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Recent Advances in Zika Virus Vaccine Development

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Abstract

Zika Virus (ZV) has infected over one million people worldwide. ZV crosses the placenta, infects developing fetus and causes Congenital Zika Virus syndrome (CZS) including microcephaly in newborns. In this up-to-date review, ZV immunology and vaccine development have been discussed with cutting edge advancement in this discipline. ZV belongs to flavivirus genus, shares structural similarities with Dengue Virus (DENV) that are the mainstay for vaccine development research and consists of positive-sense RNA, a capsid with an outer shell. Humoral immune response against ZV results in generation of neutralizing and cross-reactive antibodies. The neutralizing antibodies are produced against its pre-membrane (prM) and envelope (E) proteins, however, the cross-reactive antibodies may interact with the antigens of other flaviviruses in addition to ZV itself resulting in the antibody dependent enhancement (ADE) phenomenon, a risk for DENV immune ZV vaccine candidates. The vaccines developed against ZV include subunit and live-attenuated vaccines. Subunit vaccines are DNA, modified mRNA and virus-like particles (VLP) vaccines. DNA vaccine, developed by incorporating E protein antigen genetic code into the plasmid, is under different phases of clinical trials. Nucleoside-modified ZV prM-E mRNA vaccine, enveloped in lipid nano particles (LPN), has successfully been studied in mice and nonhuman primates. Live-attenuated 10-deletion vaccine, consisting of mutant viral RNA genome, activates cell-mediated and hum oral immune system. In conclusion, the ZV prospective vaccine must be safe enough for clinical trials, highly effective in generating neutralizing antibodies, free of ADE risk and compatible with variable transportation conditions.

Keywords: Zika Virus structure; DNA vaccine; modified mRNA vaccine; Zika Virus vaccine;

Transmission

Since April 2015, about one million people have suffered from Zika Virus (ZV) infection collectively and, approximately over 6400 cases of ZV have been reported in Americas and territories alone. [16] Main lineages of the Zika virus involved in French Polynesia epidemic and in Americas were Asian and African respectively. [11] Virus (ZV) is classified in the Family of Flaviviridae and Genus of Flavivirus virus of Arboviruses. [12] The structural similarities among Zika and other flavivirus, for example Dengue Virus [DENV], are the primary focus for the current vaccine and therapeutic research against ZV. Zika virus spreads by a mosquito, Anopheles Aegypti. [16] In addition to the mosquito bite, the vertical transmission, from mother to baby during pregnancy, and the sexual transmission have also been reported. [25] ZV infection is characterized by mild rash and fever. [22] However, where it infects the cells of nervous system may result in severe non-reversible complications. The neurological complications include Guillain-Barre (GB) syndrome characterized by neuromuscular paralysis .[24] ZV infection during pregnancy causes Congenital Zika Virus syndrome (CZS) which is a defined set of congenital conditions in newborns. CZS consists of microcephaly, decreased brain tissue, damage to the back of the eye, joints with limited movement and increased muscle tone .[7] Microcephaly, where the head circumference of newborn is less than thirty-two cms, has been the hallmark of the teratogenicity of ZV maternal infection.[2] ZV infection in pregnancy complicated with the microcephalic baby is a major cause of the anxiety and depression among the reproductive age women of ZV endemic area. [10] In adult men, ZV keeps on shedding in semen for more than two months after infection and damages spermatogonia in male reproductive organs resulting in male infertility. [19] Therefore, the consequences of ZV epidemic are affecting next human generations.

