A new replication-defective vaccinia-derived vaccine platform system co-expressing chikungunya and Zika structural genes is effective in preclinical studies

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Background

- Vaccinia virus (VACV) vaccination successfully eradicated smallpox and recombinant VACV-based vaccine vectors have been developed to target a number of diseases.
- The advantages of the VACV system include a large transgene payload capacity, minimal risk of host genome integration, induction of robust and long-lived cell-mediated and humoral immune responses and an established manufacturing process with cold chain-independent distribution capacity.
- Replication-competent VACV is not considered safe by contemporary standards
- Passage-attenuated modified vaccinia (MVA) has addressed many of the safety concerns, however MVA has some outstanding issues including evidence of viral replication in some human cell lines and dependent on chicken embryo fibroblasts for vaccine productions.
- Chikungunya virus is a mosquito-transmitted alphavirus responsible for millions of cases of arthritic disease in humans
- Zika virus is an emerging flavivirus transmitted by mosquito and non-vector borne modes of transmission and responsible for congenital Zika syndrome in humans

Aims

1. Develop a replication-defective vaccinia derived vaccine platform technology (SCV)
2. Develop a SCV cell substrate cell line that can be used for vaccine production
3. Evaluate the efficacy of SCV vaccines in preclinical mouse models of chikungunya and Zika virus

Design rationale for the SCV vaccine technology

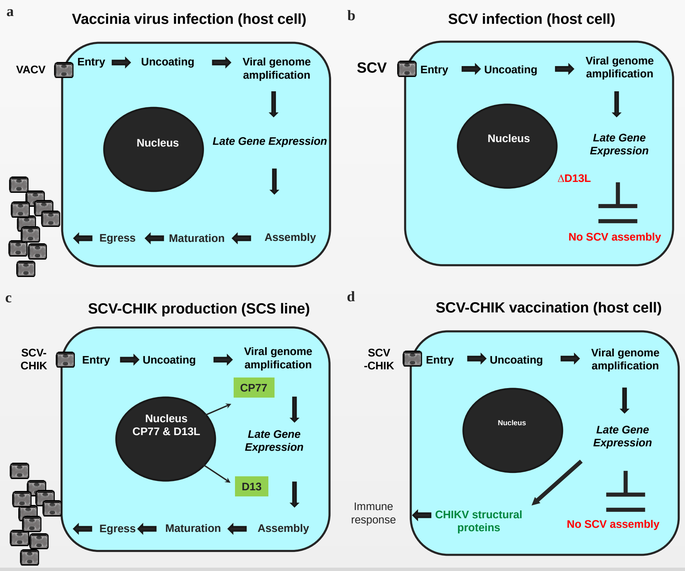

Figure 1: Rationale for the SCV vaccine platform technology. (a) VACV multiplication in the cytoplasm of host cells is able to produce infectious viral progeny as VACV encodes the essential assembly protein, D13. (b) Targeted deletion of D13L in the SCV vector prevents virion assembly, rendering SCV multiplication defective. (c) In trans provision of D13 in the CHO-based SCS line rescues viral assembly allowing production of progeny of vaccine manufacture. CP77 allows SCV (or VACV) multiplication in CHO cells. (d) After delivery of SCV-CHIK to a vaccine recipient, the genome of SCV-CHIK is amplified and late gene expression drives production of the CHIK structural proteins (vaccine antigen). In the absence of D13 protein no SCV-CHIK viral progeny are generated.

