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A lethal switch "The dengue - an inclusive review"

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Abstract

Dengue fever also known as break bone fever, is a self limited, systematic viral infection transmitted between humans by mosquitoes. It leads to a public health challenge with an economic saddle. Its symptoms include fever, headache, muscle and joint pains, and a characteristic skin rash that is similar to measles. In a small proportion of cases, the disease develops into the life-threatening dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs. Dengue fever is a serious threat to global health issues. Approximately 100 countries are endemic for dengue fever and 40% of the world's population or about 2.5 billion people in the tropical and sub-tropics have an increased risk of catching the disease. So, to the core of the review we are ought to discuss about a wide topics towards the symptoms, Mechanism of the virus in the host, penetration towards our body, Characteristic antibody responses, life cycle, various phases involved in the cycle, efficient vector control strategies, pathogenesis, Diagnosis, ongoing therapies, and its management and prevention. In addition, we ought to suggest at conclusion that would be a better way to research in this type of disease with reviews.

Keywords: Break bone fever, Dengue hemorrhagic fever, Dengue shock syndrome, flavivirus vectors.

Introduction

Dengue infections are major public health problem globally, and currently some 2.5 billion of the world's population are at risk of dengue fever, primarily in tropical developing countries. The genus *Flavivirus*, family-*Flaviviridae* represents a large number of viral pathogens causing severe morbidity and mortality in humans and animals. In endemic areas, dengue virus is transmitted by the main mosquito vector, *Stegomyia aegypti* (formerly Aedes), and circulates as a complex of 4 serotypes, DEN1 to DEN41, which all are causative agents of dengue fever, Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

The polyprotein is co-and post-translational cleaved by host cell proteases and the viral protease NS3 into three structural proteins, capsid, pre membrane and envelope protein, which are components of the virion and at least seven non-structural proteins, which are involved in replication and maturation of the virus. The virus encoded protease complex NS2B-NS3 is responsible for cleavage at the NS2A/NS2B, NS2B/NS3, NS3/NS4A and NS4B/NS5 junctions and in the capsid protein.

The genus *Flavivirus,* are enveloped viruses that contain a single-stranded positive-sense RNA genome of approximately 11kb. A single viral polypeptide was processed by viral and cellular proteases to generate three structural proteins (C, M, and E) and seven nonstructural proteins. Capsid protein C within the virus particle is involved in assembling the

nucleocapsid core (NC), which consists of multiple copies of C protein surrounding a single copy of the viral RNA genome. It thus plays an important role in NC formation and the assembly of infectious particles.

Virus structure

Dengue is part of the Flaviviridae virus family, which also includes Yellow Fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and Tick-borne encephalitis virus.

Dengue is a single stranded, positive-sense RNA genome approximately 10.6kb long, composed of an open reading frame with genes encoding structural and non-structural proteins. The genome consists of three structural proteins; capsid (C), precursor membrane (prM), and envelope (E). The envelope glycoprotein is essential for viral entry into the cell. Seven non-structural proteins are also involved in viral replication within the cell, named NS1, 2A, 2B, 3, 4A, 4B, and 5.

The E glycoprotein is responsible for viral attachment and entry into cells, and is an antiviral target of neutralizing and enhancing antibodies. This is a glycoprotein of approximately 55 kDa, with 3 distinct domains. Using X-ray crystallography, these domains have been characterized for DENV-2 and DENV-3. Domain 1 is located in the center, and domain II contains an internal fusion loop, which is involved in membrane fusion and dimerization of the E protein.

Domain III is an immunoglobulin-like domain thought to be involved in cell receptor binding. Domain II is important, as it contains mostly flavivirus group and sub-group crossreactive epitopes. This is an important finding, as cross-reactivity of non-neutralizing anti-E antibody has been attributed to a higher risk of DHF/DSS upon secondary infection with a heterologous serotype. The exact host-cell receptors involved in dengue virus binding to mammalian cells remain elusive, but they are thought to be serotype-specific.

Mechanism of infection

Upon primary infection with DENV, there is an incubation period averaging 4-7 days. In this time the virus replicates in the dendritic cells in close proximity to the bite, also infecting macrophages and lymphocytes, and finally into the bloodstream. Dendritic cells (DCs) are antigen-presenting cells that are integral to inducing an immune response. have demonstrated that the dengue virus preferentially targets DCs, specifically monocyte-derived DCs (resembling interstitial DCs) and human skin Langerhans cells (LCs). By inoculating human skin explants with DENV in vitro, they demonstrated 60–80% expressed DENV antigens, and only immature cells were permissive to infection. The cells infected consisted of a mixture of LCs and interstitial DCs.

