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Research in Pharmacy 3(2): 18-25, 2013 ISSN : 2231-539X
www.researchinpharmacy.com

Mini Review

A Mini-review of Dengue Vaccine Development

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About 100 million dengue cases are reported annually and an estimated 2.5 billion people are susceptible to the infection mostly in the tropical regions. Dengue virus is a member of the *Flavivirus* genus and consists of four serotypes (DV-1, DV-2, DV-3, and DV-4), each of which is capable of causing dengue fever and the more severe dengue hemorrhagic fever or dengue shock syndrome. There is an urgent need to develop a safe and effective vaccine that induces protective immune response to all the four serotypes overcoming antibody dependent enhancement. At present there is no licensed vaccine or specific therapeutic measures for prevention or management of the fatal infection. This mini review outlines the different vaccine candidates that are at various stages of development.

Keywords: Antigen, Bispecific antibodies, Clinical trials, dengue shock syndrome, Dendritic cell targeting, viral gene expression, Immunization, vaccine

In the last 20 years dengue has spread rapidly and is endemic in more than 100 countries. Dengue virus infection represents a major, growing public health problem with an estimated 2.5 billion people at risk of infection in tropical and subtropical countries (WHO, 2009). Early reports on dengue fever outbreaks date back to about two hundred years ago but in recent times the major burden of the fatal disease are borne particularly in south east Asia, south and central America (Whitehead *et al*. 2007; Cardosa, 1998). Dengue Fever is a debilitating and often fatal *flavivirus* infection transmitted by *Aedes* mosquitoes (Edelman, 2007). There are more than 100 million cases of dengue infections annually and about half a million reports of the more severe and threatening dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Gubler, 2002a, 2002b). There are four serotypes of dengue virus (DV1, DV2, DV3 and DV4). All the serotypes are competent in causing asymptomatic manifestations as well as the more severe and fatal DHF and DSS. Dengue virus comprises if a single-stranded, positive-sense RNA genome. Translation of the viral RNA results in a single polypeptide that is processed by proteases, generating three structural proteins and seven non-structural (NS) proteins (Lindenbach and Rice, 2001; Clyde and Harris, 2006). The structural proteins consists of the capsid, membrane and the envelope where as the non structural proteins are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. There is also a membrane precursor (prM), which assists in the folding of the envelope protein. The Envelope protein plays an important role in infection of the cells and is also reported to be the target to develop neutralizing antibodies (Mukhopadhyay *et al*. 2005; Halstead, 1989).

Received: 20.1.2013; Revised: 17.3.2013; Accepted: 20.3.2013

Dengue has become a huge burden both in terms of lives lost particularly children and also the economic setback to the emerging economies of the world to contain the disease. A primary infection with any of the serotypes would induce lifetime immunity against that particular serotype but a subsequent infection with a different serotype can increase the severity of the disease due to a phenomenon known as antibody dependent enhancement (ADE). Hence development of an effective vaccine candidate catering to all the four serotypes, which would be safe and cost effective, is of great significance particularly for children and young adults who are the most susceptible (Jin, 2009). Currently there are no licensed vaccines available for dengue although there are vaccines available for a number of closely related viruses. The World Health Organization has prioritized the development of dengue vaccine and therapeutics for a long time. But so far due to unsuccessful development of any kind of vaccine or treatment for containing dengue, the arrangements so far pursued have been vector control and individual preventive steps which are tough to sustain and also expensive (Brandt, 1990; Stephenson, 2006). The foremost issue associated with dengue vaccine development is the need for concurrent protective immunity against all four serotypes due to ADE (Guzman *et al.* 2007; Kliks *et al.* 1989; Morens and Halstead, 1990). This short article summarizes various approaches undertaken towards a successful dengue vaccine and the challenges associated with it.

