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REVIEW

THE DENGUE VACCINE CHALLENGES

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Abstract

Dengue is estimated to be the most common mosquito-borne disease globally, affecting mainly tropical and subtropical countries. It has been identified as a disease of the future, due to persistent trends in urbanization of societies, scarce water resources and changes in the environment. The four dengue virus serotypes cause from the usual mild illness to a potentially fatal syndrome associated with increased vascular permeability. The unique antigenic and biological properties of the virus have made it difficult to develop vaccines. The authors have analysed "Dengvaxia", the only licensed vaccine with partial efficacy (it protects most of the people, but makes others more susceptible to serious diseases) and complex performance. The impact of these results on the future of the dengue vaccines is discussed.

Keywords: dengue, antibody-mediated enhancement, vaccines.

List of abbreviations
DENV- Dengue virus

Résumé

Les défis du vaccin contre la dengue

On estime que la dengue est la maladie transmise par les moustiques la plus fréquente dans le monde, affectant principalement les pays tropicaux et subtropicaux. Elle a été identifiée comme une maladie du futur en raison des tendances persistantes de l'urbanisation des sociétés, de la rareté des ressources en eau et des changements dans l'environnement. Les quatre sérotypes du virus de la dengue causent de la maladie bénigne habituelle à un syndrome potentiellement mortel associé à une perméabilité vasculaire accrue. Les propriétés antigénique et biologique uniques du virus ont rendu difficile le développement de vaccins. Les auteurs ont analysé «Dengvaxia», le seul vaccin homologué avec une efficacité partielle (il protège la plupart des gens mais rend les autres plus sensibles aux maladies graves) et des performances complexes.

Mots-clés: dengue, renforcement médié par les anticorps, vaccins

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DHS- Dengue Hemorrhagic Syndrome
DSS- Dengue Shock Syndrome
IgG- Immunoglobulin G
IgM- Immunoglobulin M
PCR- polymerase chain reaction

Introduction

Dengue is the most significant and rapidly spreading infection transmitted by *Aedes* mosquitoes (*Ae. Aegypti, Ae. Albopictus*), which has adapted to living in and around people's homes. The significance of the disease in the last 50 years is determined by its increasing frequency, global spread and the appearance of severe, hitherto unknown clinical forms. The World Health Organization data on the number of countries endemic for the disease indicate from 9 before 1970 to more than 100 in May 2021 in Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific[1]. The increase in South-East Asia is at a rate not seen in other infectious diseases, and for the last 13 years alone it has been 400%[2].

The contributing factors in the 20th century are the global warming, increasing human population, overpopulation, urbanization, air transport, and the lack of effective and underfunded vector control programs in endemic countries[2,3]. In endemic countries, dengue leads to high morbidity and hospitalization of virologically confirmed dengue, which reaches 19% in Asia against 11% in Latin America[4]. Many countries in Southeast Asia are attractive tourist destinations for Europeans. Among travellers returning from low- and middle-income countries, dengue is the second most diagnosed cause of fever after malaria[1].

In the absence of large-scale effective and long-term vector control programs and the promising but initial results of the new strategy with Wolbachia bacteria, dengue vaccine is the best prophylactic intervention[5].

The authors present data on the first licensed dengue vaccine and discuss future vaccines.

Dengue – the most common arbovirus infection on the planet

The term ‚dengue' is thought to be a Spanish homonym for the Swahili phrase ‚ki denga pepo', meaning a sudden seizure similar to spasms of an evil spirit or plague[6]. The name „brittle bone fever", which is attributed to the excruciating joint pain that patients suffer from, is also often used instead of dengue.

Dengue is caused by the dengue virus (DENV), which belongs to the genus *Flavivirus*, family *Flaviviridae*[7]. Flaviviruses include the closely related viruses of yellow fever, West Nile, Zika, Chikungunya, and Japanese encephalitis.