Cell surface heparin sulfates are also involved in cell surface attachment of dengue virus to mammalian cells. Heparin sulfates are repeating disaccharides of uronic and L-iduronic acids (derived from glucosamine), and are variably O-glycosylated. Despite these efforts, the attachment and viral entry into the cell remains poorly characterized. Co-receptors for viral entry into dendritic cells have been identified and analyzed. One of the receptors is a C-type lectin, CD209/DC-SIGN, and is thought to bind to the viral E protein, aiding in entry into the dendritic cells. Another finding identified the mannose receptor present in macrophages presented evidence that dermal macrophages serve as the first innate immune cell response, with the capabilities of protecting against the dengue virus after a mosquito bite. Additionally, the C-type lectin domain family 5 A (CLEC5A) can serve as a pattern recognition receptor for macrophages interacting with dengue viruses to stimulate proinflammatory cytokines release.

Dengue infection is an immunopathological disease in which an immune response may aggravate DENV infection and cause damage to the host. This is thought to be caused by antibody-dependant enhancement (ADE), and was proposed as an underlying pathogenic

mechanism of DHF/DSS as early as 1960 .It is thought this occurs because of pre-existing sub-neutralizing antibodies and the DENV form complexes that bind to Fc-γ receptor bearing cells, which leads to increased viral uptake and replication. This increase in viral replication directly increases the amount of virus in the blood, contributing to DHF/DSS. A strong association between severe secondary infections and host genetics, as well as virulence of different virus strains may also play a role in DHF pathogenesis. Although ADE is a widely accepted theory, there is still much to be proven regarding the direct cause of enhancement.

Vaccines and antibiotics

Ideal vaccines

Dengue virus is becoming an increasing concern because of the lack of a licensed vaccine to provide protection against all four dengue serotypes. The increase in dengue infections in recent years, as well as the prevalence of all four circulating dengue serotypes has contributed to the rise in DHF. In order to design a vaccine that is protective against all serotypes but without the potential risk of enhanced disease severity, the molecular mechanisms of dengue pathogenesis must be considered. Constructing a successful vaccine for DENV has been challenging. A potential vaccine must provide a delicate balance between the level of immunogenicity it evokes and the attenuation of DENV pathogenicity. The immunogenicity induced by the vaccine should be such that the level of neutralizing antibodies produced is high enough to provide complete protection against all four serotypes, but also sufficiently attenuated so as not to cause unacceptable pathogenicity (under attenuation) or fail to induce an effective immune response (over attenuation). In order to develop a successful vaccine, a greater understanding of dengue pathogenesis is crucial. The most effective way to test the basic immunology surrounding DENV infections is to use animal models. Mice are most commonly used as an animal model before testing in non-human primates. However, this has proven to be an obstacle as wild-type mice are resistant to dengue-induced diseases.

The difficulty seems to lie in developing a mouse model in which human viral isolates of DENV strains are able to replicate well, and in which the model mice can develop signs of human DENV-induced disease. This has led to the development of a variety of different mouse models, including intercerebral infection, chimeric mice transplanted with human cells, immuno compromised mice, and immunocompetent mice. Additionally, non-human primate models have been shown historically to be the most appropriate for human vaccine development. To date, the majority of models are not considered ideal for studying dengue infections. Mouse models as well as non-human primates are essential to test the efficacy and safety of potential vaccine candidates before the use in human clinical trials. Thus, the development of a suitable non-primate model for dengue disease is crucial for future vaccine development.

Intravenous inoculation with high-titer dengue virus serotype 2 (strain 16681) was found to cause dengue hemorrhagic fever within 3 to 5 days after infection, followed by modest thrombocytopenia and noticeable neutropenia, which was associated with a decrease of hemoglobin and hematocrit levels. There was also an inverse relationship between T and B cells and a bimodal pattern of platelet-monocytes and platelet-neutrophil aggregates. This animal model may help identify the bleeding mechanisms that are a cardinal feature of DHF in human patients. Since the highest rates of infections are in developing countries, an ideal vaccine should be economical as well as highly protective. This would require the vaccine to induce an initially high level of neutralizing antibodies in response to immunization, ideally with only one inoculation. The adaptive immune response to each specific serotype protects the individual against a re-infection by the same serotype. However, this immune response is also believed to be responsible for enhancing

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the disease severity when infection occurs with a heterologous serotype. This emphasizes the complexity of an ideal DENV vaccine, as it must address both the protective immunity, as well as the role of an elicited antibody in the pathogenicity causing DHF or DSS.

Live attenuated vaccines

Live attenuated vaccines (LAV) tend to mimic the natural infection by inducing humoral and cellular responses, which induce long lasting immunity, often from a single vaccination. LAVs contain a weakened formof a live virus that allows antibodies to both the structural and nonstructural proteins of the virus to be produced. The potential for developing vaccines using live attenuated strains of all four serotypes has been widely accepted, considering this method was successful for yellow fever (YF) and Japanese encephalitis (JE) vaccines. Using this technology there are a number of vaccine candidates currently in development. Sabin first achieved dengue virus, attenuation in 1945 by passaging the virus (DENV-1) in mouse brains.