VACCINES: EFFORTS AND CHALLENGES

With the ever-increasing severity of the dengue virus and its crucial impact on loss of life, economics of the endemic region etc., the need of an effective vaccine is becoming more and more significant (Malabadi, 2008; Malabadi *et al.* 2010, 2011). The increase in dengue incidence in the past decade and the simultaneous presence of four different serotypes has posed a greater threat to the communities and has also witnessed higher volumes of the more severe forms of dengue infection. The development of an effective dengue vaccine has been not easy although focused and concerted efforts have been made for the past 70 years. Just after the end of World War II, Sabin and colleagues reported one of the first formulations of a dengue vaccine. Their work was based on live attenuated virus passaged multiple times in the mouse brain (Sabin and Schlesinger, 1945; Sabin, 1952). After about 15 passages in the mouse brain, the virus became attenuated enough for trial in humans (Edelman, 2007; Jin *et al*. 2009). Much later Halstead and coworkers made efforts by attenuating dengue virus in dog kidney cells (Thomas and Endy, 2011). Other developments are focused on inactivated and subunit virus candidates (Whitehead *et al.* 2007). The major hindrance faced by researchers in vaccine development is the phenomenon of Antibody dependent enhancement (ADE). The presence of non-neutralizing antibodies developed during a primary infection of dengue greatly increases the severity of a second dengue infection with a different serotype by formation of immune complexes or facilitating viral access to Fc**γ-**Receptor possessing cells (Calvert *et al.* 2006; Halstead, 2003). Therefore an ideal vaccine candidate should be able to circumvent the ADE phenomenon and be tetravalent in nature or a combination of four monovalent vaccines delivered as a single vaccine. The vaccine should also be able to confer immunity against all the four different serotypes. Also the need to understand the underlying molecular principles that govern pathogenesis of dengue is very important (Murrrel *et al*. 2011). Currently, though over the past decade efforts have been more aggressive to develop an effective intervention to the fatal infection with the advent of recombinant DNA technology. There are many targeting approaches and the development of nanocarrier vaccine have been reviewed (Khan *et al*. 2011, 2012). The absences of a proper animal system to test the vaccine candidates have also been a deterrent in dengue vaccine research. Emerging economies of the world bear the greatest

burden of dengue fatality so an economically affordable vaccine is necessary which would Envelope glycoprotein that is part of the virus structure is considered a major target for vaccine research. Its functionality includes virus attachment and subsequent cell entry. Domain III of the envelope protein is considered most important owing to its role in cell receptor binding (Lok *et al.* 2008). Other proteins such as the membrane and NS1 are also thought to be protective (Schlesinger *et al.* 1986). Monoclonal antibodies against Envelope and pre membrane proteins have been efficient in protecting against severe infection (Crill and Roehrig, 2001). There is also evidence that protective anti dengue antibodies are effective in children with maternal inheritance. Cell mediated immune responses have also been shown to play a role in active viral clearance (Samuel and Diamond, 2006).