DENV was first isolated in 1943 by Ren Kimura and Susumu Hotta, while studying blood samples collected from patients during the dengue epidemic that year in Nagasaki, Japan[8]. Independently from them, a year later, Walter Schlesinger and Albert B. Sabin (creator of the oral attenuated polio vaccine) also isolated DENV. DENV is an enveloped virus with a single positive-stranded RNA genome, encoding three structural (C, prM and E) and seven non-structural proteins (NS1-NS7). The latter play an important role in interacting with host proteins for successful virus replication. Currently, four closely related, but serologically different virus serotypes are known (DENV 1-4). The infection with each of them leads to intense immunity to the specific serotype (homotype), while cross-immunity to the other three serotypes (heterotype) is partial and short-lived[9]. Paradoxically, a subsequent infection with a DENV serotype other than the first can lead to a severe disease. Decreased levels of heterotypic neutralizing antibodies, instead of neutralizing the virus, bind to it in a complex, facilitate its entry into target cells and intensify the infection. This pathological phenomenon postulated by Halstead et al[10] is known as antibody-mediated enhancement of the viral replication. It causes the syndrome of increased vascular permeability, in which micromolecules and fluids leave the circulation[11]. It is observed in a small number of cases with a second heterotypic infection[12]. It is now obvious that the risk is not universal for all secondary dengue infections, but also the progression to severe forms requires proper antibody-virus ratio[12].

The increased vascular permeability syndrome has been shown to be due to circulating high levels of the non-structural protein DENV (NS1), a potent endothelial toxin[11]. It is more common in Europeans, among the Asian population, young children, adults, those with comorbidities and certain DENV serotypes[13].

Despite dengue-imported cases into the United States and subsequent small clusters over the years[14], indigenous (local) cases in Europe were first reported in 2010 in Croatia and France[15]. The first major outbreak (2000 cases) was reported in 2012 in Madeira, Portugal[16]. Dengue is a major cause of febrile illness in people returning from Southeast

Asia, ahead of malaria[17], but severe illness and death in tourists are rare.

However, there is enough historical data to show that long before the isolation of the causative agent, a large epidemic of dengue with biphasic temperature curve was registered in Europe, in the early twentieth century (in 1927-1928). The epidemic broke in Athens, Greece, affected more than 90% of the metropolitan area and resulted in more than 1,000 deaths[18]. This was related to the relatively recent entry of *Aedes albopictus* into these territories and should have served as a warning of the possibility of such a return of dengue to Europe.

Until 1950, dengue was known as a self-limiting febrile exanthematic disease, with low mortality in a few countries in Asia and Africa. In the middle of the 20th century, a new clinical syndrome appeared in Southeast Asia (Thailand, the Philippines), characterized by increased vascular permeability, a tendency to hemorrhage, organ failure, and shock, called dengue hemorrhagic syndrome (DHS). Severe dengue is observed in a second infection with a heterotypic DENV, which suggests the presence of an immunological phenomenon. A plausible explanation is the afore mentioned antibody-mediated enhancement of viral replication. In America, the first DHS epidemic was recorded in 1981 in Cuba, associated with the introduction of a new Asian serotype DENV-2, different from the American DENV-1[19]. This was followed by a dramatic spread of the disease and coverage of non-endemic countries in the United States and Europe. Globalization has led not only to the rapid spread but also to the introduction and circulation of several viral serotypes simultaneously, making most subtropical countries hyperendemic[20] (Figure 1).

According to the latest estimates, nearly half of the world's population (3.6 billion) live in areas at risk for dengue. The danger exists in 129 countries, with Asia bearing the brunt of the disease[21]. Annually, 390 million cases are registered worldwide, of which 96 million manifest clinically, 1-2 million are hospitalized with severe presentation and 0.1-5% die. The actual number is probably higher due to the predominance of asymptomatic and mild forms of infection and their misdiagnosis as another febrile illness. Data for Africa are inaccurate because of laboratory capacity constraints and many outbreaks are considered malaria[22].