However, the degree of attenuation seemed to vary depending on the strain of the virus, leading to many human volunteers developing a reaction in the form of a rash. This complication was addressed by with the discovery that dengue virus could be propagated and attenuated by serial dilutions in primary dog kidney (PDK) cells. However, viral attenuation by serial passage in cell culture has the disadvantage that it leads to unpredictable molecular changes. Now, attenuation is often attained by introducing a specific genetic mutation into the genome of the virus, to interfere with the virus's ability to replicate.

Recurring problems have prevented many vaccine models from advancing past clinical phases. Difficulties lie in achieving optimal attenuation of each of the four DENV serotypes, which are needed to provide a minimal level of reactogenicity and maximum immunogenicity. Problems can occur when individual, attenuated serotypes are combined into a tetravalent formulation. Difficulties may occur in maintaining the balance in attenuation and immunogenicity of monovalent components that can sometimes revert to wild type sequences. To address this problem, have used reverse genetics to attenuate the dengue virus with mutations in the 3'UTR region. These deletions introduce a greater genetic stability, and do not affect the gene translation, allowing the wild type proteins to induce the full immune response.

The interference of viral replication among the four DENV serotypes can result in immune imbalance in the hosts that may even elicit an unwanted immunopathogenic response. Replication interference could result from loss or increase of infectivity of the four DENV serotypes, so that the dose formulation of the tetravalent vaccines may overcome the potential problems of viral interference and balanced immunity.

Recombinant chimeric vaccines

Another approach to building a DENV vaccine has been to utilize molecular genetics. A recombinant chimeric vaccine is constructed by using the “backbone” of a related flavivirus (yellow fever, attenuated DENV strain), and replacing the prM-E genes with corresponding genes from DENV. The backbone would still contain the capsid and non-structural proteins and the 5′- and 3′-UTRs. The objective is to retain the attenuation properties from the “backbone” viral vaccine, and incorporate dengue antigenicity. A recombinant chimeric vaccine candidate was developed by Acambis and licensed to Sanofi Pasteur.

This vaccine uses the genes of Aventis–Pasteur's 17D yellow fever virus vaccine as its backbone. Vero cells were transfected with DENV strains using electroporation with RNA transcripts from viral cDNA from each of the 4 strains A tetravalent ChimeriVax- DEN1-4 candidate was tested. It showed poor levels of replication in the vector, Clinical trial data also indicated that the sero-conversion rates after 3 dose immunization of ChimeriVax-DEN1-4 vaccine were 100% in US adults, 77-92% in Mexican subjects 2–45 years and 88%-100% in

Mexican subjects 2-5 years. After receiving three doses over 15-months, all participants were seroconverted against all four serotypes.

Recent data showed that the ChimeriVax-DEN1-4 vaccine did not induce significant YF 17D NS3- specific CD8 and dengue serotype-specific T-helper responses in vaccines. If ongoing phase IIb/III trials are successful, Sanofi Pasteur is prepared to obtain the licensure from health authorities within 5 years.

The Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) in the USA have employed a different method of attenuating dengue virus based on the infectious cDNA clone derived from DEN-4 814669 strain. Reverse genetics was used to mutate the 3′ un-translated region in cDNA clones. The deletion of 30 nucleotides, referred to as Δ30, resulted in a successful balance between immunogenicity and attenuation for DENV-1 and DENV-4 in both monkeys and humans.

The Center for Disease Control (CDC) of the USA, Inviragen, and Shantha Biotechnics have also produced a chimeric vaccine candidate, DENVax, using the infectious cDNA clone derived from DENV-2-PDK- 53 strain developed at Mahidol University in Thailand. Using DEN-2 PDK-53 as the genetic backbone, the intertypic dengue viruses were constructed by replacing the preME structures of DEN-1, DEN-3, and DEN-4. The recombinant chimeric viruses were transfected into Vero cells. However, three sequential plaque purifications of each virus were performed to reduce variation and ensure safety of master seed virus. The Inviragen's DENVax tetravalent vaccine with several formulations was safe and immunogenic as tested in AG129 mice and cynomolgus monkeys. These chimeric viruses have been successfully re-derived under GMP conditions and characterized, and are currently being investigated as potential vaccines by human clinical trial.

Cell culture bioprocessing for live-attenuated and recombinant chimeric vaccine production

All live attenuated and recombinant chimeric vaccine candidates should be propagated in an approved cell line such as Vero (kidney cells from African green monkey), MRC-5 (normal diploid cells from human lung), or FRhL (normal diploid lung cells from rhesus monkey) for human vaccine production. Since Vero cells are derived from a continuous line from the African green monkey (*Cercopithecus aethiops*), the cell growth for human vaccine production is restricted to 150 generations due to its potential to become tumorgenic.

However, compared to MRC-5 and FRhL cells, which have a finite life span, Vero cells, can be grown indefinitely and can easily be adapted to serum-free cultivation. Serum-free microcarrier cultures have been reported to produce four serotypes of high-titer DENVs. However, the vaccines produced from Vero cells may require more extensive purification step(s) to remove cellular proteins and residual cellular DNA. According to current regulations for cell-derived vaccine products, the residual DNA content produced from Vero cells should be less than 10 ng per dose andwith a size of DNA less than 200 base pairs. Regulations for vaccine products obtained fromhuman diploid cell lines such as FRhL and MRC-5 cells are less stringent in their allowable residual cellular protein and DNA content.

