Meeting report

Review on flavivirus vaccine development
Proceedings of a meeting jointly organised by the World Health Organization and the Thai Ministry of Public Health, 26–27 April 2004, Bangkok, Thailand

Abstract

In light of the continuous spread of human pathogenic flaviviruses, in particular the mosquito-transmitted species, vaccine development remains a high priority on the public health agenda. On 26–27 April 2004, a conference was held in Bangkok, Thailand, to review current status of flavivirus vaccine development and related issues, focussing on dengue (DEN) and Japanese encephalitis (JE). This event, co-sponsored by the World Health Organization (WHO) and the Thai Ministry of Public Health, reviewed the progress made with vaccine development, sero-epidemiological studies and other accompanying activities critical for vaccine development and vaccination. The considerable interest in and awareness of the flavivirus diseases and their prevention by public health decision makers, as well as the establishment of two dedicated programmes for dengue and Japanese encephalitis vaccine development raise hopes that new or improved vaccines will become available in the coming years.

Keywords: Flavivirus vaccine; Human pathogenic flaviviruses; Sero-epidemiological studies

1. Introduction

The genus Flavivirus of the family Flaviviridae consists of approximately 70 viruses of which 50% are transmitted by mosquitoes and 25% by ticks. Half of them have been associated with human diseases and several are important human pathogens including the mosquito-borne viruses responsible for the diseases yellow fever (YF), dengue (DEN), Japanese encephalitis (JE) and West Nile fever (WN). All four are considered to be emerging diseases due to their geographic spread and intensified transmission over the past years or decades. This spread, often caused by a combination of reduced vector control and increased presence of amplifying hosts as well as travel of viremic persons, has put management and control of flavivirus disease high onto the public health agenda of disease endemic countries.

There is no specific treatment available for any flavivirus infection, and for some diseases, such as JE, case fatality rates can be as high as 30%. While vector control represents an important preventive measure against mosquito-borne flavivirus diseases, it has proven difficult to sustain, and vaccination appears the most promising avenue to control these important mosquito-borne diseases. Furthermore, experience gained with YF vaccine shows that protection of the population can be highly efficacious, if routine immunization is practiced and implemented according to Expanded Programme on Immunization (EPI) recommendations.

On 26–27 April 2004, the World Health Organization (WHO) and Thai Ministry of Public Health co-sponsored a conference to review the current status of vaccine development against DEN and JE, and the future challenges with respect to clinical evaluation of these much-needed vaccines. Additional sessions were on WN, which reviewed experi-
Dengue is a disease caused by four serologically related viruses, termed DEN-1, DEN-2, DEN-3 and DEN-4, and represents the most important arthropod-borne viral disease in humans in terms of morbidity and mortality, affecting over 100 countries and threatening a population of 2.5 billion people. There is a spectrum of disease, ranging from relatively mild dengue fever (DF) to dengue hemorrhagic fever and shock syndrome (DHF/DSS). Recent spread of the disease, in particular in the Americas, is a major concern to global public health.

While vaccine development is an undisputed public health priority, it remains a considerable scientific challenge due to the disease being caused by four serologically related viruses. One of the most intriguing problems for the development of a DEN vaccine has been the sequential infection hypothesis as a risk factor of DHF/DSS. Experiences from prospective cohort studies and natural epidemics suggest that secondary infection by a heterologous DEN virus is the main risk factor for the severe disease [2,3]. This susceptibility has been observed up to 20 years following primary infection. Lack of an animal model that reproduces the disease has limited our understanding of pathogenic mechanisms.

Based on these observations, most researchers agree that an effective and safe DEN vaccine should confer long-lasting protective immunity against all four DEN viruses. Towards that goal, a series of approaches are pursued, including live attenuated and live recombinant vaccines, recombinant subunit vaccines (E, coli, yeast, baculovirus), as well as DNA vaccines. Most advanced in their clinical development are the live attenuated candidates. Many of these strategies were discussed at the conference.

