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Chikungunya, is a mosquito borne viral disease. The virus (CHIKV), an alphavirus of the family Togaviridae was first isolated during the 1952-53 epidemics in Tanzania, Africa. The virus is transmitted by mosquitoes (genus Aedes). CHIKV is a positive sense, single stranded RNA virus of approximately 12 kb genome and has been classified in 3 genotypes (names). Importantly, a single serotype exists.

The 21st century witnessed resurgence of Chikungunya. Several Indian states experienced massive outbreaks of Chikungunya since October 2005 and are continued till date. Mortality among patients with underlying medical condition and a substantial proportion exhibiting continued arthralgia for several years were/are noteworthy. Considering the widespread infections and associated sequelae/mortality, the need for an effective vaccine was obvious. With the availability of newer platforms for vaccine development, different approaches have been tried. So far, none of the vaccines have been licensed.

We attempted to develop (1) recombinant E2 protein-based and (2) Whole virus-inactivated vaccines.

CHIKV (ECSA genotype) isolated from a human serum sample collected during the 2006 CHIKV outbreak in Andhra Pradesh, India was used for the preparation and assessment of the vaccines. The Asian strain originally isolated from Calcutta during the 1963 outbreak, passaged 12 times in infant mice and once in Vero E6 cell line was used as the heterologous strain for neutralization test.

Viral RNA was extracted, converted to cDNA and PCR amplified using gene specific primers (E1 and E2 genes). The PCR product was purified, digested by restriction enzymes and cloned in pET15b vector. The clones were confirmed by restriction digestion and sequencing. Only rE2 protein (rE2p) could be expressed in BL-21(DE3) RIL Codon plus competent cells and was used for all further experiments. This protein specifically reacted with sera from chikungunya patients.

Following infection in Vero cells, CHIKV was harvested and purified by ultracentrifugation at 35,000 rpm at 4°C through 30% glycerol cushion. Virus
titre was determined by 50% tissue culture infectious dose (TCID$_{50}$) in Vero cells. Virus was inactivated by 0.1% formalin and BPL (1:3500). Immunoreactivity of inactivated virus was checked by western blot. Complete inactivation of the virus was achieved by 12 hr and 4 hr treatment with formalin and BPL at 4°C respectively as confirmed by absence of CPE in Vero cells and loss of virulence in infant mice.

Six to eight weeks old female BALB/c mice (6 mice per group) were immunized intramuscularly with two doses of 10µg, 20µg and 50µg of different vaccine formulations with and without adjuvants, 2 weeks apart; rE2p (alum/ MW/ CadB adjuvants), formalin inactivated CHIKV (alum/ MW adjuvants) and BPL-inactivated CHIKV (alum).

For the respective detection, titration and isotyping of anti-CHIKV antibodies in the mice groups receiving chemically inactivated and rE2p based vaccines, whole CHIKV-based and recombinant protein based ELISAs were done. For the detection and quantitation of neutralizing antibodies (nAb), CPE-inhibition-based neutralization assay was carried out. Cytokine analysis from stimulated splenocytes culture supernatants of vaccinated mice were done by Cytokine Bead Array (CBA).

100% sero-conversion (ELISA-based) was observed two weeks after 1st dose for all the formulations. Percent nAb seroconversion was dose-dependent and 100% seroconversion after 1st dose was noted only with 50µg alum adjuvanted killed vaccines and BPL-inactivated virus without any adjuvant. CadB+rE2p formulation led to highest (83.3%) seroconversion for the recombinant vaccine. Two weeks post-second dose, 100% seroconversion was recorded for all the adjuvanted formulations, irrespective of the dose.

The antibody titres were immunogen concentration dependent, 50 µg yielding maximum titres and were boosted by the 2nd dose. The peak antibody titres were observed 2 weeks after the 2nd dose in each mice group. When ELISA titres were compared, irrespective of the type of the immunogen, highly significant antibody-enhancing effect was evident for all the adjuvants evaluated. No significant difference (P=0.07 - 0.35) was noted when antibody titres for all the 3 rE2p-adjuvants were compared. Formalin-inactivated, alum-adjuvanted vaccine yielded higher IgG-anti CHIKV titres
when compared to the MW-adjuvanted product \( (p=0.0013) \). With BPL-inactivated virus, only alum was used. Though BPL-inactivated virus resulted in higher anti-CHIK titres when compared to the formalin-inactivated \( (p=0.003) \), alum adjuvanted preparations did not differ in the induction of antibody titres in mice \( (p=0.273) \). The antibody titres in mice immunized with rE2p were comparable when rE2p or CHIKV were used as coating antigens. Overall, alum was a superior adjuvant for rE2p and whole virus-based vaccines employing both inactivating agents.

Purified rE2p adjuvanted with alum and CadB skewed host immune response towards Th2 type whereas same immunogen adjuvanted with MW modulated it towards Th1 type. In contrast, formalin inactivated CHIKV adjuvanted with alum gave a balanced immune response while MW adjuvant led to Th2 type. In BPL inactivated CHIKV receiving mice groups, addition of alum did not alter the type of immune response generated \( i.e. \) it remained Th2 type.

rE2p+CadB and BPL inactivated CHIKV+alum immunized mice groups induced higher (~13.6 and 18.2 fold respectively) levels of IFNG (Th1 cytokine). For other formulations, balanced cytokine response was noted. For the recombinant protein, cytokine response was adjuvant-driven.

As far as the neutralizing antibodies were concerned, though the pattern of antibody response was similar to ELISA (immunogen concentration dependent seroconversion rates and titres), overall titres were low. With alum, highest reciprocal titres were 320 (rE2p), 1280 (formalin-inactivated virus) and 2560 (BPL-inactivated virus), at 2 weeks after the second dose. MW-adjuvanted, formalin-inactivated vaccine induced 2-fold lower titres. Overall, BPL-inactivated vaccine led to highest ELISA as well as nAb titres.

The antibodies generated by all the formulations could neutralize the Asian genotype showing 10 amino acid substitutions in the E2 region of the African genotype virus used for vaccine preparation. Again, the titres were immunogen concentration dependent. Overall, the titres were ~ 4fold lower with the Asian genotype.

Protection was judged by the reduction in viral replication as measured by quantitative real time PCR in muscle, spleen, brain and serum of the immunized mice, challenged with the homologous virus. Complete protection
was offered by the alum-adjuvanted rE2p and both the inactivated vaccines as no virus was detected in the tissues and blood. The PBS-immunized, virus challenged mice exhibited high viral load in muscles, the target organ as well as brain, spleen and blood. Thus, these vaccine candidates generated sterilizing immunity inhibiting CHIKV replication. On the 2nd day post-challenge, very low replication levels were noted in the mice immunized with CadB-adjuvanted-rE2p; no virus was detected on the 4th day.

To assess the longevity of protective antibodies, groups of immunized mice were challenged 20 weeks after the second dose. At 20 weeks, a 3-8 fold decline in nAb titres was noted. Mice immunized with rE2p alone exhibited ~12-fold lower titre. Again, both the inactivated vaccines and alum-adjuvanted rE2p formulation yielded sterilizing immunity. In mice immunized with CadB+rE2p, no virus was detected in the tissues. However, viremia was recorded on the 2nd day post-challenge (100 fold reduction in viral RNA titre). Incidentally, reciprocal nAb titre in this group was the lowest (40).

To conclude, three vaccine candidates were successfully developed. Depending on the facilities available with the vaccine manufacturers in the developing countries and economics, any of these alternatives can be chosen and evaluated further.