Dengue – a three-phase disease

Infection with any of the four DENV serotypes can lead to a disease ranging from mild febrile illness to severe, even fatal disease. Asymptomatic infections predominate (75-80%). World Health Organization

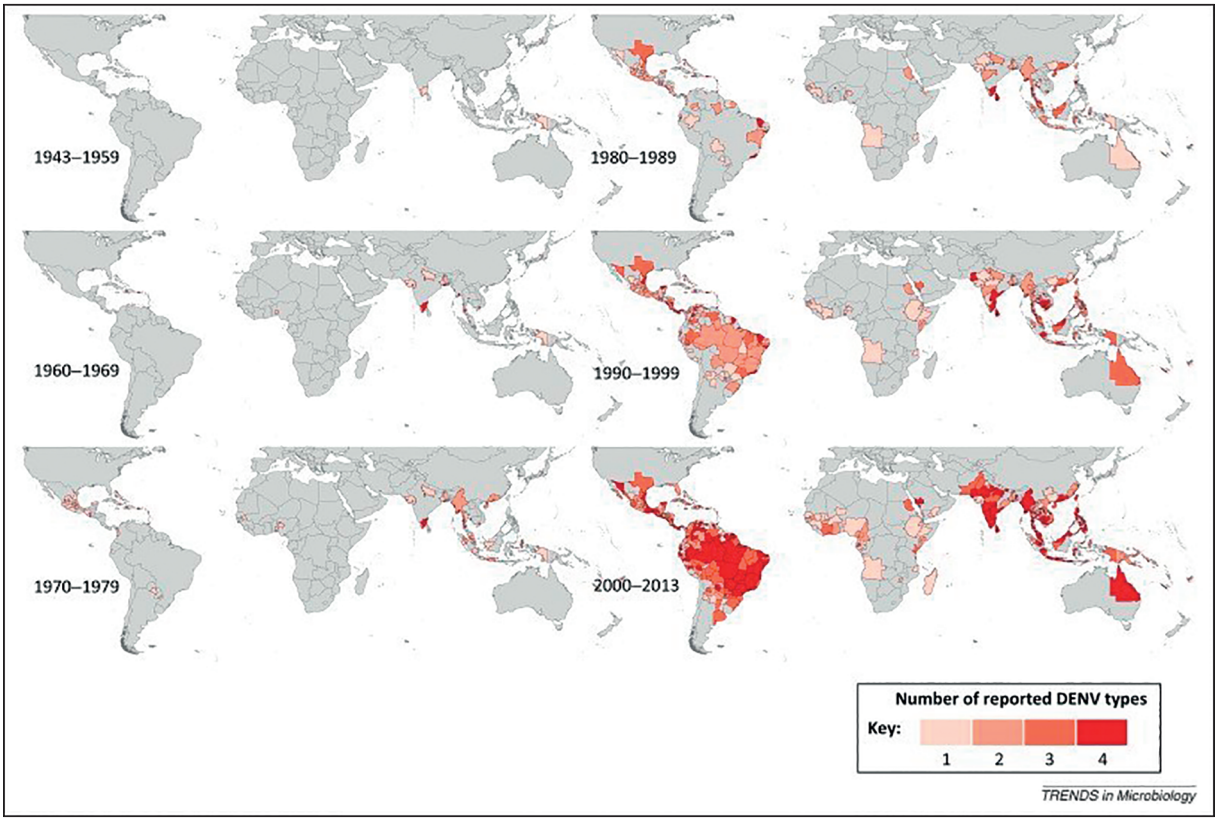

Figure 1. Spatial distribution of reported confirmed cases of DENV since 1943[20]

classifies clinically manifested dengue into two categories: classic dengue (with/ without warning symptoms), and severe dengue in 5% of patients, commonly collectively referred to as dengue hemorrhagic syndrome/dengue shock syndrome (DHS/DSS)[23]. The disease manifests 3-14 days (average 4-7 days) after a mosquitoe bite and is characterized by sudden onset, fever, headache, retrobulbar pain, muscle and joint pain, flushing on the face and trunk, transient macular rash**,** nausea, vomiting and petechiae – spontaneous or provoked (Tourniquette test). The blood tests show leukopenia, to a lesser extent thrombocytopenia and moderately elevated aminotransferases. This phase (febrile) lasts around 2-7 days and usually ends with recovery. In a very small proportion (5%) of patients around the time of defervescence the second (critical) phase follows, lasting 24-48 hours. The syndrome of increased vascular permeability is manifested. Although short-lived, it can lead to plasma leakage into the interstitium, accumulation of fluid (polyserositis), respiratory distress, and hypovolemic shock[24]. Hemorrhages and severe thrombocytopenia have been rarely reported. Signs threatening the progression to severe dengue include increasing abdominal pain, persisting vomiting, increasing hepatomegaly, and lethargy. The increased permeability normalizes after 24-48 hours. Timely and adequate therapy leads to the recovery of a significant proportion of patients. The recovery (reabsorption) phase follows with improvement of the condition and an increase in diuresis. Rehydration should be performed with caution due to the risk of fluid overload and the development of pulmonary edema.