The phenomenon of Antibody Dependent Enhancement of infection (ADE) is an important risk for the sample selection in clinical trials for monoclonal antibodies. Antibody Dependent Enhancement of infection is the phenomenon that results in magnifying the host cell infection by the viruses of same family due to the presence of cross-reactive antibodies. The respective antibodies recognize the receptors on the viral particle and the host cell membrane. These antibodies may be produced against one type of virus, for example Dengue Virus and can cross-react with the same epitopes present on the Zika Virus (ZV). There are three proposed mechanisms of 'ADE, the first one being recognition of the receptor on the surface of virus particle, inducing a conformational change in the structure of the receptor that increases its affinity for a corresponding receptor on the host cell membrane (Figure 1 A). In the second mechanism, one component of the antibody binds to the receptor on the virus particle and other on the cell surface receptor, thus a single antibody bringing together the virus to the cell surface (Figure 1 B). While the third and the main mechanism is the cross-reactive antibody binding to the virus particle and its fixed or Fc component being attached to the receptor on the cell surface, thus promoting the endocytosis of the virus (Figure 1 C). [30]

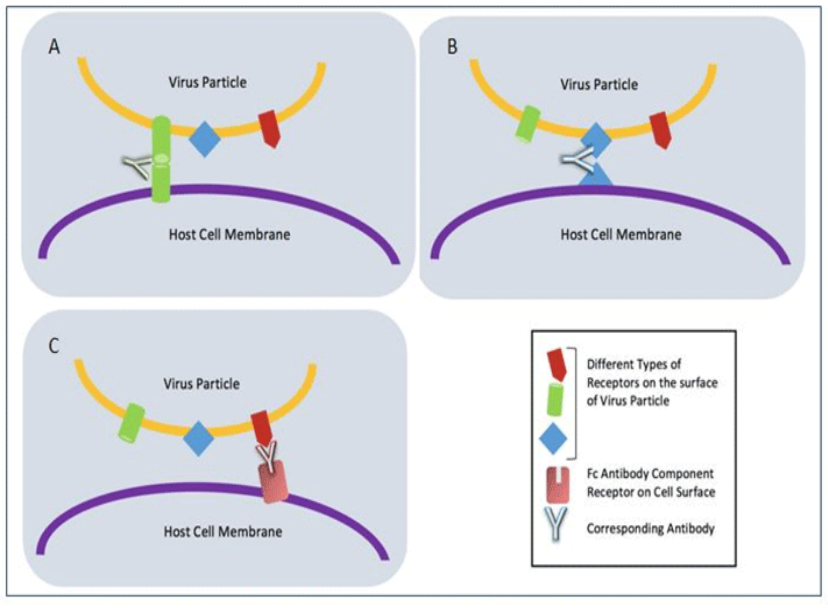

Figure 1: Three different mechanisms of ADE (Antibody-dependent enhancement): A-receptors on virus particles bound by cross-reactive antibody get conformational change in structure that results in high affinity with the corresponding receptor on the host cell membrane. The corresponding receptor was not able to recognize the viral receptor before this conformational change. B- The cross-reactive antibody binds to viral and host cell membrane receptors at the same time bringing the viral particle near to the host cell. C- The Fc component of the cross-reactive antibody is recognized by the corresponding receptor on host cell membrane being the predominant mechanism of ADE phenomenon.

Vaccines are used to generate acquired active or acquired passive immunity depending upon the type of vaccine. [3] To prevent ZV complications, world needs effective vaccines and therapeutics. Understanding the structure and immunology of ZV is a stepping stone towards the successful designing of an effective vaccine. The present review is meant to provide an update of recent advancements in ZV vaccine development with the background information about Flaviviruses, host immunity, vaccines, types of vaccines and structure of the Zika Virus.

Flaviviruses

Arbovirus or Arthropod-borne viruses include 545 species out of which about 150 are of clinical significance being related to Flaviviridae, Bunya viridae and Togaviridae families. [6] These viruses are prevalent in developing countries, spread through their specific arthropod reservoirs and are transmitted to human species. [30] The Arboviruses or arthropods-borne viruses are included in six different taxonomic families grouped according to the type of their genome: *Togoviridae* and *Flaviridae* – single-stranded positive-sense RNA; *Bunyaviridae, Rhabdoviridae* and *Orthomyxovidae* – single-stranded negative sense RNA and Reo viridae – double-stranded RNA . [30] The Flaviridae Family includes *Flavivirus* genus having more than 70 viruses. [14] In humans the *Flaviviruses* cause a broad spectrum of clinical conditions ranging from rash with mild fever, hemorrhagic disease, encephalitis and even death. [20] Although, there are 53 species in total responsible for human diseases, albeit, five of them i.e. Dengue Virus (DENV), West Nile Virus (WNV), Japanese encephalitis Virus (JEV), Yellow Fever Virus (YFV) and recently added Zika Virus (ZV) contribute towards the most diseases. [12] The effective vaccines are available against the YFV and JEV but not yet against the DENV and ZV. [30] In general, the viruses of *Flaviridae Family* have single-stranded, positive sense, 9.5-12.5 kb RNA genome in a nucleo capsid of ~30nm formed by capsid (C) protein. These are enveloped viruses with the core surrounded by an icosahedral shell of ~ 40-60 nm in diameter. [30]