2.2. Status of vaccine candidates in pre-clinical development

Dr. L. Markoff (US FDA) reviewed progress made towards the development of rationally attenuated mutant F' vaccines [4]. DEN mutant F' viruses have been derived by site directed mutagenesis of infectious DNAs for the four DEN viruses. They contain a specific set of mutations in the lower part of the long 3' terminal stem and loop structure, the 3'SL. This structure is required for virus replication and mutations may affect the host-range of flaviviruses. Candidate mutant viruses are screened for restricted replication in mosquito cells and reduced neurovirulence. Mutant F' mutations inserted into the genome of a DEN-1 strain Western Pacific (WP) produced an attenuated (i.e. lower and shorter viraemia observed compared to wild type), immunogenic and protective virus in a monkey model. By a similar strategy, DEN-3 and DEN-4mut F' viruses were created and shown to be restricted for replication in mosquito cell line C6-36. To complete a tetravalent vaccine candidate, a DEN-2mutF' will be generated starting from a non-virulent DEN-2 virus.

Another strategy to develop a live DEN vaccine consists in creating DEN/DEN chimeras. Dr. R. Kinney (CDC, Ft Collins) presented on the strategy to develop such a tetravalent vaccine, based on the DEN-2 live attenuated vaccine candidate (strain 16681, PDK-53) co-developed at Mahidol University, Thailand and licensed to Aventis Pasteur, France. Here, attenuated DEN-2 PDK-53 is used as backbone for the structural prM-E genes of the other dengue viruses for making a DEN/DEN chimeric tetravalent vaccine. The DEN-2 vaccine is highly attenuated and the major attenuating mutations appear to be located in two non structural genes and the 5'-noncoding region. The vaccine has shown good immunogenicity and safety features in cynomolgus monkeys [5].

A recombinant, subunit, tetravalent vaccine is under development by Hawaii Biotech Inc. The vaccine is composed of the ectodomain of the envelope (E) protein (termed 80%E) and the non-structural protein NS1 expressed in Drosophila S2 cell expression system. The rationale for including NS1 emerges from mouse protection studies. Using a proprietary adjuvant, four different formulations were evaluated in Rhesus macaque, as reported by Dr. C. Weeks-Levy. Monkeys received four doses over an interval of 28 days. Three out of the four formulations generated a robust virus neutralizing titre antibody response to all four DEN viruses with titres varying from 1:100 to >1:1000, although a variable cellular immune response was observed depending on the vaccine formulation. Careful antigen dosage and adjuvant selection are required to optimize both humoral and cellular response. Challenge studies in Rhesus macaques are ongoing.

2.3. Characterization of the antibody neutralizing response

Three studies were presented that aimed at providing an in-depth characterization of antibody responses to DEN. The
first one, presented by Dr. W. Sun (Walter Reed Army Institute of Research (WRAIR), Washington), had the objective to characterize the protective neutralizing antibody response. This study was conducted in human volunteers vaccinated with a candidate DEN live attenuated vaccine that is licensed to and co-developed with GSK, and later on challenged with low-passage wild type virus. Ig class, subclass, affinity and neutralizing antibody titre were measured in sera of the vaccinated volunteers. Biacore technology was used to measure antibody affinity using recombinant DEN E protein as antigen. Preliminary results suggest that the relationship of association/disassociation and neutralizing antibodies have some predictive value for protection capacity after vaccination. Low levels of IgG3 may be associated with lack of protection. While the technology appears powerful, more studies from natural primary and secondary infections are needed to confirm findings.

The second study, conducted and presented by Dr. M. Guzman (Havana), took advantage of the unique DEN epidemiological situation in Cuba. In a longitudinal study, homotypic and heterotypic neutralizing antibodies were measured in DEN-1 immune individuals up to 20 years after infection. Reduced heterotypic neutralization titres were observed against DEN-2 genotypes, corroborating findings that antibody responses become more monotypic with time and would lose heterotypic neutralization capacity. It remains to be confirmed if this relates to increased risk of severe dengue in secondary heterotypic infections.