Live attenuated vaccine development efforts are considered one of the effective approaches. A wild type dengue virus can be attenuated by serial passages in tissue culture to be used in humans maintaining its ability to generate an immune response. In another embodiment a chimeric vaccine candidate is generated by modification of a licensed yellow fever virus vaccine with dengue proteins. Another important feature of a live attenuated vaccine is its ability to induce sustained immune responses very closely mimicking a response incase of a natural infection. Sanofi Pasteur has developed a chimeric vaccine based on the yellow fever vaccine by incorporating dengue premembrane and envelope genes of dengue (Guy and Almond, 2007; Murphy and Whitehead, 2011). Recently, efficacy trial of the vaccine in Thailand demonstrated impressive safety profile along with the ability to protect against three of the four-dengue serotypes. Further clinical trials are underway to validate vaccine efficacy and safety (www.sanofipasteur.com). Inactivated virus vaccines are also in preclinical stages of development and one of the dengue serotype 2 vaccines manufactured by the Walter Reed Army Institute for Research is scheduled to begin clinical trials shortly. Inactivated vaccines have certain advantages over the attenuated ones including inability to become pathogenic and also being able to induce immune response in all four dengue serotypes due to immunogenic equality (Whitehead *et al.* 2007). Recombinant subunit vaccine candidates have also been developed primarily based on the envelope protein. There has been a study by the Pedro Kouri Tropical Medicine Institute where the domain III of the envelope protein was fused with a carrier protein and monovalent constructs were made for all the four serotypes. Each of the constructs was able to induce neutralizing immune response and also demonstrated protection against viral challenge (Hermida *et al.* 2006). Another bivalent effort is based on a domain III and STF2D fusion protein conjugate. There is also a tetravalent conjugate of Domain III and Ag473 fusion protein being evaluated in animals (Das *et al.* 2009). A lot of Dengue vaccine efforts based on virus like particles (VLP) are in preclinical phases. Viruses like particles lack replication material but are able to mimic antigen presentation like a natural viral infection (Jennings and Bachmann, 2008). A tetravalent vaccine candidate have been developed by Cytos Biotechnology by chemically attaching envelope domain III to an *E.coli* expressed VLP. Another monovalent candidate has been in development in ICGEB, India where the basis is the envelope domain III and *P. pastoris* expressed VLP (Schmitz *et al.* 2011). The Carolina vaccine Institute (CVI) has been a pioneer in the development of a alpha virus replicon vector expressing envelope protein. This strategy was demonstrated by immunizing monkeys resulting in the generation of neutralizing antibodies and protection from viral challenge as well. GenPhar Inc., developed bivalent vaccine candidates based on an adenovirus construct. Challenge studies resulted in absolute protection against dengue type 1 and 3 whereas significant reduction in viremia was observed against type 2 and 4. Another strategy adopted by the Pasteur Institut is centered on a tetravalent dengue antigen from a live attenuated measles vaccine vector. The

construct expresses domain IIIs of all the four serotypes along with the membrane protein ectodomain. Analysis in murine models showed induction of neutralizing antibodies against all the serotypes. Clinical trials of the construct have been planned in the near future (Schmitz *et al.* 2011). With genetic engineering and recombinant technology rapidly making progress, concept of DNA vaccines have been gaining considerable momentum. Studies have been conducted with monovalent constructs of dengue in macaques that resulted in production of neutralizing antibodies. It also conferred protection to the vaccinated macaques when challenged with a wild type dengue virus strain (Raviprakasha *et al.* 2000). A follow up study was done by the same research group, to enhance the neutralizing efficiency of the earlier DNA vaccine construct. Neutralization efficiency increased significantly from the earlier construct and was stable for 6 months post vaccination. All the monkeys barring one were also completely protected from a virus challenge (Raviprakasha *et al.* 2000; Konishi *et al.* 2006). These important findings led to the transition of DNA based dengue vaccines towards clinical trial (Danko *et al*. 2011). Advantages of DNA based strategies involve its ability to induce both humoral and cell mediated immune responses, lack of complicity like other vaccine approaches and technically simpler to develop. On the contrary there are also some drawbacks associated like the level of immunogenicity obtained from clinical studies have been slightly low. Another limitation is that of insufficient cell uptake (Murrrel *et al*. 2011) leading to reduced protein expression. Nonetheless, promising DNA based strategies are being explored at preclinical and clinical phases with varied outcomes. Further studies on DNA based vaccination including design, adjuvant selection, delivery methodology are needed to overcome the current limitations as it has the potential of being an effective strategy to counter dengue and other infectious agents owing to being cost effective, technically easier to manufacture, stability and the ability to deliver multiple agents in one single construct.