DNA and virus-vectored vaccines

Vaccines based on vectors using recombinant DNA technologies have recently been undergoing animal trials. DNA vaccines have the advantages of inducing intracellular antigen processing for adaptive immunity, being stable (meaning they could not revert to a pathogenic phenotype), and easy manufacture. DNA vaccines do not have the complications associated with live replicating viruses, namely problems of combining monovalent formulations to form tetravalent vaccines, thus inducing serotype competition or dominance. The disadvantage of DNA vaccines includes low immunogenicity elicited in immunized human hosts. Concerns arise in

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regards to the ability of a DNA vaccine to induce immunity to the inactive viral proteins that would be used to make the vaccine.

Some DNA vaccines have been reported to be successful in primates. A DNA vaccine with a plasmid that encodes the prM and E genes, and two non-structural proteins, was reported to be protective in animal trials with no interference from competing monovalent components. Another group has developed a non-replicating DNA vaccine and a recombinant viral vector vaccine for dengue with the possibility of enhancing gene delivery and immunogenicity. In an attempt to reduce the complexity of the vaccine, used an adenovirus vector that allowed them to make multivalent recombinant constructs. The constructs, named CAdVax- Den12 and CAdVax-Den34, each express prM and E proteins from their respective serotypes. Following successful tests showing that the two bivalent constructs produced neutralizing antibodies, a tetravalent vaccine using the adenovirus vector was made, CAdVax-DenTV. This vaccine was tested and found to produce high titers of neutralizing antibodies to all four serotypes in rhesus macaques.

Venezuelan Equine Encephalitis (VEE) virus has also been used to develop a dengue vaccine. The VEE vector expressing the prM and E gene of dengue 1 virus was capable of forming virus replicon particles. Three-dose immunization of the VEE replicon particles induced potent neutralizing antibody titers in macaques but only offers a partial protection after live dengue virus challenge. Heterologously priming with two-dose naked DNA, followed by boosting with the VEE replicon dengue particles at the third dose was demonstrated to induce a complete protection in immunized macaques. Thus developing a new vaccine using a heterologous prime boost regimen can offer another means to improve the existing immunity in vaccinated hosts.

Live-attenuated measles viruses (i.e.) Schwarz/Moraten, AIK-C, and EZ vaccine strains) have shown excellent results in inducing the life-long immunity of children after one or two-dose immunization. The current reverse genetics technology has offered the ability to engineer the measles virus genome to deliver single or multiple antigens and can be used as a vaccine vector to induce the life-long memory and, more broadly, protective immunity. The live-attenuated Schwarz vaccine strain of measles viruses has been engineered to encode the domain III and/orM ectodomain of DENV. Immunization by priming the DENV-measles virus vector and boosting with the recombinant protein was capable of inducing long-term specific neutralizing antibodies in mice. This approach has the advantage of developing a single-dose combination vaccine against measles virus and DENV infection.

Recombinant protein vaccines

The envelope E protein of DENV has been selected as the major antigen for recombinant protein vaccine development. Expression of the DENV E protein usually requires the expression of prM protein which may act like molecular chaperone to help the correct folding of the E protein. In addition, the prM-E protein expressed intracellularly will be automatically cleaved into prM and E by cellular furin. Studies have also demonstrated that the expression of 80% E (r80E) at the N-terminal can assist the E protein secretion in cultured cells. The r80E protein of DENV-2 has been successfully expressed in fruit fly cells, purified in a high yield and formulated with AS04, AS05, and AS06 adjuvants as the subunit DENV vaccine.

Two-dose immunization in rhesus macaques with the r80E subunit vaccine was found to induce comparable levels of neutralizing antibodies and protection against viremia after DENV challenge . The DENV E protein contains three distinctive domains (domain I, II, and III). Domain III contains the receptor binding sites for DENV and can be independently folded as an individual structure. The domain III protein of DENV is also responsible for inducing the serotype-specific antibodies.

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Several strategies have been used to improve the immunogenicity of the DENV domain III antigen, including fusions to the Staphylococcal A protein, the Maltose Binding Protein of *Escherichia coli* and the P64K protein from *Neisseria meninglitidis* . Incorporation of the capsid protein of DENV beside the domain III in a highly aggregated form can further induce cell-mediated immunity. The chimeric protein, formed by fusing the domain III of four serotypes, can be obtained by over-expression in P. pastoris, and the immunization with the chimeric domain III protein elicited all four-serotype neutralizing antibodies in mice .