Construction of the SCV-CHIK vaccine

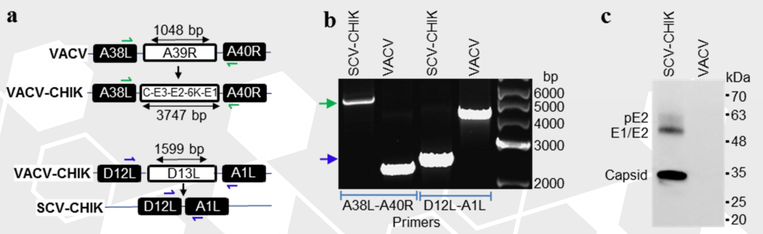

Figure 2: Construction and in vitro characterisation of the SCV-CHIK vaccine. (a) Schematic representation of SCV-CHIK vaccine construction. The CHIKV structural polyprotein cassette (C-E3-E2-6K-E1) was inserted into the A39R locus of VACV to generate VACV-CHIK. The D13L gene was deleted from VACV-CHIK to generate SCV-CHIK. (b) Viral DNA from parental VACV and SCV-CHIK was subjected to PCR analysis using site-specific primers shown in (a) to confirm insertion of (C-E3-E2-6K-E1) (green arrow) and deletion of D13L (blue arrow). (c) Immunoblot analysis of VACV and SCV-CHIK infected SCS cells using mouse polyclonal anti-CHIKV antisera confirming expression of processed CHIK antigens; pE2 (E3-E2), E1 and E2 and capsid.

Production and morphology of SCV-CHIK in SCS cells

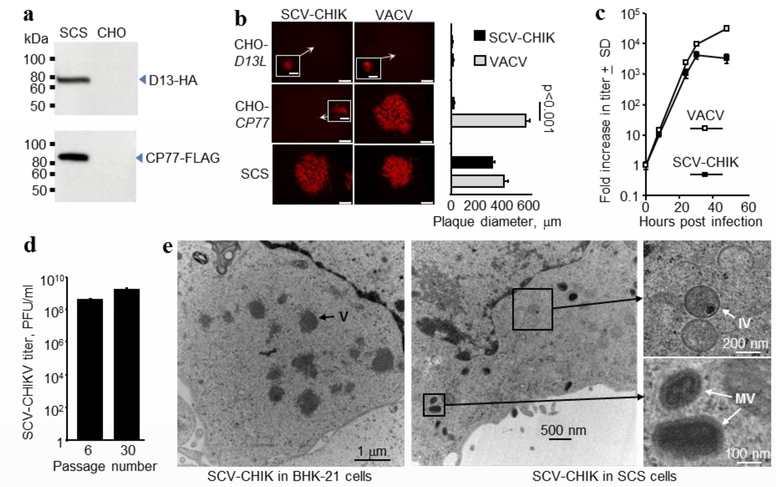

Figure 3: (a) Immunoblot analysis confirmation of the HA-tagged D13 (assembly protein; 56kDa) and FLAG-tagged CP77 (host range protein; 77kDA) in the SCS line using anti-HA and anti-FLAG antibodies. (b) Monolayers of CHO-D13L, CHO-CP77 and the SCS line were infected with the VACV or SCV-CHIK (MOI 0.01 pfu/cell). After 48hrs, cells were fixed and viral plaques were examined by immunofluorescent staining using polyclonal anti-VACV antibody. Representative plaque images (scale bars 200μm) and single infected cells (inserts; scale bar 10μm) are shown. Bar charts show mean plaque size (μm ± SD; n=16-25) for the two viruses. (c) SCS monolayers were infected with SCV-CHIK and VACV (MOI 0.01 pfu/cell) and at the indicated times virus titres were determined by plaque assay. Data presented as fold increase in viral titre over the original inoculum (pfu ± SD; n=4). (d) SCS cells, passaged 6 or 30 times, were infected with SCV-CHIK (MOI 0.01 pfu/cell) and after 48 hrs titres were determined (n=4). (e) Electron microscopy examination of virus morphology in SCV-non permissive BHK-21 and SCV-permissive SCS cells infected with SCV-CHIK (MOI 2pfu/cell). SCV-CHIK production was blocked at the viroplasma stage in BHK-21 cells, whereas virion assembly and maturation was observed in the SCS line. V-viroplasma; IV immature virions; MV-mature virions.

SCV-CHIK does not replicate in human cell lines

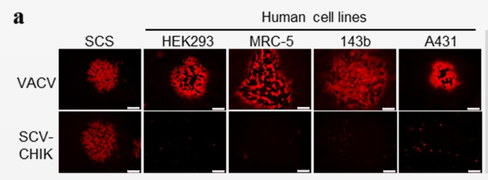

Figure 4: Monolayers of the indicated cell lines were infected with VACV or SCV-CHIK (MOI 0.01 pfu/cell) and fixed after 48hrs and cells examined by immunofluorescent staining using polyclonal anti-VACV antibody.

