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Review

Chikungunya virus vaccines: Current strategies and prospects for developing plant-made vaccines

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ABSTRACT

Chikungunya virus is an emerging pathogen initially found in East Africa and currently spread into the Indian Ocean Islands, many regions of South East Asia, and in the Americas. No licensed vaccines against this eminent pathogen are available and thus intensive research in this field is a priority. This review presents the current scenario on the developments of *Chikungunya virus* vaccines and identifies the use of genetic engineered plants to develop attractive vaccines. The possible avenues to develop plant-made vaccines with distinct antigenic designs and expression modalities are identified and discussed considering current trends in the field.

1. Introduction

Q4 *Chikungunya virus* (CHIKV) causes an infection typically characterized with fever, skin rash, incapacitating arthralgia, and severe synovitis [1]. This virus is transmitted by the *Aedes* mosquitoes and its name derives from the Swahili or Makonde word Kun qunwala that translates to "walk bent over", which describes the posture of infected persons experiencing severe joint pain. Chikungunya is easily confused with dengue as they share the same vectors, symptoms, and geographical distribution; but differs in the absence of headache, and retro-orbital eye pain [2]. CHIKV, first isolated in 1952 from a febrile patient during an outbreak in the Makonde Plateau in the southern province of Tanzania (formerly Tanganyika), is a prevalent pathogen in tropical and subtropical regions of Africa, the Indian Ocean Islands, and south and southeast Asia among the Makonde tribe [2].

CHIKV is an enveloped alpha virus belonging to the family *Togaviridae*, whose genome consists of a single-stranded positive-sense RNA of approximately 11.8 kb. The genome is capped at the 5′ end and polyadenylated at the 3′ end. The genomic structure of CHIKV encodes for the following: one open reading frame (5′ORF) yielding four non-structural proteins (nsP1–4) at the post-translational level which participate in genome replication, RNA capping, polyprotein cleavage, and other functions required for viral replication; and another ORF that yields three major structural proteins (Capsid, E1, and E2) and two small cleavage products (E3 and 6K) [3]. The mature virion is 70 nm in diameter and contains 240 heterodimers of E2/E1 arranged as trimeric spikes on its surface. These heterodimer spikes are inserted into the plasma membrane of infected cells after transported through the secretory pathway. Cytoplasmic nucleocapsids containing the genomic RNA and 240 copies of the capsid protein bud from the cell surface to acquire the virion envelope and envelope protein spikes. The E1 and E2 glycoproteins form heterodimers that associate as trimeric spikes on the virion surface while E3 and 6K were demonstrated to act as helper proteins in the budding and maturation process of the virion envelope [4,5].

CHIKV is believed to be originated in Africa where two genetically distinct lineages have been identified: one containing all isolates from western Africa and the second comprising all southern and East African strains, as well as isolates from Asia. West African lineages caused multiple CHIKV epidemics in East Africa,

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the Indian Ocean Islands, and many parts of South East Asia [6–11]. Before 2000 C.E., large outbreaks of CHIKV were rare but become more frequent afterwards. More recently new episodes of CHIKV have been reported in the Americas, further broadening the geographical spread of the disease, which has been associated with an emerging genetic variability [12,13] leading to hypotheses on possible mechanisms of evolutionary adaptation of the virus to the mosquito vector [14,15].

The main preventive strategy against Chikungunya is mosquito control, but this has proven to be difficult especially in poor countries; thus new strategies to fight the disease are needed. Currently the only treatments for CHIKV-disease symptoms are non-steroidal anti-inflammatory drugs. The re-emergence of CHIKV has led to the assessment of several potential treatments including ribavirin [16,17], chloroquine [18,19], and CHIKV antibodies [20–23]. In addition the peptides Latarcin (LATA) and Thanatin (THAN) as well as the protein PAP1, all of which having antiviral activity [24,25], have shown a promising potential to protect against CHIKV [26]. Among the explored strategies, vaccination is considered the ideal intervention to prevent the CHIKV infection; however no licensed vaccines for human use are available yet. Despite the development of several animal models, few of them have met the requirement to be used in pre-clinical studies to assess potential therapeutics. Recent epidemiological data showed the increasing importance of antibody-mediated protection against CHIKV [21–23], highlighting the feasibility of using anti-CHIKV antibodies as a passive immunotherapy or as a prophylactic treatment. However, information about the exact target of the adaptive immune response either in human or in animal models remains limited. In addition the cost for immunotherapies produced under conventional platforms should be considered, which is prohibitive for massive use in developing countries.