Dr. P. Keelapang (Chiang Mai) presented the third study which examined the role of anti-prM antibodies in neutralization of DEN virus [6]. During virus formation, both prM and E proteins are associated in the endoplasmic reticulum as heterodimers. The pr peptide prevents premature conformational change of the E protein during transport through the cell and is cleaved off just before the virions are released. This prM-M conversion in DEN viruses appears to be inefficient and the role of prM in the virion is not clear. Anti-prM monoclonal antibodies were used to examine the role of these antibodies in the neutralization of high and low passage DEN strains of all four viruses. Results point towards a limited role in virus neutralization, but plaque size reduction was observed, indicating that anti-prM antibodies might interfere with intracellular virus formation.

2.4. Vaccine clinical trials

Dr. A. Sabchareon (Mahidol) presented an update on long-term surveillance of individuals vaccinated with a candidate live attenuated tetravalent vaccine manufactured by Aventis Pasteur [7]. Given the theoretical link between antibody-dependent enhancement (ADE) and the development of the severe disease, long term immunogenicity and safety data in dengue vaccine recipients are being compiled. The subjects of two clinical trials, one in adults in 1998 and one in children 5–12 years of age in 1999 are being followed. Neutralizing antibodies are measured yearly and febrile illness and hospital admissions are monitored. In the last 2 years, two vaccine recipients (one child and one adult who received vaccine formulations 3313 and 3212, respectively) developed the clinical picture of DF. DEN-1 or DEN-2 viruses were detected in these cases, demonstrating breakthrough infection despite the presence of vaccine-induced neutralizing antibodies of 1:35 and 1:25, respectively. In other subjects asymptomatic infection was demonstrated. None of the infections resulted in DHF/DSS.

The report from the WHO Dengue Clinical Trials Task Force meeting held in December 2003 was presented by Dr. I. Kurane (Tokyo). The Task Force was established to accelerate the development, evaluation and introduction of DEN vaccine candidates with the main objective to analyze results on safety, immunogenicity and efficacy of available vaccine candidates in clinical trials. The progress in clinical trials of two live attenuated and one chimeric vaccine candidates, as well as the characterization of dengue antibodies and standardization of dengue reagents and assays were discussed.

Mahidol University, in collaboration with Aventis Pasteur, initiated sero-epidemiological studies to describe age-specific and serotype-specific prevalence of DEN antibodies in infants living in Bangkok including the evaluation of the kinetics of transplacentally transferred DEN antibodies and incidence of infections in infants. Nearly, all pregnant women included in the study had antibodies to all four DEN viruses and DEN antibody titres declined steadily in infants after birth and by 9–12 months were below the level of detection. The data corroborate the epidemiological finding that showed increased DEN susceptibility by the age of 1 year.

The WRAIR/GSK candidate tetravalent live attenuated DEN vaccine has been tested in 164 vaccinees without eliciting any serious adverse events [8]. For clinical studies, a re-actogenicity index has been computed by evaluating graded scoring and duration of fever or chills, headache or retro-orbital pain, myalgia or arthralgia, nausea or vomiting or abdominal pain. Two studies have been undertaken, one to evaluate two vaccine formulations and another to evaluate protection in vaccinated individuals after challenge with low passage virus. An enrollment strategy was presented for paediatric studies in Thailand, which have started in the meantime.

The ChimeriVax strategy by Acambis to develop a live DEN vaccine is based on generating DEN antigenic chimeric viruses using prM and E protein genes of DEN viruses in the attenuated YF virus (YF-Vax) genetic backbone [9]. A randomized, double-blind phase I clinical trial was initiated for ChimeriVax-DEN-2 both in naive and yellow fever immune individuals. The vaccine was well tolerated with no serious adverse events. Post infection serum neutralizing antibody titres were generally high for all groups receiving the vaccine with 100% seroconversion at 12 months from either dose. A ChimeriVax DEN tetravalent formulation was tested in cynomolgus monkeys. 100% seroconversion was achieved after single dose. After challenge all monkeys were protected against DEN-2 and DEN-3 and 83% were protected against DEN-1 and DEN-4 viruses. Based on these findings,
Acambis has initiated a phase I study of the Chimeric Vax DEN tetravalent vaccine candidate in adults.

