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A Rapporteur's Summary: Research on Dengue Vaccine

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Abstract: Dengue virus is a member of flavivirus group. Diseases caused by flaviviruses have been a scourge of mankind for long history of human kind; with yellow fever at the top, followed by dengue fever, Japanese encephalitis (JE) and Russian spring-summer encephalitis (RSSE). Due to the dvevelopment of a safe and efficacious live-attenuated vaccine against yellow fever as a first laboratory-designed virus vaccine, this disease is no longer a threat in countries where adequate vaccination is practiced. Similarly, safe and efficacious inactivated vaccines against JE and tick-borne encephalitis (TBE) have also been developed, resulting in dramatical reduction in the disease incidences. In spite of these successes, the development of vaccines against other pathogenic flaviviruses, such as dengue fever and RSSE have not been successful. Control of dengue virus became rather urgent because of the emergence of new syndrome, dengue hemorrhagic fever (DHF), which causes high mortality among infants. The present review attempts to summarize the characteristics of dengue fever and DHF, common features of flavivirus infections as the basis vaccine development and recent advances and problems encountered in dengue vaccine research.

1. Diseases Caused by Flaviviruses.

Diseases caused by flaviviruses have been recognized as severe public health problems in various parts of the world for centuries. Yellow fever was first identified in 1667 in Barbados and continued to be a major threat to public health in the Americas throughout the nineteenth and the early part of the twentieth century. In Africa, yellow fever has still been causing periodic epidemics with the most recent occurring in Gambia and Ghana in 1978. Dengue fever has been recognized since 1780 and has since been described in every continent including Europe and Americas. Dengue fever continues to be a serious threat with outbreaks occurring almost annually in south-east Asia, causing several hundred deaths, particularly of young children. A severe, occasionally fatal form of the disease was reconized in Asia in 1957. This new type of dengue infection, called DHF, has since been recognized in Caribbean during the 1970's, and in 1981, a large outbreak occurred in Cuba. There is a hypothesis that DHF has an immunopathological basis and occurrs in individuals previously sensitized by infection with a heterologous dengue serotype[1].

JE became a serious public problem in the mid 1930 in Japan and recently finding further dissemination among south and south-east Asian countries like India and Thailand.

been developed by incorporating the recombinant DNA technology. A dengue 4 baculaovirus recombinant expressed both E and NS1 proteins in insect cell host, and the protein products induced protective immunity in mice[13]. Purified NS1 protein from dengue-infected cells was also shown to induce protective immunity in mice[14]. This new approach to immunization without involvement of virion antigen, which is related to the induction of enhancing antibodies, may be of great theroretical importance, in special referenc to the control of DHF. Recombinant vaccinia virus expressing dengue 4 structural and non-structural proteins were also active in immunizing mice against the same type of dengue virus[15]. The most possible combination of dengue virus immunogen seems to be E+NS1 in serotypes so far tested. WHO is coordinating a collective efforts of flavivirus experts to develop the dengue vaccine based on 4 approaches: a. identification of protection-inducing antigen or determinant, b. development of most effective expression system of that antigen, c. identification of pathogenicity gene, and d. development of artifically produced attenuated virus, including the recombinant virus[16].

4. Problems to be Considered in the Dengue Vaccine Development.

The ability of specific antibodies to enhance the replication of viruses in cell culture system has been described for a variety of cells and viruses. Most work on this phenomenon of antibody-dependent enhancement (ADE) has been performed in macrophages or macrophage cell lines. The mechanism of ADE *in vitro* has been elucidated, i. e. IgG antibody molecules bind to virus particles and the virus-antibody complex can then bind to the Fc receptor on the macrophage (or any Fc receptor-bearing cell). Thus virus particles which normally cannot infect these cells are able to use the Fc receptor as a virus receptor. Another mechanism utilizing the binding of virus-IgM complexes to the C3 receptor was also reported with flavivirus[17].