Patients with DHS or DSS go through all three stages. The critical phase is bypassed by those with dengue fever.

The clinical suspicion of dengue by day 7 is confirmed by virus detection (culture, detection of its nucleic acid or NS1), and after day 7 by serological tests. Cross-reactivity with other local flaviviruses should be considered in the interpretation. In endemic countries, the simultaneous determination of NS1 and IgM/IgG by rapid tests is recommended, which offers a prolonged diagnostic window[25].

There is still no etiological treatment. Rehydration (oral or parenteral) and avoidance of non-steroidal anti-inflammatory drugs (aspirin, ibuprofen), which increase the tendency to bleed, are essential[26]. In endemic regions, dengue patients undergo triage in specifically designated hospitals. Patients referred for outpatient treatment should be informed of the threatening signs and when to seek help.

The vast majority of third or fourth infections are mild or asymptomatic.

Dengue vaccines – the best preventive intervention

Historically, flavivirus live attenuated vaccines have proven their safety and long-term efficacy. Examples are the 17D yellow fever vaccine and the Japanese encephalitis vaccine. Viruses in live vaccines multiply in the recipient and, resembling wild viruses, stimulate both humoral and T-cell responses[27].

However, the development of a dengue vaccine faces a number of challenges and obstacles, such as the lack of an appropriate experimental animal model and insufficient data on immune correlation, predicting protection or exacerbation of infection. In addition, even though it is widespread in developing countries, dengue does not attract sufficient financial resources for research. In many endemic countries, several DENV serotypes are circulating simultaneously and the vaccine has to provide protection for all. The biggest challenge is the immunological interaction between serotypes and the potential to increase the severity of the disease. For complete protection at the individual level, a tetravalent immune response is required, and each serotype in the tetravalent attenuated vaccine must independently stimulate four different homotypic antibodies. Unfortunately, this is very difficult to achieve[28,29].

Although for 30 years various vaccine platforms have been developed in search of a suitable vaccine, only live attenuated vaccines have reached the third stage of clinical trials. Three of them are in the final stages of research led by the pharmaceutical companies Sanofi and Takeda, as well as the National Institutes of Health, USA. Only one – Sanofi Pasteur – CYD-TD vaccine under the trade name Dengvaxia, has passed stage 3 (including subsequent post-marketing 5-year follow-up) and has been licensed.

The path of Dengvaxia ("the road to hell is paved with good intentions")

Dengvaxia is a live attenuated tetravalent chimeric vaccine in which the structural genes of the four DENV serotypes are incorporated into the genome of the yellow fever vaccine virus (the so-called "genetic backbone")[30]. However, the results of a phase 3 study of Dengvaxia in Asia and South America have showed an unexpectedly contradictory efficacy depending on the serotype, baseline serostatus for DENV, and age at infection[31]. Additional tests have found an increased risk of severe disease in vaccinated people who were seronegative before vaccination, compared with seronegative unvaccinated. At the same time, this confirms the long-term seroprotection in seropositive people[30].

A plausible explanation is that in seronegative individuals, Dengvaxia stimulates an immune response, predisposing them to severe disease, like what is seen in natural secondary dengue. Dengvaxia causes a "primary-like" silent infection (which live attenuated vaccines commonly do). A subsequent infection is the first encounter with the wild dengue virus and would be "secondary-like", leading to a more serious illness. In fact, not the vaccine itself, but rather the vaccine's induction of an immune response is the one that increases the risk of subsequent more severe infection. Dengvaxia does not contain NS1, which generates cell-mediated response and cross-protective antibodies. Its absence is probably an explanation for the vaccine's suboptimal protection.