SCV-CHIK protects against CHIKV challenge

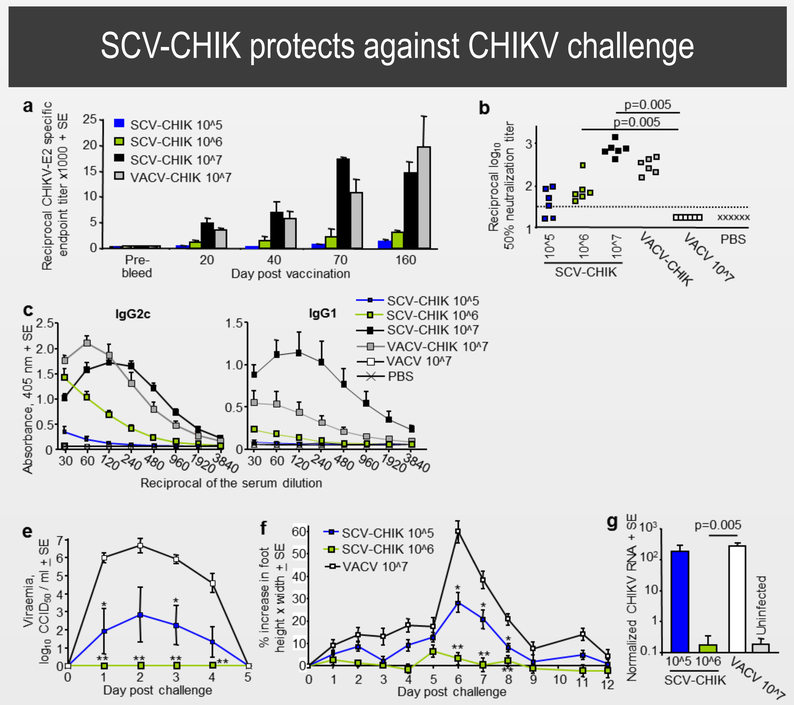

Figure 5: (a) Groups of 6-8 week old female C57Bl/6 mice (n=5 per group) were vaccinated with the indicated doses of SCV-CHIK or VACV-CHIK. At the indicated times serum anti-CHIKV E2-specific IgG endpoint titres were determined. (b) Day 30 post-vaccination CHIKV neutralising antibody responses were assessed. Dotted line indicate limit of detection (1 in 30 serum dilution). (c) Day 30 post-vaccination anti CHIKV IgG2c and IgG1 antibody levels were measured. (d) At the indicated times post-vaccination anti-CHIKV IgG2c levels in mice vaccinated with SCV-CHIK or VACV-CHIK (both 107 pfu) were examined by ELISA. (e) Vaccinated mice (SCV-CHIK at 105 and 106 pfu, and VACV at 107 pfu were challenged with CHIKV (Reunion isolate) and viraemia measured over time. Statistics by Kolmogorov-Smirnov compared with VACV 107 pfu/group. (f) foot swelling after CHIKV challenge of mice in (e). (g) Persistence of CHIKV RNA in the feet of vaccinated mice after challenge (described in (e) was quantified by qRT-PCR (normalised against RPL13A) 30 days post-challenge.