Types of Immunity

Broadly, the immune system is divided into an innate and acquired immune systems, each having humoral and cellular components. *Humoral immunity,* achieved by the antibodies, is the antibody-mediated while the cell-mediated immunity involves the array of immune cells including phagocytes, T-lymphocytes and the chemical mediators called cytokines. As the body is exposed to an antigen, the response of the immune system results in the active immunity known as natural or acquired, depending on the type of exposure . [3] On the other hand, the *passive immunity* is achieved by protecting the body through prepared antigen-specific immunoglobulins. The IgG antibodies cross the placenta providing the natural passive immunity to developing fetus.

Flavivirus family viruses' immunology

The humoral or antibody-dependant immunity against flaviviruses is generally conferred by the production of antibodies against envelope (E) protein present in the outermost shell, premembrane (prM) protein and nonstructural NS1 protein. [34] These antibodies are of various types including cross-reactive, cross-neutralizing, neutralizing and non-neutralizing antibodies. *Cross-reactive and cross-neutralizing antibodies* interact with the antigens that have triggered their production, as well as, with the ones that are similar to triggering antigens but are not responsible for their initial production. Thus, these antibodies may confer immunity against multiple viruses of the same family. *Neutralizing antibodies* are the antibodies that bind with the same epitopes on the viral surface and the cell membrane of effector cell. These antibodies are capable of attenuating the biological effects of the pathogen without the involvement of immune cells or mediators. These antibodies are usually against the envelope proteins and lead to the viral clearance. On the other hand, the *non-neutralising antibodies* have different epitopes on the pathogens and the effector cells. These make immune complexes with the pathogens and help them be endocytosed by the cells with corresponding receptors. [4]

The cross-reactivity of monoclonal antibodies against DENV may be used to target the ZV but the downside is the *antibody dependent enhancement* (ADE). In this phenomenon, the non-neutralizing antibodies cross-react with the antigen making an antigen-antibody complex. The Fc components of these antibodies recognize the receptors on cell surfaces and help the virus to enter into all the cells having their specific receptors. This results in increasing the viral infectivity many-folds without generating an optimal immune system response. [21]

Vaccines and types of vaccines

A vaccine may consist of immuneglobulins or any other form of antigen and the excepients. The excepients are the substances in a vaccine other than an active ingredient including an adjuvant, the stabilizer and a carrier substance. The stabilizer acts as a preservative to let the vaccine stable enough during fluctuating conditions of storage and transport. The carrier is the vehicle for delivering the antigen to the site of action by increasing its solubility. [3]

The vaccines are classified according to the type of the antigen. There are four main groups: live attenuated, Toxoids, killed or inactivated and subunit vaccines. *Live attenuated* vaccines consists of an infectious agent weak enough not to cause the disease in the host. Toxoids are modified toxins with molecular conformational changes that are produced by formaldehyde treatment to the toxin molecules. [3]*Inactivated* viral vaccines contain attenuated or killed whole viral particles that are not capable to replicate inside the host but are immunogenic in nature. [8]