Dr. S. Whitehead presented the strategy of NIAID/NIH to develop a live DEN vaccine [10,11]. Attenuation is achieved by removal of 30 nucleotides from the 3’ NTR of respectively 4 virus. The rDEN-Δ30 has been shown to be safe and immunogenic in Phase I and II clinical trials. Two strategies based on Δ30 attenuating mutation are being pursued: in one strategy, the Δ30 mutation was used to attenuate wild type clones of DEN-1, DEN-2 and DEN-3 viruses; in the other strategy, chimeric viruses have been generated replacing the prM/E genes of the vaccine candidate DEN-4.Δ30 with the corresponding genes derived from the other DEN strains. rDEN-1.Δ30, rDEN-2.Δ30 and chimeric viruses rDEN-2/4.Δ30 and rDEN-3/4.Δ30 were significantly attenuated in rhesus monkeys. Two tetravalent formulations, the Δ30 and Δ30/chimeric formulations were tested in rhesus monkeys. The first consisting of rDEN-1-4.Δ30 showed a decrease in viraemia and peak titres compared to the wild type formulation. Monkeys immunized with the second formulation consisting of rDEN-1.Δ30, rDEN-2/4.Δ30, rDEN-3/4.Δ30 and rDEN-4.Δ30 showed complete protection against DEN-1, -2 and -4 viruses. Additional formulations are being studied in rhesus monkeys, and phase I clinical trials are in progress for DEN-1 and -2.

2.5. Sero-epidemiological studies, normative activities and others

Dr. T. Endy (WRAIR) provided an update on a prospective study of DEN virus transmission and disease in 2000 primary school children in Thailand. During the period 1998–2000, yearly serological studies and acute illness case surveillance were undertaken. Unapparent DEN infections were identified by a fourfold rise in DEN antibody titre without fever. The average incidence of unapparent and symptomatic dengue for all years were 3.9% and 3.4%, respectively. The cost of pre-hospitalization illness, hospitalization and parent work-loss days allowed estimating the economic impact of DEN illness. This type of study provides an opportunity to determine disease severity, disease incidence and circulation of a particular DEN virus in a cohort population, and also provides an opportunity to determine the economic impact of DEN disease.

The availability of reference materials facilitates the standardization of antibody assays for the detection of specific antibodies to both DEN and JE. These materials can be used in the assessment of antibodies induced in vaccine trials as well as naturally infected individuals. Dr. M. Ferguson (NIBSC) reviewed the progress of the standardization of DEN reagents and assays. A master cell bank and three working cell banks of Vero cells have been prepared and characterized. DEN candidate standards (both viruses and plasma) were prepared. A collaborative study is ongoing to identify suitable sera samples which will be assigned a unitage in International Units in order to establish international standards.

Discussion concluded with an update on the Paediatric Dengue Vaccine Initiative (PDVI) [12]. The PDVI is an alliance of stakeholders with the goal to facilitate and accelerate the development of safe, effective and affordable DEN vaccines for children. At present several research approaches are under evaluation with the immediate priorities of establishing field sites to perform both disease surveillance and clinical trials, to estimate the burden of illness, study vaccine biology and to increase dengue laboratory and scientific capabilities especially in endemic countries. Other research priorities include the definition of DEN target cells, mechanisms of virus entry, mechanisms of neutralization and ADE. A PDVI-WHO co sponsored laboratory workshop was being conducted concomitantly with the meeting.

3. Japanese encephalitis

3.1. Vaccine development

While there are several vaccines against JE that have been successfully used to control the disease, there remain limitations to their use on a broader public health scale due to either absence of international registration, high cost or unsuitable vaccine characteristics, requiring multiple rounds of immunization. Thus, while the feasibility of JE vaccination has been demonstrated, there is still a need for safe, affordable and efficacious vaccines that meet international regulatory standards and are suitable for large scale public health use. The session covered the development of new vaccines, improvement of existing ones as well as considerations for clinical evaluation of JE vaccines.