TARGETING STRATEGIES FOR DENGUE: A VIABLE ALTERNATIVE

Dendritic cells (DCs) are a unique class of antigen presenting cells that have an important role in both innate and cell mediated immunity. DCs have significant role in the activation of T- and B-cell immune responses and are deemed to be more efficient than other antigen presenting cells. Targeting of DCs via the surface receptors may be an alternative strategy for development of low dose targeted dengue vaccines. DC targeting greatly enhances antigen delivery efficiency to the DCs than traditional vaccination methods wherein enzymes and other cells might have adverse exposure before being taken up by the DCs (Wang *et al*. 2009; Tacken *et al.* 2006). DCs internalize antigens using its many cell surface receptors including Gb3, CD40, Fc receptors, C-type lectin receptors etc and present them to both MHC I and II pathways (Figdor *et al*. 2002; Tacken *et al.* 2007). A lot of studies have been conducted on DC cell receptors and antigen targeting. DEC-205 is a part of the C-type lectin receptor family that has been extensively studied. It is present on both mature and immature dendritic cells and targeting DEC-205 results in improved cross-presentation of antigens compared to other receptors (Bozzacco *et al.* 2007; Jiang *et al*. 1995; Banchereau and Steinman, 1998). Reports illustrate that DEC-205 targeting resulted in improvement in antigen presentation efficiency to T-Cells (Wang *et al*. 2005). We have developed bispecific antibody based delivery vector to target DCs with biotin labeled antigens that could be proteins, DNA, peptides, gangliosides etc. (45, Wang *et al*. 2005). In this strategy, the antibody has dual specificity i.e. one arm can bind to any biotinylated antigen and the other arm is specific to the DC receptor DEC-205. Targeting resulted in reduction in antigen dosage by approximately 500 fold when compared with non-targeted antigens. Animal studies demonstrated targeted strategy along with co-stimulatory anti-CD40

monoclonal antibody significantly enhanced both humoral and adapted immune responses for SARS-CoV, Ebola GP1, MUC-1 peptide (Wang *et al.* 2009). Another study targeting mucosal DCs in murine model with SARS NP DNA induced strong immune response. When CD40 monoclonal antibody as a DC maturation stimuli was used, the responses were improved considerably. Dengue specific DC targeted vaccine candidates are currently investigated (unpublished data). DC targeted systems involving bispecific antibodies could be potentially a viable alternative for the development of dengue vaccines which have been long overdue (Bonifaz *et al.* 2004; Malabadi *et al.* 2012) Exhaustive research on alternative approaches needs to be done to come up with potent strategies to counter dengue fatalities that have so far taken millions of lives around the world.

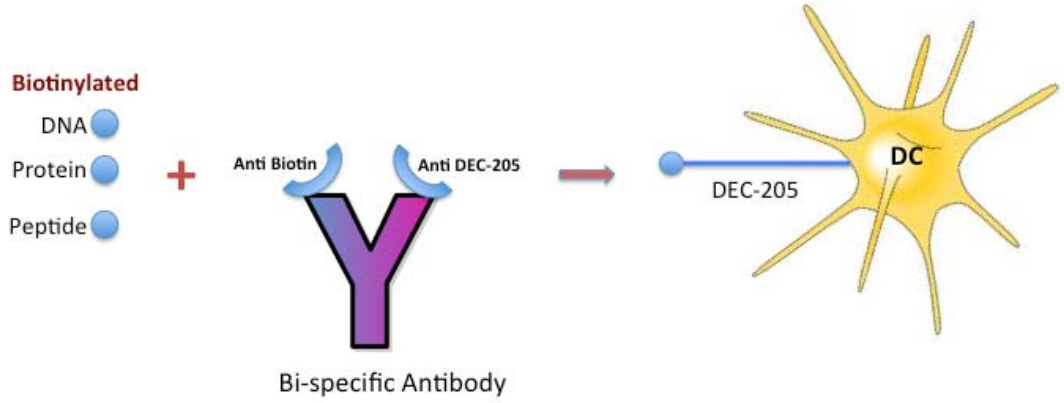

Fig: Schematic representation of DC Targeted Strategy

Acknowledgements

This work was supported by research grant from The Natural Sciences and Engineering Research Council of Canada (NSERC-Strategic). AG is a Ph. D graduate student and RBM is a Research Associate.

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