Live attenuated vaccines

Live attenuated vaccines consist of an infectious agent weak enough not to cause the disease in the host. The attenuation approaches for developing live attenuated viral vaccines may include growing the virus in a foreign host or in an artificial growth medium at a lower temperature than that of human body. [3] The attenuated viral particles stimulate both humoral and cell-mediated immune pathways. Dendritic cells phagocytose the particles, process the antigens and stimulate T cells in the draining lymph nodes where there is also production of plasma cells, memory B cells and neutralizing antibodies. The vaccine particles infect the muscle or skin cells at the site of injection, their peptides are processed, presented on the surface of infected cells onto the major histocompatibility complex type I molecules and attract the cytotoxic T cells (Tc). Cytokines released by Tc lead to apoptosis or programmed death of infected cells.[1] The live-attenuated vaccines bear the risk of causing the disease in the host either due to insufficient attenuation or the compromised immune system. [3]

Subunit vaccines

Subunit vaccines are developed by identifying the particular antigen of a pathogen having an epitope with the ability to stimulate the production of neutralizing antibodies in the host. The subunit vaccines are further subdivided into virus-like particle (VLP), DNA vaccines and modified mRNA vaccines. [8] Virus-like particle vaccines are concentrated viral structural proteins that are antigenic, display the specific epitopes and are able to stimulate the production of neutralizing antibodies.[8] DNA and modified mRNA vaccines have the genetic codes for the desired antigens and are transcribed inside the host cell environment. Subunit vaccines consist of a 'subunit' or an 'antigen only' of a pathogen. The antibodies produced in response to the subunit or antigens are potent enough to prevent the infection. Subunit vaccines may have single antigen molecules or in combination. [1] The immune response to these vaccines may be T cell dependent in case of protein subunits or T cell independent against polysaccharide antigens. [3] Subunit vaccines are safe because the antigen does not multiply in host but is only there to generate immune response, therefore, cannot cause disease. These vaccines are very compatible with variable storage and transport conditions, remaining stable while being used in far off geographical areas. Under no circumstances these vaccines may affect or infect the unimmunized members of the community. The tradeoffs include the type III or Arthus reaction at the injection site by the adjuvant in the vaccine and multiple dose regimen. [3]

ZV structure

Being a member of Flaviridae Family, ZV consists of positive-strand RNA genome in the core surrounded by capsid, a lipid bilayer and an outermost shell. RNA genome is the code for ZV proteins and is translated into a long polyprotein strand in the cytoplasm of host cell. This polyprotein strand is further cleaved into the individual proteins inside the cell cytoplasm. ZV proteins are of two types, structural and nonstructural. The three structural proteins include the precursor membrane (prM) protein, envelope (E) protein and capsid (C) protein.[32] These proteins form the outer shell of the virus and are the first to be recognized by the host immune system, thus playing the key role in the viral binding on the host cell surface . [13] E protein is a glycoprotein having three domains - DI, DII and DIII, joining to form one monomer where DI is in the center and the other two domains are on each side. This monomer combines with another monomer

creating a dimer that looks like a finger. The three dimers align parallel to each other with a diamond shaped symmetry that can further be subdivided into two symmetrical triangles, each having half of all the three dimers .[32] In DII domain, there is a 'Fusion Loop' present in a small gap where DII joins with DIII completing the E protein dimer. [15] Fusion Loop consists of highly conserved, 98-110 glycine-rich, hydrophobic amino acid residues that interact with the endosomal membrane during fusion of the viral envelop with the host cell membrane, hence called 'Fusion Loop' .[30] The nonstructural proteins are seven in number: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. These are responsible for polyprotein strand processing, regulating the genome replication and manipulating the host responses for safe viral escape. [32]. [13]

Zika Virus Vaccines

Effective vaccine against ZV needs to be developed as soon as possible because of the devastating neurological and pregnancy complications. Therefore, the main objective is to design a vaccine that could primarily protect the brain and uterus from viral infection. To-date subunit and live-attenuated ZV vaccines have been developed in animal models. The subunit vaccines against ZV include DNA and modified mRNA vaccines. The National Institute of Allergy and Infectious Diseases (NIAID) is supporting the development of multiple ZV vaccines. NIAID works under the National Institutes of Health (NIH), a part of the U.S. Department of Health and Human Services. [23] An experimental DNA vaccine is now-a-days in Phase 2/2b clinical trial stage that aims to determine the effective dose and route of injection for this vaccine. [27] NAID is also funding the Phase 1 trials of a whole-particle inactivated ZV vaccine developed by the scientists at Walter reed Army Institute of research (WRAIR) [20]. Currently, a live-attenuated ZV vaccine is under-development at the NIAID Laboratory of Infectious Diseases, Bethesda, Maryland campus of NIH. [23]

DNA vaccine.