Development of a preclinical model of congenital Zika syndrome

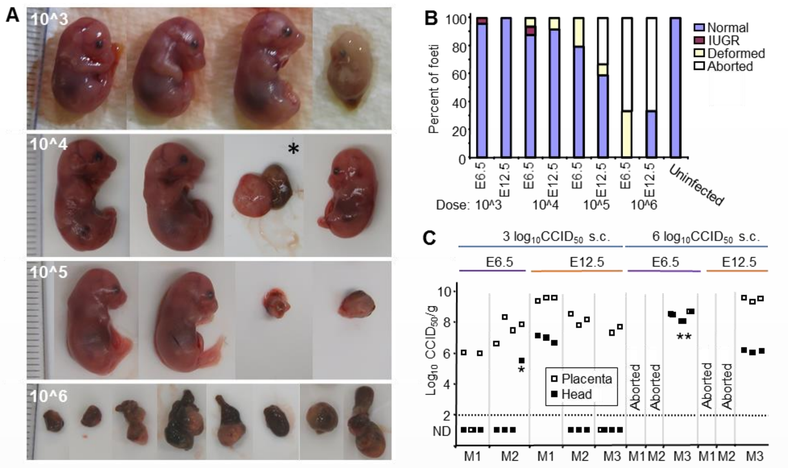

*Figure 6: Pregnancy outcomes of ZIKANatal-infected IFNAR-/- dams. (A) Examples of E17.5/E18.5 fetus from IFNAR-/- X IFNAR-/- mating after sc infection of dams (>8 weeks of age) at E6.5 (or E12.5 [*]) with the ZikaNatal doses indicated. Fetuses with clear signs of IUGR are shown facing left. * severely deformed fetus and placenta. Severely deformed fetal/placental masses are also shown in the right two images in row 105 and all the images in row 106. (B) The percentages of foetuses that appeared normal, showed IUGR and were severely deformed are shown. Data are from 3 pregnancies per group (mean of 7.78 ± 1.55 SD foetuses per pregnancy. White bars indicate that one (33%) or two (66%) of the dams aborted. (C) Placenta and foetal head virus titres after ZIKVNatal infection of dams at E6.5 or E12.5. *fetus with IUGR shown at the top right of panel A; **, severely deformed fetal/placental masses. ND, not detected.*

SCV-CHIK/ZIKA induces antibodies post-vaccination

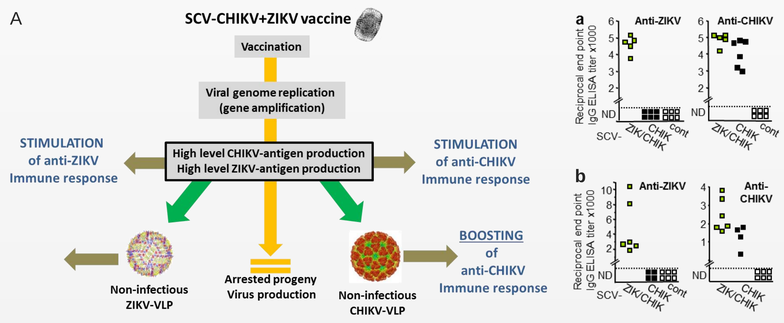

Figure 7: SCV-CHIK/ZIKA efficacy. (A) Schematic of how a single vectored SCV-CHIK and ZIKA vaccine works (B) CHIKV and ZIKV responses in C57BL/6 mice (a) End point IgG ELISA titers against ZIKV and CHIKV post vaccination with the indicated SCV vaccine. Limit of detection 1 in 30 dilution; ND – not detected. n=6, except SCV-CHIK n=4, mice per group. SCV-ZIK/CHIK vaccinated mice had higher titers than SCV-control vaccinated mice, both p=0.005, Kolmogorov-Smirnov tests. (b) ZIKV and CHIK responses in IFNAR-/- mice. End point IgG ELISA titers against ZIKV and CHIKV post vaccination with the indicated SCV vaccine. Limit of detection 1 in 30 dilution; ND – not detected. n=5/6 mice per group. SCV-ZIK/CHIK vaccinated mice had higher titers than SCV-control vaccinated mice, both p=0.009, Kolmogorov-Smirnov tests.

Conclusion and future works

- Innovative vaccinia virus-derived vaccine platform (SCV) was developed
- Multiplication deficient by targeted deletion of the essential viral assembly gene, D13L
- SCV cell substrate line developed for SCV vaccine production in CHO cells
- Benefit - scale up capacity for manufacturing
- Single shot vaccination of SCV-CHIK provided protection against CHIKV challenge, preventing both viraemia and arthritis
- A single vectored SCV-CHIK/ZIKA vaccine candidate is currently being evaluated in preclinical mouse models of disease, including models of Zika congential syndrome and testis infection

References:

1. Eldi P, Cooper TH, Liu L, **Prow NA**, Diener KR, Howley PM, Suhrbier A, Hayball JD. Production of a chikungunya vaccine using a CHO cell and attenuated viral-based platform technology. *Mol Therapy. Accepted 18th June, 2017*
2. Setoh YX*, **Prow NA***, Peng N, Hugo LE, Devine G, Hazlewood JE, Suhrbier A, Khromykh AA. De Novo generation and characterization of new Zika virus isolate using sequence data from a microcephaly case. mSphere 2(3). pii: e00190-17
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