Dr. V.K. Naka is presented on efforts by Acambis using the Chimeric Vax platform to develop a live recombinant JE vaccine, which is based on the YF 17D vaccine strain. In Chimeric Vax-JE, prM and E protein encoding sequences of live JE vaccine strain SA 14-14-2 virus are exchanged into the YF 17D backbone. The vaccine candidate has undergone phase I-II clinical evaluation in adults and showed promising results in terms of safety and reactogenicity, immunogenicity and the propensity to mount anamnestic responses [13]. The company now plans to initiate a first clinical trial in a disease-endemic country that will be conducted as an age de-escalation study in Thai children. Safety and immunogenicity will be compared to commercial inactivated JE vaccine.

There were two presentations by Japanese manufacturers that updated the audience on their efforts to produce JE inactivated vaccine in a continuous cell line. Dr. S. Manabe reported on Biken’s candidate BK-VJE which is produced in Vero cells. With phase I studies completed, the company has moved directly into a paediatric phase III to assess safety, immunogenicity and establish the booster immunization schedule. Licensing is foreseen on the basis of non-inferiority to existing mouse-brain derived vaccine, and could be as early as 2005. Kaketsuken pursues a similar strategy to adapt mouse-brain derived inactivated JE vaccine of

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Beijing strain to Vero cells. Phase I studies have been completed and phase III studies are underway to assess safety, immunogenicity and efficacy in children of 6–90-month age, using a two dose immunization schedule followed by one booster.

Dr. Y. Yu of the National Institute for Control of Pharmaceutical and Biological Products, China, gave an update on production and quality control of a live attenuated vaccine SA 14-14-2 at ChengDu Institute in China. ChengDu has upgraded facilities to WHO Good Manufacturing Practices standard and established a Specific Pathogens Free facility for breeding of hamsters to provide controlled kidney cell substrate for production. Other measures to assure safety, quality and consistency of production have been implemented to meet international regulatory requirements.

Strategies to assess JE vaccine efficacy through surrogate markers were discussed by Dr. L. Markoff. Clinical efficacy trials are an unlikely option for new JE vaccine candidates, as placebo controlled studies would be unethical and comparative trials to existing vaccine appear impractical. The measurement of virus-neutralizing antibodies would represent a possible option, in particular as this measure is already accepted for YF vaccines. For licensure, a neutralization antibody test would need to be standardized and validated to show a correlation between the test result and protection in a suitable animal model. Once the protective efficacy of neutralizing antibodies as measured by a standardized test was established, efficacy of a novel vaccine in humans could be assessed by a “head-to-head” immunogenicity trial using the licensed JE vaccine as control [14].

3.2. JE disease burden and current vaccination strategies

The status of JE immunization and disease burden assessment was discussed by Dr. Z.-Y. Xu (IVI, Seoul). Detailed studies on disease burden have been conducted in Indonesia which aimed at measuring mortality and long-term disability caused by JE disease. The study revealed disability in some 43% of confirmed clinical cases, underlining the devastating impact of the disease. Data from China also suggest significant long-term disability. Therefore, disease data should capture both mortality and morbidity figures to provide a more meaningful picture of the disease-burden due to JE. In line with these findings, Chinese data also suggest that JE vaccination is highly cost-effective, and can actually be cost-saving for the health care system [15].

Dr. S. Chunsuttivat (Bangkok) presented on JE disease control efforts in Thailand. Through successive introduction of JE vaccination, which now has become EPI practice throughout the country, the incidence of clinical JE has been substantially reduced, with about 15% of viral encephalitis cases being attributable to JE compared to 40% previously. In addition to vaccination, it was noted that other factors, such as changes in agricultural practice have contributed to this drop in JE incidence as well, whereas little impact has been observed as a result of direct vector control measures. Vaccination coverage is currently at 87% for the first two doses and 76% for booster immunization.

Dr. J. Jacobson concluded the session on JE by presenting the newly established JE program at the Program for Appropriated Technology in Health (PATH) [16]. The JE program has a 5-year plan and is funded by a grant by the Bill & Melinda Gates Foundation. The program has identified four major goals: (1) Improve disease surveillance, including improved diagnostics, (2) advance an improved vaccine, (3) introduce and integrate the vaccine in routine immunization programmes, and (4) advocate and promote JE control, especially immunization. In close co-ordination with government health authorities and WHO, the program aims to demonstrating its activities in selected countries, including those which are using current vaccine and others which so far have not been using JE vaccine.