Earliest DNA vaccine was formulated in early 1990s and by now many have been approved in the veterinary medicine discipline [36]. DNA vaccines are created by incorporating the genetic codes of the specific proteins, the antigens, in the DNA plasmid. [29] The plasmid enters into the various cells, depending on the site of injection, including keratinocytes or skin cells, myocytes or muscle cells and the local antigen presenting cells or APCs. The DNA transmitted as plasmid incorporates itself into host cell nucleus DNA and the encoded antigen genes result in the synthesis of proteins in the host cell cytoplasm. These antigens are presented on the surface of cell membrane or are excreted out in the extracellular environment (Figure 2). The APCs with antigens and MHC complexes travel to the lymph nodes, stimulate the cell-mediated and humoral immunity systems and confer the immunity against the antigen. In nutshell, the DNA vaccine is able to confer both types of immunity. [36] The vaccine plasmid is delivered by deep intramuscular injection in humans, although the future looks forward to many improved ways of delivery modes. The prospective methods may include topical patch, jet-injector, ultrasound-guided injection, transcutaneous micro needle and gene gun. DNA vaccines have the advantages of being specific and safe enough. Larocca et al., were able to show the successful immunization of susceptible mice with plasmid DNA ZV vaccine directed to encode the pre-membrane and envelope (preM-Env) proteins. The mice were injected 50 μg DNA vaccine in saline, via intramuscular route, followed by viral load challenge and successful isolation of specific IgG. Vaccine successfully conferred immunity as evidenced by absence of viremia after challenge and, the successful passive protection by isolated IgG in

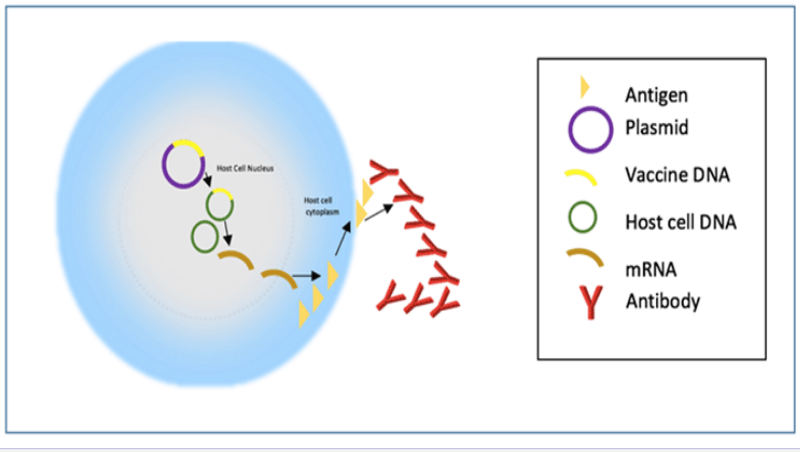

Figure 2: DNA vaccine plasmid is incorporated into the host cell genome, transcribed into mRNA that is translated in the host cell cytoplasm producing antigen proteins which are expressed on the cell surface. The neutralizing antibodies are produced against these antigens conferring the acquired immunity to the host.

modified mRNA vaccines.

mRNA vaccines consist of the modified mRNA molecules encapsulated in optimized lipid nano particles (LPN). In LPN method of delivery the mRNA molecules are surrounded by lipid bilayer that has been evidenced as safe and well-tolerated in clinical trials. [7] (Figure 3). Specifically designed mRNA molecules enter into the host cells at the site of injection as well as immune cells and translate into the antigenic proteins (Figure 4).