JE-CV vaccine

The vaccine virus for JEV (designated as JE-CV) was constructed by using the PrM/E genes from the commercially available liveattenuated Chinese vaccine (SA14-14-2 strain). There are 10 amino acid differences in the E protein (derived from the SA14-14-2 virus) of JE-CV when compared to the wt JEV Nakayama strain and the SA14 strain, the parental strain from which, SA14-14-2 vaccine was derived by empirical passages. At least 6 of these 10 amino acids (E107, 138, 176, 279, 315, and 439) are believed to be critical neuroattenuation determinants based on sequence comparisons with wt strains.

To determine the importance of these and other residues for the attenuation of JE-CV, a series of chimeric viruses were constructed with either single or multiple amino acid substitutions that systematically reverted the JEV SA14-14-2 E protein to that of the wt JEV Nakayama strain. Theconstructed revertants were tested in a mouse encephalitis model by i.c. inoculation of highly sensitive young mice [40], and then classified into three groups: attenuated (no mortality), sublethal (mortality rates 13%-38%) and lethal (mortality ratio > 89%) revertants. No single reversion significantly altered the attenuated phenotype, at least two reversions were required to partially restore the neurovirulent phenotype; three reversions to the wt (Nakayama) sequence at positions E107, 138, and 176/177 were required for restoration of a highly neurovirulent phenotype but four or more reversions in at least three clusters were required to confer full neurovirulence for mice.

Furthermore, reversion of all SA14-14-2 JEV residues to wt (Nakayama strain) sequences in the JE-CV virus did not result in a full reversion to neurovirulence of the parental virus (i.e. the phenotype of the virus was similar to that of the parental YFV 17D virus rather than the wt Nakayama JEV) [17].

This proves that in combination, the YFV 17D backbone and the chimerization process contributes to, and stabilizes the attenuation of the chimeric viruses.

During a decade long effort to develop a JE-CV vaccine, multiple viruses were constructed and evaluated in non-clinical studies. Two were selected to progress into clinical trials; pilot JE-CV and large-scale JE-CV. The pilot JE-CV virus was not plaque purified and used as a liquid formulation in Phase 1 and 2 clinical trials. For Phase 2 and 3 stages, large-scale JE-CV production utilized SF Vero cells transfection and clonally derived virus following genetic stability passages and plaque purifications. The new JE-CV vaccine in a lyophilized formulation presenting an amino acid change at position 60 in the M protein (M60C). This mutation was first identified at P5 and is believed to be an adaptation to SF media as was observed with WN02 virus (M66P). The JE-CV M60C virus replicated to higher titers than the original JECV virus, but retained the immunogenicity and neuroattenuated phenotypes, and was therefore selected for further vaccine development.

Preclinical evaluation of safety and immunogenicity

Before entering clinical trials, all candidate vaccines must be tested for safety and immunogenicity in preclinical studies. Such evaluations can be conducted both in vitro on primary or transformed cells – including human cells – and in vivo in animal models, in particular non-human primates (NHP). In the case of arboviral vaccines, studies with

Fig.1. Immunopathogenesis of dengue virus infection (Huan-Yao Lei et al., 2001)