The serious role of ADE has been demonstrated in humans infected with dengue virus. Early observations of children with the more severe forms of dengue virus infection, i. e. dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS), indicated that children with antibodies to one serotype (either from a previous infection or from maternal antibody) had an increased risk of contracting DHF/DSS when an epidemic involving another serotype appeared. Antibodies induced by virus of one serotype would bind to virus of another serotype (as they are antigenically related), but as these antibodies cannot neutralize the new virus, it will be able to bind to and infect macrophages and thus destroy a crucial component of the immune system. This hypothesis has been supported by a series of animal experiments[1], but its impacts on the grand design of vaccines preventing dengue fever and DHF have not yet been elucidated quantitatively.

As all flaviviruses contain cross-reacting epitopes on their major envelope glycoprotein (E), it is possible that vaccination against one virus may enhance the infection with another, or even worse change a non-virulent vaccine strain into a virulent virus. So far, the possibilities were not materialized, in fact, the opposite effect has been observed between different flaviviruses. Previous vaccination of human volunteers with yellow

relationships among subtypes and other flavivirus members are being revealed by the introduction of monoclonal antibody method, e. g. between dengue 2 and 4 and between dengue and JE and TBE complex viruses. Geographic distribution of 14 variants of dengue 2 virus differentiated by monoclonal radio-immunoassay (RIA) correlated clearly with the genomic variation demonstrated by RNA oligonucleotide fingerprinting[6].

In spite of the world-wide prevalence of dengue fever, the development of vaccines against this virus have not been successful. The only vaccines available against dengue fever at present are for serotypes 1 and 2 and all of these are still to be called the experimental vaccines. Further, there is no record of any inactivated vaccines being developed, principally because all dengue serotypes grow poorly in cultured cells.

The first vaccine against dengue type 1 was proposed by Sabin's group. This was a live attenuated vaccine derived by repeated passage of the Hawaian isolate in mouse brain and was shown to be protective in human volunteers[7]. A similar vaccine against dengue 2 was later developed from the New Guinea B isolate by a similar procedure and produced a good serological response in human volunteers. Similar vaccines were prepared by adaptation to avian fibroblast culture with similar limited success to mouse brain vaccines. Despite earlier promising results, works on these vaccines were discontinued due either to limited immunogenicity or to relatively high frequency of adverse effects.

Recent successful studies with dengue vaccines have been based upon a small-plaque, temperature-sensitive (ts) clone, PR-159/S1, derived from a dengue-2 virus isolated in Puerto Rico from a patient of dengue fever. The virus was shown to be attenuated for humans, but caused limited adverse reactions with fever, rash and leukopenia in some recipients[8]. Double-blind, placebo-controlled trials in human volunteers revealed that this vaccine caused slightest vaccine-related symptomes in some individuals. An interesting feature of this vaccine was that seroconversion, as measured by the development of neutralizing antibody was as low as 61% in nonimmune volunteers, whereas individuals who has record of previous vaccination against yellow fever gave seroconversion rates of 90%[9]. The synergistic effect of flavivirus vaccinations was confirmed by several workers and may pose double-edged considerations in both benefitial and adverse directions.

One of the most promising candidate dengue 2 vaccines has been developed recently by a group in Thailand using a human isolate in Thailand (16681) adapted to growth in primary canine kidney cell cultures[10]. The virus strain 16681-PDK53 demonstrated many *in vitro* markers for attenuation and protected human volunteers without the adverse effect seen in earlier vaccines. The vaccine was immunogenic even in individuals without other flavivirus immunity. These workers also report that a similar vaccine to other serotypes being developed in their laboratory, and a tetravalent vaccine composed of Den-1-PDK-13, Den-2-PDK-53, Den-4-PDK-48, and Den-3-PGMK-30 (passaged through primary African green monkey kidney cells), was reported to be safe and immunogenic with minimal febrile reaction and good antibody response in both adults and infants[11].

An experimental vaccine against dengue 4 has been described by an American team[12], details of the efficacy and safety seem yet to be seen. Various strategies have also

St. Louis encephalitis first became a serious public health problem in the continental USA in 1960s and sporadic outbreak continues to be a cause of concern.

