YMTHE, Volume *25*

Supplemental Information

Production of a Chikungunya Vaccine Using a CHO Cell and Attenuated Viral-Based Platform Technology

Preethi Eldi, Tamara H. Cooper, Liang Liu, Natalie A. Prow, Kerrilyn R. Diener, Paul M. Howley, Andreas Suhrbier, and John D. Hayball

Supplemental Figures

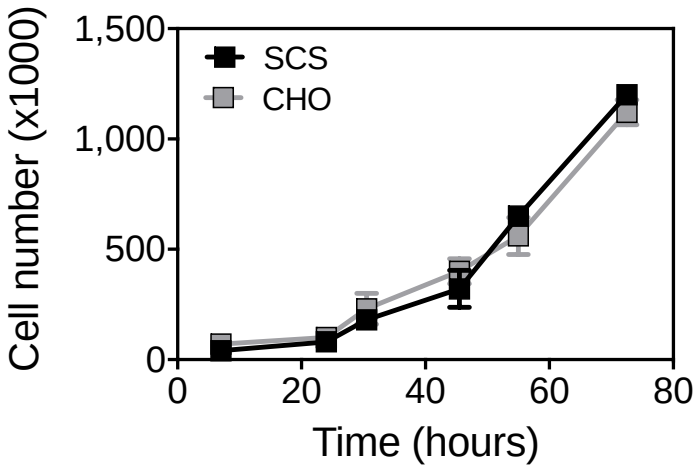

Figure S1: Comparison of growth kinetics between parental CHO cells and the SCS. Cells were plated at a seeding density of 2 x10^4 cells per well in a 6 well plate and at time-points indicated, cells were harvested and cell counts determined. No differences in the growth rate could be detected between the two cell lines. Data expressed as mean ± SEM and is representative of two independent experiments.

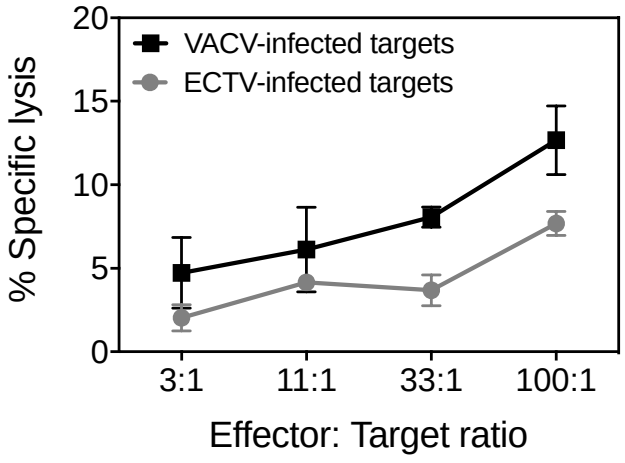

Figure S2: Induction of virus-specific cytolytic CD8 T cell effector population post-vaccination. BALB/c mice vaccinated with SCV-CHIK (10^7 PFU) were sacrificed 8 days post-vaccination and virus-specific (VACV and ECTV cross-reactive) splenic cytolytic activity was determined *ex-vivo* by ^{51}Cr-release assay. Data represented as mean percent specific lysis ± SEM for indicated effector (splenocyte): target (radiolabelled, virus infected P815) cell ratio.

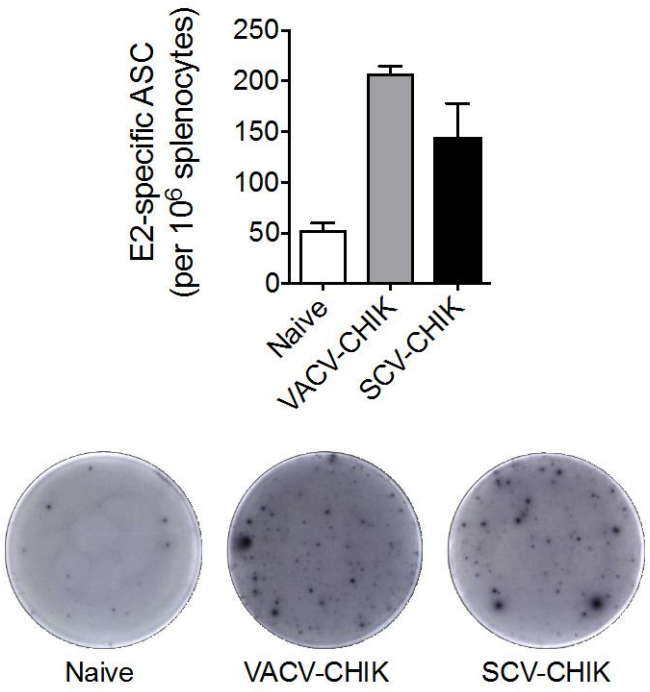

Figure S3: Analysis of vaccine-induced CHIKV-E2 specific IgG producing antibody secreting cells 1 year post-vaccination. Groups of C57BL/6 mice (n= 3 mice per group) were vaccinated with VACV-CHIK or SCV-CHIK at 10^7 PFU and the number of splenic CHIKV-E2 specific ASC were enumerated by ex-vivo ELISPOT assay one year post-vaccination. Data represented as mean ± SEM of E2-specific IgG producing ASC per million cells. A representative well image from each group is included.