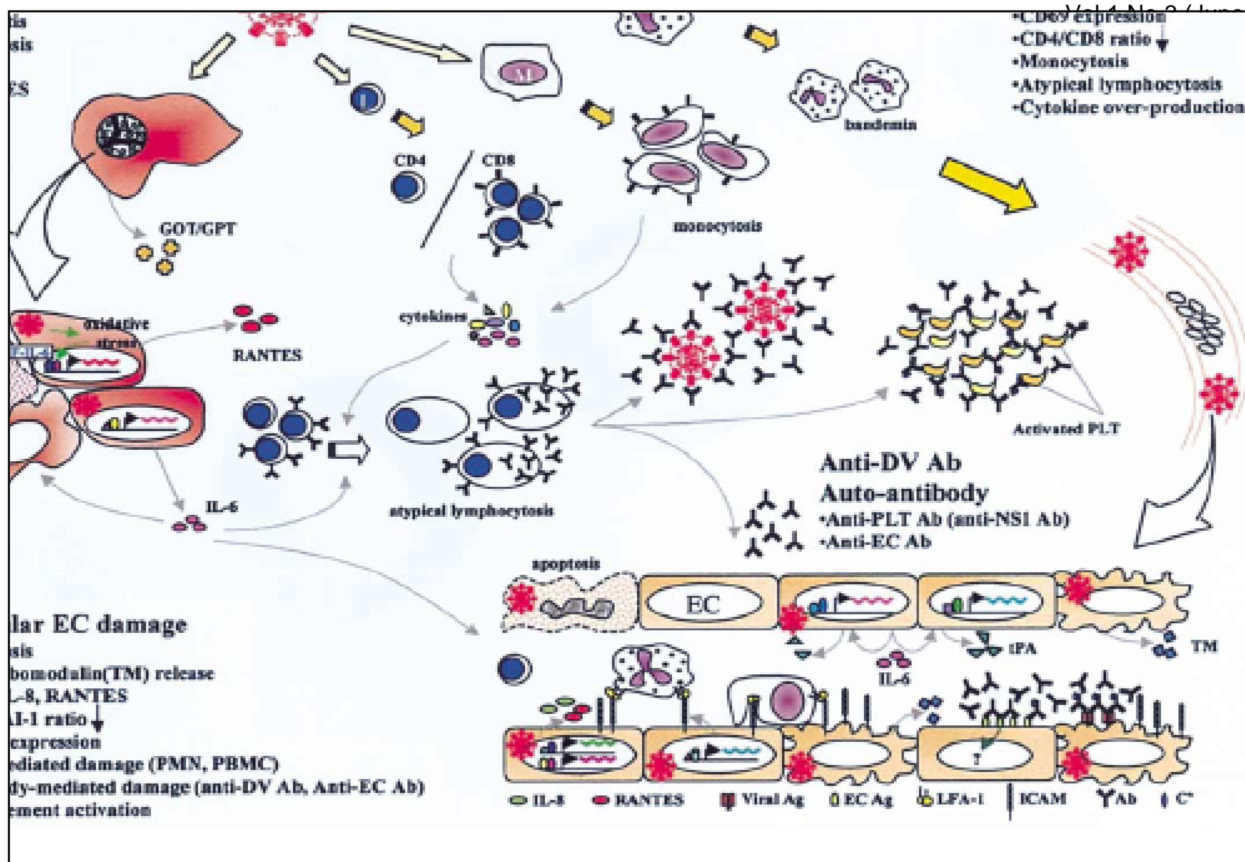
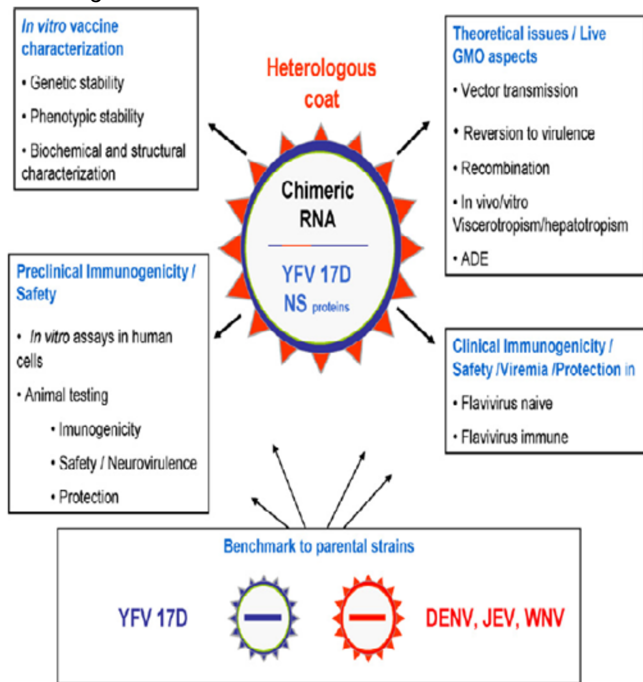


Fig.2. Evaluation of chimerVax-based vaccines



Different tools, assays and strategies have been used to characterize vaccine candidate, at the chimeric nucleic acid, heterologus structural/17D non-structural (NS) proteins and overall viral levels phenotypic and genetic properties/stability, preclinical invitro or animal evaluation, and clinical testing. The theoretical issues linked to the live GMO nature of the ChimericVax-based vaccines have also been addressed (Guy *et al.,* 2010).

potential vectors also have to be standard (Table.1 & 2).

Dengue vaccine

Particle content per dose

The surrogate assay for vaccine potency of a live vaccine is in vitro infectious titration, assuming that protection induced by the vaccine is linked to the infectious dose of virus injected.The total number of particles injected has also to be carefully monitored. In the case of the YF vaccine, Stamaril For DEN vaccines based on the YFV 17D backbone, a quantitative, realtime, reverse transcriptase PCR (qRT-PCR) system was developed: one assay targets the NS5 gene in the YFV 17D backbone, and four assays target the E/NS1 junction of each chimeric virus. The RNA copy number per infectious unit consistently ranged between 3.0 and 3.3 log10, similar to what has been found in commercial YFV 17D vaccine lot. In addition, viral particles produced along the vaccine development pathway (cell monolayers, biogenerators, serum-free process) consistently exhibited by cryo-electron microscopy the same typical flavivirus morphology: round, smooth particles of about 52– 54nm and spiked or partially spiked

particles of about 54–56nm representing various degrees of maturation of the virus

Genetic stability

It is critical to address genetic stability in the course of RNA virus vaccine development. For live-attenuated strains obtained empirically on several cell substrates, the emergence of mutants with potentially increased or decreased virulence is unavoidable across multiple passages, even after further adaptation to Vero cells. In this regard, the seed lot strategy has been remarkably successful in maintaining the attenuated phenotype of YFV-17D vaccine over decades (Fig.1).