The tick-borne flaviviruses, like their mosquito-borne counterparts, are amongst the earliest known causes of infectious diseases. Ovine encephalomyelitis (louping ill) among sheeps and RSSE were described in the nineteenth century. TBE was first recognized in 1930's in central Russia and far-eastern subtype of TBE virus continues to be a major public health problem in eastern Russia with a high mortality rate and frequent long-lasting neurological lesions. The western subtype of TBE, prevalent in east and central Europe, is closely related to louping ill, but with less severe clinical symptoms and much lower mortality rate. Apart from TBE, there are relatively few tick-borne flaviviruses; the most important are Omsk hemorrhagic fever of central Russia and Kyasanur forest disease of India.

2. Structure and Replication of Flaviviruses.

The flaviviruses have the structure of enveloped, spherical nucleocapsid, with unknown symmetry, of the size 40-50 nm in diameter. The genome consists of one molecule of positive-sense, infectious single strand RNA, size 10 kb. The viruses have three major structural polypeptides[2], one of which is glycosylated. Genome sequence has been elucidated on several mosquito-borne viruses[3] but very little data are available on the tick-borne flaviviruses. The sequence data suggest that there are ten virus-specified proteins and this is in close agreement with what is found in cells infected with mosquito-borne and tick-borne viruses.

The minor component of the envelope is a small non-glycosylated polypeptide (M) which is sometimes found in virion particles as a glycosylated precursor, pre-M. The major protein of the virus envelope (E) is present as an oligomeric complex and contains all the observable biological properties associated with the virus particle, i. e. antibodies directed against epitopes on the E polypeptide are responsible for plaque-reduction neutralization, hemagglutination inhibition, passive protection, complement-fixation and antibody-dependent enhancement. Sera from animals infected with flaviviruses or vaccinated against them contain antibodies to a non-structural glycoprotein NS1. The precise functions in virus infection or immunity of non-structural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 are unknown at present, although some or them are suggested to be involved in RNA replication.

The genome structure of the flavivirus is very similar to that of the picorna-viruses, and their protein synthesis strategies are expected to be very similar. But there has been little evidence indicating the presence of precursor molecules in favivirus-infected cells.

3. Vaccines against Dengue Virus.

Four serotypes (types 1-4) are recognized on the basis of plaque reduction neutralization tests[4], and constitute a distinct antigenic complex. Dengue types 1 and 3 form a subcomplex defined by monoclonal and polyclonal antibodies[5]. Additional antigenic

fever vaccine increased the seroconverion rate of an experimental dengue-2 vaccine and a similar synergistic effect was observed between yellow fever vaccination and TBE vaccines. Although ADE does not appear to be the problem with flavivirus vaccines at present, dengue vaccine should be developed with special vigilance in this respect.

Flaviviruses are characteristic in that the major virion structural protein E may not be present on the surface of the infected cell, but non-structural protein NS1 is. It implies that if a vaccine contains only components from E, free virus particles will be eliminated, but infected, virus-producing cells would not be. In the similar case of inactivated measles vaccine, the consequences were tragical. The converse situation could be feasible if a vaccine contained only components from NS1. In this case, infected cells may be killed but virus which had escaped would be free to travel throughout the host and infect other tissues. It is therefore essential to ensure that new component vaccines against flaviviruses should contain components from both E and NS1 proteins.

5. Conclusion.

Although ADE is a theoretical concern in developing the flavivirus vaccine, there has been no evidence that it is a problem with flavivirus vaccines so far developed. But, as the pathogenesis of DHF strongly suggests the involvement of ADE, development of any dengue vaccine should maintain the vigilance against this mechanism. Development of animal model reproducing human DHF should be given the top priority from this point of view. After establishing the pathogenic mechanism of DHF, programmed vaccines containing protein components may not be difficult. Development of artificial attenuated live vaccine may be feasible after the elucidation of gene product(s) involved in the genesis of DHF. Recombinant or chimeric viruses may be possible to be constructed by advanced recombinant DNA technology.

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