2. Immununopathogenesis of CHIKV infection and animal models

Deciphering CHIKV specific molecular features and how the virus interacts with its host are key aspects to prevent, treat, or cure the infection. However, the knowledge of human CHIKV infection immunology is limited to small animal models (mouse) [27] in which muscle and joint disease were recently achieved in C57BL/6 mice [28,29]. Although the mouse model is useful at pre-clinical level for vaccine development, CHIKV disease mice models (young or immunodeficient mice) do not fully recapitulate human disease patterns in terms of infectivity and immune responses. Therefore, Labadie et al. [30] proposed a model for CHIKV infection in adult immunocompetent cynomolgus macaques (*Macaca fascicularis*). CHIKV pathogenesis using this animal model seems to resemble the viral, clinical, and immunopathological features observed in the human disease; and, interestingly, macrophages were identified as the main cellular reservoirs during the late stages of CHIKV infection in vivo. Overall, the inflammatory response to CHIKV infection in humans clearly contributes to virus elimination since the viral load has been associated to the serum levels of proinflamatory mediators such as IFN-alpha, IFN-gamma, IL-1-RA, IL-6, MCP-1/CCL-2, IL-12, IP-10/CXCL-10, IL-18, and IL-18BP [31,32]. However it is important to point out that proinflamatory mediators are orchestrated and depend largely on the stage of the viral pathogenesis, as demonstrated in cynomolgus macaques after CHIKV experimental infection [30]. Nevertheless, the beneficial or deleterious effects of inflammation on viral persistence remain unclear even though CHIKV infection-associated markers have been described [33,34]. In general, T and B cells have been associated to the clearance of CHIKV since reduced immune responses in mice models and aged NHPs promoted long-term virus persistence [35,36].

3. Production of vaccine candidates against CHIKV

Based on the hypothesis that an efficacious CHIKV vaccine should resemble the viral infection to provide accurately immunoprotection against CHIKV disease [37], several promising vaccines have been recently evaluated. For example the immunization with virus-like particles (VLPs) in a monkey model elicited neutralizing antibodies against envelope proteins from different CHIKV strains, mediating protection against viremia when challenged with a high dose of CHIKV; moreover, the transfer of these antibodies into immunodeficient mice conferred protection against a subsequent lethal CHIKV challenge [38]. In addition, purifying human anti-CHIKV antibodies from patients in the convalescent phase exhibit a high in vitro CHIKV neutralizing activity and a powerful prophylactic and therapeutic efficacy against CHIKV infection in a mouse model, which correlates with the fact that infected individuals are in general protected against reinfections [20]. Therefore, the protection against CHIKV disease is considered to be primarily mediated by humoral responses. This knowledge have supported the development of immunization approaches resembling the natural infection process as close as possible through the use attenuated CHIKV strains or VLPs, which mimic the infection mechanism and induce antibody-mediated. Among the technologies that have been explored for the development of CHIKV vaccines stand-out: formalin-inactivated viral vaccines [39,40], live-attenuated viruses [41–43], alpha virus chimeras [44–46], consensus-based DNA vaccines [47–50], and recently virus-like particle (VLP) vaccines and recombinant subunit vaccines. A detailed scenario on protein subunit vaccines development is provided below.

CHIKV structural proteins form enveloped VLPs (eVLPs) when expressed alone in eukaryotic expression systems [51,52]. The first CHIKV eVLP-based vaccine candidate was reported in 2010 by researchers of the NIH and has become the most promising VLP-based vaccine against CHIKV. DNA transfection of a plasmid comprising the full-length CHIKV structural coding region C-E3-E2-6K-E1 into human HEK293 cells successfully resulted in CHIKV VLPs assembly. The viral glycoproteins in the VLPs are organized in 240 E1–E2 heterodimers, which form 80 spikes on the VLP surface, resembling replication-competent alpha viruses. These eVLPs were isolated from the supernatant of transfected mammalian cells, purified, and used to immunize mice and nonhuman primates. Vaccination of rhesus macaques with 3 doses consisting of 20 μg of eVLPs at 0, 4, and 24 weeks induced an antibody response that was sufficient to confer protection upon a high-dose CHIKV challenge 15 weeks after the last boosting [53]. These results demonstrated that immunization with these VLPs elicited neutralizing antibodies directed against envelope proteins and protected NHPs against a subsequent lethal CHIKV challenge, indicating a humoral-mediated mechanism of protection. The next developmental step for this vaccine consisted on performing a Phase I dose-escalation clinical trial under a 3-dose vaccination scheme (weeks 0, 4, and 20) of up to 40 μg of eVLPs per administration. This vaccine was safe, well tolerated and immunogenic [38]. Another VLPs-based promising vaccine development has consisted on a measles vaccine expressing CHIKV VLPs. A single immunization with this vaccine fully protected mice from a lethal CHIKV challenge [54]. This vaccine induced high titers of neutralizing CHIKV antibodies although specific cellular immune responses were also elicited.

CHIKV eVLPs have also been expressed in insect cells. Research performed since 2011 demonstrated that the expression of the structural coding regions C-E3-E2-6K-E1 in Sf21 insect cells led to the assembly of VLPs [55–57]. Interestingly, these eVLPs displayed

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