Clinical evaluation

The three different chimeric vaccine candidates against DENV, JEV and WNV have now been tested extensively in clinical trials, in volunteers ranging from children to the elderly. All results obtained so far demonstrate their excellent safety profile, with low/absent viremia of short duration [41,62]. Satisfactory immunogenicity is obtained after a single dose (JEV,WNV) or after 2–3 doses (DENV). The following paragraphs will describe the main find ings and conclusions obtained from these trials Immunogenicity is first measured by neutralizing antibody levels through the Plaque Reduction Neutralization Test (PRNT) assay.

Dengue

The first clinical evaluation of a ChimeriVax DEN vaccine was conducted with a monovalent ChimeriVax-DEN2 (CYD 2). The safety profile of YF-17D (YF-VAXTM) and CYD2 were similar. Both types of vaccines induced transient low-level viremia (less than
1.4 log pfu/ml). More naïve subjects inoculated with ChimeriVax- DEN2 developed viremia than with YF-VAX and the number of subjects with viremia tend to be higher in YF-immune subjectswho received the high dose of ChimeriVax-DEN2 vaccine. The values were however low, transient, did not associate with any adverse events and were not statistically significant in this trial. Most participants sero converted to DENV2 strain 16681, and prior YFV 17D immunization had no negative effect on the DENV2 sero conversion response. On the contrary, prior immunity induced both stronger, broader (cross protective), and long lasting responses.

No significant serious adverse effects (SAE) related to vaccination were identified. The chimeric
Vaccines did not even induce the mild dengue-like syndrome associated with some whole virion live-attenuated candidate dengue vaccines. *Dengue-specific issue: anti body dependent enhancement ADE).* As stated previously, the most often proposed markers of protection are sero neutralizing antibodies, as measured for instance by the PRNT50, although titres required for protection are yet to be determined. Cross-protection between DENV serotypes is limited and antibody dependent enhancement (ADE) has been proposed to constitute one mechanism leading to severe dengue disease, e.g. DHF or DSS pre-existing non-neutralizing heterotypic antibodies, instead of being protective, would enhance infection through the binding of virus: antibody complexes onto the surface of cells possessing receptors for the Fc part of IgG antibodies (Fc_R). Similarly, enhancement may take place if homotypic antibodies want to sub-neutralizing levels (like in infants born from dengue seropositive mothers). We took these potential concerns into account and developed early in our DENV vaccine research a sensitive and reproducible in vitro assay using Fc_RII positive-K562 cells and flow cytometry. Sera from Thai children vaccinated with first-generation LAV candidates were analyzed using this assay and correlated with a low/absent risk linked to ADE activity in vitro, despite very diverse immune profiles, from low to high PRNT levels against one or several dengue serotypes. Specifically, in vitro ADE was absent in the presence of broad neutralizing response against all four DENV serotypes.

Table.1 Dengue serotype association to disease severity. (Vaughn et al.,2000; Fried et al.,2010)

Dengue serotype	Association to dengue disease
DENV-1	Primary infection results in frequently more severs disease, when compared toDENV-2 or DENV-4
DENV-2	Secondary infection associated with more severe disease (twice as likely to result in DHF that DENV-4)
DENV-3	Primary infection results in frequently more severe disease, When comparedtoDENV-4, a secondary infection, is twice as likely to result in DHF than DENV-4
DENV-4	Least associated with severe dengue disease

Table 2.Preclinical and clinical development (Bruno Guy et al., 2010 ; Ietson et al., 2010)

	In vitro preclinical evalution	*Invivo preclinical evaluation*	*Clinical evalutation*
DENV	ChimeriVax-DEN1-4 show similar growth kineticsw to their parent viruses (wt DEV and YFV 17D) in DCs. Such DCs infection Induces maturation and a controlled response, accompanied by limited inflammatory cytokine production and consistent expression of anti-viral type 1IFN.ChimerVax-DEN1-4 grew to significantly lower titers than YFV 17D viruses in human hepatic cell lines THLE-3 and HepG2, but not in Huh7 cells.	TV ChimeriVax-DEN1-4 vaccine is immunogenic in monkeys, induce limited viremia and protect against wt DENV1-4 challenge (aviremia) Interferences between serotypes can be overcome by relative serotype dose and schedule adjustment eg., complementary bivalent administration at different anatomical locations.	ChimeriVax-DEN2 is immunogenic and safe in humans. Yf17D pre immunity is linked to higher and broader neutralixing Ab responses(PRNT50) Tv ChimeriVax is safe and immunogenic after three doses all vaccines seroconverted against all four who reference strains(unpublished data) Cell amediated immunity: TV ChimeriVax vaccine Induces dengue serotypes specific CD4(Th1) and Yf17D NS3-specific CD8 responses. Dengue pre-immunity or booster immunization broadens CM1 responses no cross reactive 17D/DEN NS3-specific responses. Stage of development: phase 2B/3
WNV	Efficient growth in vero cells serum free vero cells select for a small size plaque variant with a mutation at M66 chimeriVax-WN01 (Vet) grew in THLE-3 to a similar level as of the YfV-17D virus. ChimeriVax WNO2(human)grew significantly to a lower titer than YFV 17D virus in these cells (unpublished data)	ChimeriVax-WN is safe, immunogenic and protective in mice, hamsters and monkeys ChimeriVax-WNO1 (vet) virus vaccine is less neurovirulent than YFV 17D vaccine in mice and monkeys and safe and immunogenic in horses. ChimeriVax WNO_2 (human) is safe and immunogenic in monkeys, prominent sites of replication are skin and lymphoid tissues generally sparing vital organs. Viruses were cleared from blood by day 7 and from tissues around day	ChimeriVax WNO2 is safe and immunogenic in humans; Transient viremia was detected in most subjectsw. After a single dose, all subjects developed neutralizing antibodies to WN The majority of vaccines developed WN specific T cell responses. Stage of development : phase 2