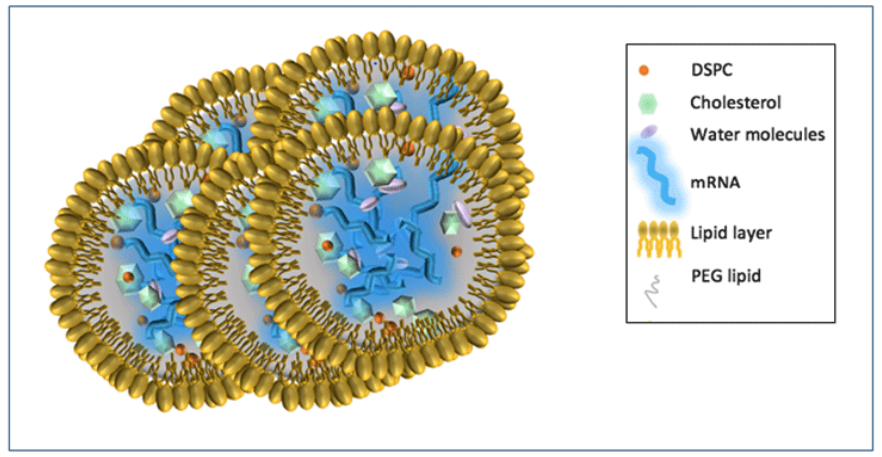

Figure 3: mRNA vaccine lipid nano particles (LPN) structure. mRNA is incorporated in the core with the water molecules, cholesterol; disteraroyl phosphate dylcholine (DSPC) surrounded by lipid layer with polyethylene glycol (PEG) molecules.

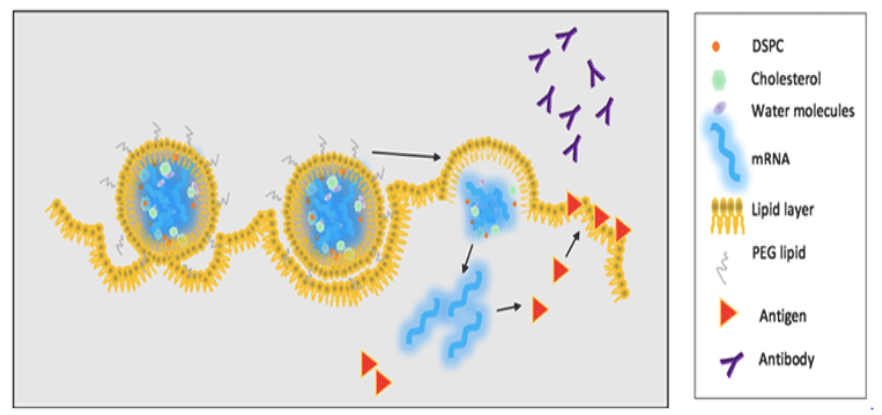

Figure 4: mRNA vaccine lipid nano particles (LPN) outer lipid shell diffuses with the host cell membrane, the core contents are released and mRNA molecules enter into cell cytoplasm where these are translated into the antigens further expressed on cell surface and generate antibodies subsequently by immune system.

Richner et al., experimented with ZV modified mRNA vaccine, encoding ZV prM-Env as an antigen, delivered in two doses. The mRNA molecules were encapsulated in optimized lipid nano particles (LPN) and delivered via intramuscular injection to mice. [28] Data were encouraging as the vaccine generated high neutralizing antibody titers. To minimize the risk of ADE, Richner et al., created another modified mRNA vaccine or mutant vaccine deficient in 'Fusion Loop' sequence in DII domain of E protein. The mutant vaccine was effective enough to generate high neutralizing antibody titers and to reduce ADE to DENV-1 in cell cultures. It also prevented the ZV dissemination in the mice brain and uterus. The brain and uterus are primary target organs to be protected by ZV infection due to its neurological and pregnancy related complication.[28]