In 2018, World Health Organization revised its 2016's recommendations, according to which in endemic countries the vaccine is recommended only for individuals with evidence of dengue (with a positive serological test or documented laboratory confirmed infection) or for individuals without pre-vaccination screening in areas with documented high seroprevalence for dengue (at least 80% by age 9 years). Unfortunately, at this stage there is not enough specific serological test for a previous dengue infection[32].

The vaccine is registered in 20 endemic countries, is indicated for patients > 9 years of age, and is administered in a three-dose regimen subcutaneously every 6 months. It has been included in large-scale programs in only 2 countries – Brazil and the Philippines. After a media release in 2017 about the safety concern for seronegative persons, the Philippines suspended its program, while Brazil completed it, but has not expanded it. This media information resulted in a huge public discussion in the Philippines, with anxiety and lack of confidence around the vaccine. This led to the subsequent resurgence of measles, reflecting the global resurgence of measles[33].

Second generation dengue vaccine

Two other live attenuated chimeric vaccines are in phase 3 clinical trials. One of them has been developed by Takeda – TAK-003 (or DENVax), in which the structural proteins of DEN –1, DENV-3 and DENV-4 are included in the attenuated DENV-2 serotype ("genetic backbone"). It is administered in two doses, three months apart. The results published in 2020 have shown a sustained immune response against the four DENV serotypes, regardless of the dengue serostatus of the vaccinated, as well as efficacy in dengue-seronegatives[34,35]. In March 2021, the European Medicines Agency has adopted a review package for the candidate vaccine TAK-003, intended for markets outside the European Union.

The third vaccine (TV-003/005) has been developed by the National Institutes of Health, United States and is in Stage 3 in Brazil, but is also licensed by Merck and other manufacturers for further development outside Brazil. It contains a mixture of an attenuated version of each of the 4 DENV serotypes and is administered once[36].

The advantages of the second-generation vaccines are the inclusion of NS proteins in their composition and the more convenient dosing, with a reduced number of doses. Whether they will provide balanced high protection against the four serotypes and thus overcome the serostatus-dependent problem of Dengvaxia remains unknown. These questions can only be addressed to the long-term results of forthcoming tests in phase 3.

Dengvaxia and travellers to endemic countries

Dengue is more common than other travel-related vaccine-preventable diseases such as yellow fever, hepatitis A and Japanese encephalitis. Although licensed in the European Union by the European Medicines Agency (2018), in the United States by the Center for Disease Control and Prevention (2019) and in Australia, the vaccine has not been approved for the prevention of travellers. Dengvaxia is indicated only for seropositive individuals, and most of them have not had dengue disease and are seronegative. The scheme of three applications every six months is practically inapplicable to them. Until an effective vaccine appears, regardless of serostatus and with fewer applications, travellers to endemic countries should use personal mosquito repellents and be informed of the risk of dengue.

Conclusions

Rarely is a vaccine, other than rotavirus and the coronavirus disease 2019 vaccine, accompanied by so many safety issues and controversies. According to World Health Organization, even in the Philippines, the overall impact of the vaccine is positive and age-dependent. At this stage, the best approach is to limit the vaccine to young children to prevent the risk of vaccine-related disease. At the same time, the incidence of Dengue can be significantly reduced through carefully thought-out and improved vaccination programs, and finally, but not least, through improved dengue vaccines. Ideal features of such vaccines are the stimulation of a sustained homotypic immune response to the all four DENV serotypes and in all age groups, regardless of serostatus, administered in two or one dose. The inclusion of other antigens (capsid, other non-structural) would stimulate

a more intense T-cell response, adding to the efficacy of the vaccine.

Authors contributions

R.K. conceived the original draft preparation. A.K., P.V., and V.R., were responsible for conception and design of the review. R.K. and A.K. were responsible for the collec-tion and assembly of the articles/published data, and their inclusion and interpretation in this review. R.K, A.K., P.V., and V.R. contributed equally to the present work. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

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