Table .2a Preclinical and clinical development (Bruno Guy et al., 2010; Ietson et al., 2010)			
JEV	Efficient growth in vero cells, serum free vero cells select for a small size plaque variant with a mutation at M66 chimeriVax-WN01 (Vet) grew in THLE-3 to a similar level as of the YfV-17D virus. ChimeriVax-WNO2(human)grew significantly to a lower titer than YFV 17D virus in these cells (unpublished data)	JE-CV is protective in mice inoculated IP with wt JEV	ChimeriVax-JE safety and immunogenicity is similar to that of YfV 17D. Neutralizing antibody seroconversion to ChimerVax-JE was 100% seroconversion to wt JE strains was imilar or lower than to the homologous (vaccine) virus.
		JE-CV is not neurovirulent for 3 to 4 week old mice at doses <6 log10 pfu inoculated by IC route, to the difference of YfV 17D Passive protection with mouse sera raised against JE-CV against all four JE genotypes.	JEV specific antibody levels were higher in YfV immune than in naive subjects
		A single dose of JE-CV conferred a better protection against MVE virus in mice than 3 doses of an inactivated JEV vaccine, JE-VAX	Repeated administration of JE-CV did not show a real benefit over a one dose schedule
		A single dose of JE-CV conferred a better protection against MVE viruse in mice than 3 doses of an inactivated JEV vaccine, JE-VAX	The long term follow up of up to 5 years after JE-CV vaccination still ongoing but data up to 4 years support the persistence of the immune response after a single dose of JE-CV
		A single SC dose of JE-VC Indusces short lastin glow viremia and protects monkeys against IC challenge with a heterologous JEV JE-CV was safe when inoculated by SC and IC route4s in monkeys. Its biodistribution was limited to the injection site found on day 4 no replication in liver and kidneys virus was not found on day 22 in blood, urine, feces, injection site swabs and saliva samples (unpublished data)	Three pediatric studies are currently ongoing in India, Thailand and the Philippines JEV-specific cellular responses were induced in vaccines (unpublished data) Stage of development: in registration.

Finally, JE-CV is the chimeric vaccine candidate closest to registration. Preclinical characterization of the various JE-CV viruses obtained by transfection of different cell lines demonstrated that these viruses are highly attenuated. Protective efficacy of JE-CV was demonstrated in mice and monkeys upon immunization with a single dose and comprehensive protection against a severe i.e. challenges with virulent JEV strains. JE-CV will be effective as a vaccine against all circulating JEV strains representing all four major genotypes and should provide a convenient, affordable, single-dose vaccine (Fig.2).

Detection of dengue virus specific antibodise

Dengue virus specific antibodies IgM and IgG were tested by Dengue blot kits (Genelabs Co. Ltd, Singapore) and ELISA kits (IBL Co. Ltd, Germany).

Phylogenetic analyses

Sequences were aligned using the ClustalW multiple sequence alignment function of MacVector® version 8.0 (Accelrys, Cary, NC) with default gap penalties. Phylogenetic analyses of the aligned genomic sequences were estimated with maximum likelihood and distance/neighbour joining using the PAUP* program version 4.10 (D.L. Swofford, Illinois Natural History Survey, Champaign) under the general time-reversible model of nucleotide substitution. The parameter values used for the substitution type, optimal base composition, proportion of invariable sites, as well as the shape parameter of the distribution of rate variation among sites were estimated from the data and are available upon request. We also utilized Bayesian analysis (MrBayes v 3.1.0) where 4 MCMC tree searches of 4 million

generations each were run simultaneously sampling 1 in 100 trees and computing a 50% majority-rule consensus tree out of the last 9800 sampled trees, where the initial 10% of trees removed as burn in. Bootstrapping obtained by the Bayesian analysis was used to place confidence values on grouping within the consensus tree (Felsenstein, 1985). Character evolution was traced using Maclade 4.08 (Sinauer Associates, Sunderland, MA).

Discusssion and contribution

A comprehensive review heads us about the empowering threatening sickness to our awareness and the future endemic economic universal issue to be solved either by therapies and research oriented methods and a better knowledge towards its prevention. we also state the study to this particular area to be accelerate and must cover a wide examine to beat the health issues and which also illustrates the importance for a serious proposal towards a wide research. And also our earliest way could head us towards a picky site specific strategy.

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