3.3. West Nile fever and yellow fever

A final session provided an update on WN epidemiology and efforts to develop a vaccine, and concluded with a discussion on YF vaccination practice.

Dr. J. Roehrig (CDC) reported that during 2003 WN virus continued to spread throughout the Western Hemisphere, resulting in almost 10,000 human infections and 262 deaths in the US alone. Since its introduction into the region, WN virus has been identified in 255 bird species, 37 mosquito species, and at least 13 other species of mammals, including 5251 US equine cases in 2003. In 2003, the US initiated nationwide WN virus screening of blood donations. Over 1000 presumptive viraemic blood donors were identified, and blood products from these donors were removed from the national blood supply.

Human WN vaccine development continues with phase I clinical trials ongoing for the Acambis ChimeriVax-WN vaccine. Other vaccine candidates are still at pre-clinical development stage, which include a recombinant subunit vaccine from Hawaii Biotech, WN/DEN chimera (WN/DEN-4Δ30, WN/DEN-2) from the NIH and DNA vaccines [17].

Dr. A. Dabbagh (WHO) reminded participants that disease control can remain a formidable challenge even when an efficacious, safe and affordable vaccine is available. Today, there are some 33 countries at risk of YF, with some 200,000 cases and 30,000 fatalities per year WHO’s recommended strategy builds on routine infant immunization concurrently with measles immunization and preventive mass campaigns in high risk areas [18]. These measures need to be complemented by strengthened case-based surveillance and improved laboratory confirmation, and better outbreak response capacity. Although there is an efficacious vaccine, research on vaccine safety and efficacy in HIV positive individuals, as well as on rare severe adverse events involving viscerotropic and neurotropic disease following YF vaccination remains to be done.
3.4. Challenges and opportunities

A major practical challenge on the path to a safe and efficacious DEN vaccine is the understanding of the mechanism of virus neutralization and the development of an in vitro assay that would be a meaningful surrogate for protection. In fact, the presence of neutralizing antibodies to a particular DEN virus may not always imply protection against that virus, as shown in prospective studies carried out in Kamphaeng Phaet, Thailand. This observation is corroborated by the finding that DEN candidate vaccine recipients developed clinical DEN fever with DEN viraemia, despite having pre-existing neutralizing antibodies to the virus. These findings underscore the need for additional research into protective immune mechanisms, including cell-mediated protection.

Another challenge is to understand the relevance of ADE in increasing risk of severe DEN and, more importantly, to define the role of the secondary immune response to DEN viruses in protection and pathology. As the development of DEN vaccines moves toward phase III clinical trials, guide-lines for the design, implementation and follow-up needs to be in place with due consideration of ethical and safety issues [19].

Efficacious vaccines against JE are already available. However, current JE vaccine production is unable to meet the requirements of vaccinating the population at risk in Asia with routine and catch-up vaccinations, which would be needed for disease control. New vaccines need to be at least as safe and efficacious as the existing ones, and be suitable and affordable for developing countries. Vaccine developers need to assess efficacy and safety also against the background of other circulating flaviviruses, in particular DEN. As for DEN, improvements of classical neutralization tests for JE virus would be highly desirable.

The Bangkok meeting provided an important forum for exchange of information between scientists, clinical specialists, public health experts and vaccine developers, helping to shape activities for the coming years. Triggered by the spread of disease, but also by real opportunities to develop these much-needed vaccines, there is renewed interest into this field, as shown by the creation of PDEV and the JE program at PATH. Priorities of the WHO steering committee are evolving and cover vaccine evaluation with particular emphasis on safety, support to clinical and laboratory capacity for trials, and standardization activities. The committee will continue to foster exchange of information and help to build capacity for research. Rational co-ordination and sharing of experiences will help to accelerate vaccine development, and make vaccination a reality in the foreseeable future.

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References


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