vaccine antigen or combination of antigens is required to sufficiently mimic natural immunity and protect its recipient from disease following infection.

2. Dengue vaccine development pipeline

2.1. General

There are numerous dengue vaccine candidates in the pipeline spanning the spectrum from pre-clinical to advanced clinical development. Vaccine candidates are diverse, representing classic live attenuated and inactivated constructs along with approaches utilizing technologies to produce recombinant antigens, DNA constructs, vector-based expression, and virus like particles (VLPs). Each approach attempts to induce immune responses to individual or combined structural and non-structural protein targets encoded by the dengue genome believed to play an essential role in DENV infection or replication once infection occurs.

The DENV envelope (E) protein attaches to host cell receptors, facilitates endosomal membrane fusion, and displays sites mediating viral neutralization [9]. Because of this, all dengue vaccine candidates in clinical development contain at least a portion of each DENV serotype's E protein (DENV-1-4). Where the candidates differ is their inclusion of DENV non-structural (NS) proteins. Certain candidates contain no NS proteins, some contain NS proteins from each serotype, others contain the NS proteins from a single serotype with different pre-Membrane (preM) and E proteins representing all serotypes, and one candidate uses the NS proteins from Yellow fever 17D vaccine with the preM and E proteins from each DENV serotype [10].

2.2. Pre-clinical development tools

Pre-clinical vaccine development activities are intended to recreate the human clinical and immunologic response to infection, immunization, and immunization followed by infection (challenge studies). The intent is to utilize small animal and non-human primate models of infection and disease to inform vaccine development decisions regarding the selection of viable antigen platforms, dose selection and formulation, and administration methods, routes, and schedules. Most importantly, animal models provide developers with an early indication of the safety profile of any particular candidate, its ability to induce a relevant immunologic response, and whether there is the potential the immunologic response could confer some clinical benefit to the recipient in the form of preventing infection, preventing disease, or significantly attenuating disease following infection. Models are intended to de-risk development programs.

Mouse models of DENV infection and pathogenesis have improved significantly and experienced increased use for early investigations of candidate vaccines or anti-dengue antivirals. In addition to increased availability and lower cost relative to non-human primates, they have improved their ability to mimic humanized immunologic responses to infection. There are numerous mouse models, each with strengths and weaknesses.

Wild type mouse models have demonstrated central nervous system findings following DENV infection but lack many classic dengue clinical features [11]. The propensity to experience neurologic abnormalities may become increasingly important as central nervous system insults are being reported more often in the dengue clinical literature [12,13]. AG129 mice lack IFN-α/β and–γ receptors and readily support DENV infection and viral replication [14]. However, like wild type mice, AG129 mice fail to demonstrate a complete spectrum of human disease phenotypes. Mice lacking only IFN-α/β receptors ($IFNAR^{-/-}$) retain IFN- γ and the neuroprotective action of $CD8^+$ T cells. Similar to AG129 mice, $IFNAR^{-/-}$ mice support DENV replication resulting in select, but not comprehensive, human disease features [15]. Disease phenotypes of both AG129 and $IFNAR^{-/-}$ mice have been made more severe by infecting mice in the presence of sub-neutralizing or enhancing antibody preparations [16].

"Humanized" mice have been developed by engrafting various human cell lines into severely immunodeficient mice. Severe combined immunodeficient (SCID) mice lacking B and T cells, irradiated non-obese diabetic (NOD)/SCID, NOD/SCID/IL-2Rγ-null, and $RAG2^{-/-}/\gamma c^{-/-}$ mice have been engrafted with human hepatocarcinoma or leukemia cells (HepG2 or K562, respectively), human $CD4^+$ hematopoietic stem cells, cord blood hematopoietic stem cells, and fetal thymus and liver tissue [17–23]. Fever, rash, viremia, cytokine production, and development of anti-dengue IgM following experimental infection have all been reported in these models. Although encouraging, there is inter-mouse performance variability and, similar to the "non-humanized" models, the full complement of the human immune system is not represented and may impact disease expression and/or vaccine efficacy. Practical considerations of cost and availability of sufficient numbers are also of concern.

Despite sharing a close genetic relationship to humans, non-human primates are disappointing models of dengue disease. Non-human primate species are permissive to DENV infection but typically do not display clinical pathology [24–26]. A recent literature review provides a comprehensive summary of primate species' virologic and clinical responses to infection with a variety of DENV strains [27].

The general observation is that non-human primates, following viral inoculation, experience infection, develop measurable peripheral viremia, and a neutralizing antibody and cellular immune response [28,29]. A recent literature review explored estimates of the time to detectable viremia following DENV inoculation and the duration of viremia among a number of Old and New World non-human primate species. The time to viremia in rhesus macaques ranged from 2.63 to 3.32 days for DENV-2 and -1 and the duration from 3.13 to 5.13 days for DENV-4 and -2. Differences in time to viremia and viremia duration were not significantly different between non-human primate species [30]. Quantitative measures of non-human primate viremia reveal peripheral RNAemia values to be lower than what is found in humans experiencing dengue disease, offering one explanation of why non-human primates do not develop clinical disease consistent with human dengue illness. To overcome this limitation investigators have explored intravenously inoculating non-human primates with DENV and claim the method produced "classic" hemorrhagic manifestations. This model has not been widely reproduced [31].

It is possible that additional alterations in study methodology may improve the non-human primate dengue model. Exploring new infecting viral strains, incorporating mosquitoes into the process of virus inoculation, increasing the viral concentration of the inoculation, or trying to create an immune environment conducive