Pardi et al., Designed another modified mRNA vaccine that also encoded pre-membrane and envelope (prM-Env) glycol proteins of ZV H/PF/2013 strain. The vaccine molecules were encapsulated in LNP and the vaccine was delivered through intra dermal injection in a single dose of 50μg to C57BL/6 mice [26]. The ZV-specific neutralizing IgG were quickly developed with an endpoint titer of 180,000 or 90μg/ml within 8-12 weeks. The immunity was confirmed by follow-up ZV intravenous challenge. This nucleoside-modified ZV prM-E mRNA-LNP vaccine was studied in rhesus macque nonhuman primates in the same study, delivered via single intradermal dose and tested with subcutaneous challenge of the virus. Subcutaneous route was selected to simulate mosquito bite. Effective neutralizing antibody titers developed and vaccine was concluded as effective, safe and single-dose prospective ZV vaccine candidate. [26] In summary, the modified mRNA vaccines are easy to design and deliver as well as cost-effective candidate group of vaccines. Moreover, the mRNA molecules do not integrate into the genome of the host cell thus, ruling out any chances of mutations that may occur in case of DNA vaccines. [26]

ZV live-attenuated virus.

Shan et al., have developed live-attenuated ZV vaccine by deletion of 10 nucleotides resulting in the mutant viral RNA genome. Vaccine delivered subcutaneously in a single dose to mice reported to stimulate both humoral and cell-mediated immune pathways, indicated by high neutralizing antibody titers as well as high interferon (IFN)- mutant-virus immune CD4+and CD8+cells response. Subsequent experiments indicated that mutant virus was 1000 fold less neurovirulent. Moreover, the attenuated 10-del ZV did not infected the experimental urban mosquitoes; therefore, the vaccinated hosts would not disseminate the virus to the vector. Both of these characteristics were indicative of a better safety profile for a prospective live-attenuated ZV vaccine in view of clinical trial safety. [31]

DENV-specific cross-reactive monoclonal Antibody.

The DENV monoclonal antibodies (mAb) that cross-react with ZV antigens may be potential candidates for ZV vaccine to confer passive immunity or as a therapeutic. ADE is a risk in DENV immunized host or in case of future infection by DENV due to these cross-reacted mAb. [21] The cross-reacted or ADE responsible antibodies are the ones directed against E protein and prM epitopes of flaviviruses. [30] 2A10G6 and SIgN-3C are two such monoclonal antibodies under investigation.

2A10G6 combines with the Flavivirus Family viruses including Dengue, West Nile and Yellow Fever viruses. The cross-reactive property of 2A10G6, a novel monoclonal antibody, is due to its binding capacity to the 'Fusion Loop' DII domain of envelope (E) protein. [9]

Kam et al., investigated DENV-specific mAb, SIgN-3C, for cross-reactivity with ZV. SIgN-3C significantly reduced viral load in Zika infected pregnant IFNAR -/ mouse model in placenta and the fetal organs with attenuation of fetal growth retardation. [33] The cross-reacted SIgN-3C antibody recognized the three epitopes on ZV outer shell, all being present on the E protein including the fusion loop of DII, a confirmation-specific epitope and a quaternary epitope .[17]

Conclusion

The target population to be vaccinated against ZV may include men and women of reproductive age, immune compromised and old age people as well as pregnant women at risk. ZV prospective vaccine is supposed to be specifically designed for reproductive age women to prevent pregnancy complications of this virus. Therefore, the conferred immunity must be effective enough, not only to neutralize the viral infection but, to protect the developing fetus from the teratological effects of ZV infection. The men of reproductive age need to be vaccinated against ZV to prevent male infertility, shedding of virus in semen and its sexual transmission. The ideal ZV vaccine is expected to generate neutralizing antibodies in the host to avoid the complication of ADE. These vaccines need to be equally effective and safe for DENV immune and non-immune individuals in view of risk of ADE. Subunit vaccines, DNA and modified mRNA, seem highly promising in view of their safety profile, low dose regimens and stability over variable transportation conditions. However, the live-attenuated vaccine must have high safety profile, before being proposed for clinical trial, to avoid the potential neurological and pregnancy complications in case of infection by re-activated virus in the live-attenuated vaccine.

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