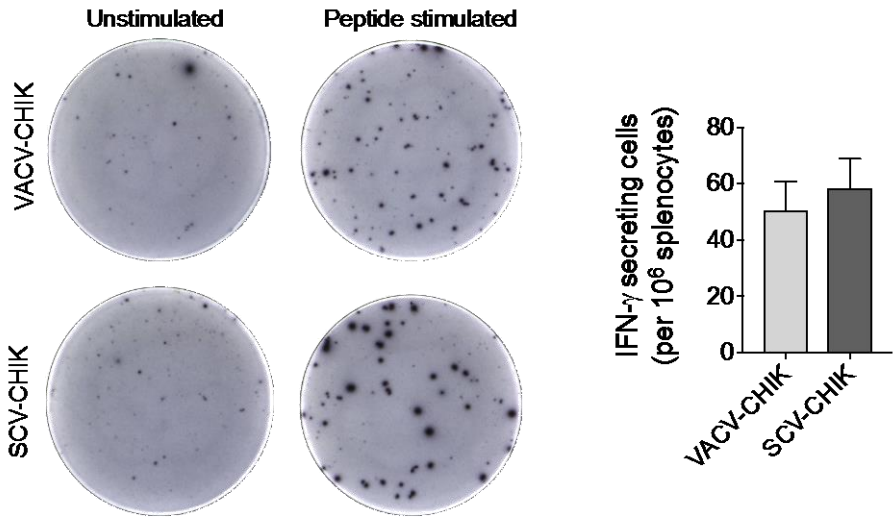

Figure S4: Analysis of vaccine-induced IFN-γ secreting cells by ELISPOT. Groups of C57BL/6 mice (n= 3 mice per group) were vaccinated with VACV-CHIK or SCV-CHIK at 10^7 PFU and the number of CHIKV-Capsid/ E2 and E1-specific IFN-γ secreting cells were enumerated by *ex-vivo* IFN-γ ELISPOT assay one year post-vaccination. Data represented as mean ± SEM of IFN-γ secreting cells per million cells. A representative well image from each group is included.

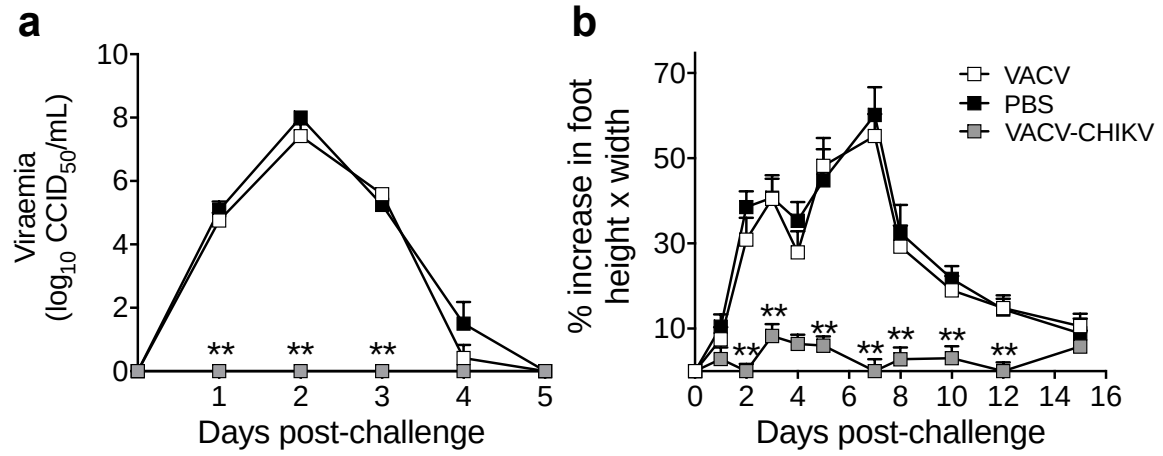

Figure S5: Replication competent VACV-CHIK provides protection against CHIKV challenge. Groups of 6-8 week old female C57BL/6 mice (n=6 mice per group) vaccinated with VACV (10^7 PFU), VACV-CHIK (10^7 PFU) or mock-vaccinated with PBS vehicle were challenged s.c. with CHIKV (10^4 $CCID_{50}$) into the ventral side of both hind feet. **(a)** Mice were bled at time-points indicated and viral titers determined by serial dilution of serum on C6/36 cells and expressed as log_{10} $CCID_{50}$ per mL. Data expressed as mean ± SEM and statistical analysis done using Kolmogorov-Smirnov test; **p=0.005. **(b)** Post-challenge, the height and width of the perimetatarsal area of the hind feet was monitored using Kincrome digital vernier calipers, Data expressed as mean ± SEM and statistical analysis performed using Mann-Whitney U-test; ** p≤0.002.

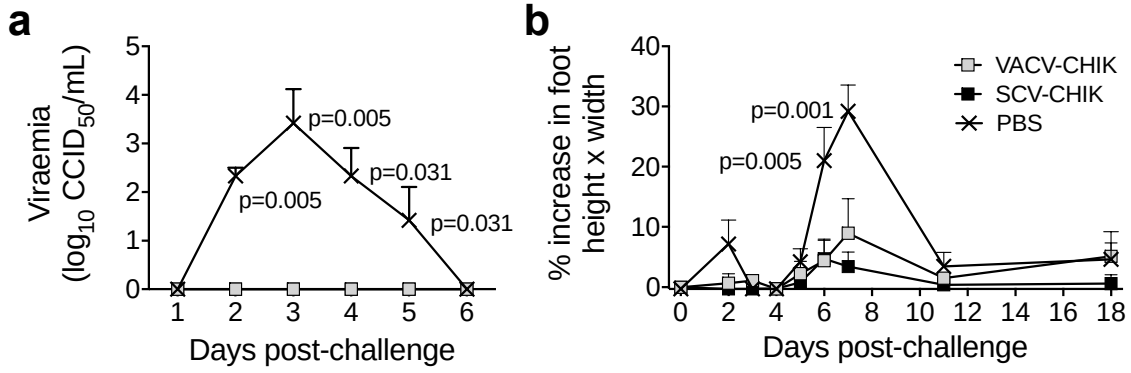

Figure S6: Long-term protection against CHIKV challenge. Groups of 6-8 week old female C57BL/6 mice (n=6 mice per group) vaccinated with SCV-CHIK (10^7 PFU), VACV-CHIK (10^7 PFU) or mock-vaccinated with PBS vehicle were challenged s.c. with CHIKV (10^4 $CCID_{50}$) 1 year post-vaccination. **(a)** Mice were bled at time-points indicated and viral titers determined by serial dilution of serum on C6/36 cells and expressed as log_{10} $CCID_{50}$ per mL. Data expressed as mean ± SEM and statistical analysis done using Kolmogorov-Smirnov test. **(b)** Post-challenge, the height and width of the perimetatarsal area of the hind feet was monitored using Kincrome digital vernier calipers. Data expressed as mean ± SEM and statistical analysis performed using Mann-Whitney U test.

Supplemental Tables

Table S1: Viral load in VACV and SCV-CHIK infected SCID mice.

	Ovary		Spleen		Liver		Lungs		Heart	
	VACV	SCV-CHIK	VACV	SCV-CHIK	VACV	SCV-CHIK	VACV	SCV-CHIK	VACV	SCV-CHIK
Day 1	++	-	+	-	++	-	+	-	+	-
Day 2	++++	-	+++	-	++	-	+	-	+	-
Day 3	++++	-	++	-	++	-	++	-	+	-
Day 5	++++	-	+++	-	++	-	++	-	++	-
Day 10	++++	-	+++	-	++	-	+++	-	++	-
Day 14	++++	-	+	-	+	-	+++	-	+	-

Groups of 6-8 week old female SCID mice (n=3 mice per group) infected with 10^7 PFU of VACV or SCV-CHIK as indicated in Fig. 4 were humanely killed at time-points indicated and organs harvested. Viral load in the organs was determined by viral plaque assay and presented as mean PFU/organ: 1-10^2 PFU (+); 10^2-10^4 PFU (++); 10^4-10^6 PFU (+++); > 10^6 PFU (++++); no plaques detected (-).

Table S2: Viral load in SCV-CHIK and VACV-CHIK vaccinated mice following lethal ECTV challenge.

	Liver	Spleen	Lungs	Lymph node	Ovary
Mock-vaccinated (PBS)	+++	+++	++	++	++
VACV-CHIK (10^7 PFU)	-	-	-	-	-
SCV-CHIK (10^5 PFU)	-	-	-	-	-
SCV-CHIK (10^6 PFU)	-	-	-	-	-
SCV-CHIK (10^7 PFU)	-	-	-	-	-

Groups of 6-8 weeks old ECTV-susceptible BALB/c mice (n=5 mice per group) were vaccinated with VACV-CHIK (10^7 PFU), SCV-CHIK (10^5, 10^6, 10^7 PFU) or mock-vaccinated with PBS vehicle as indicated in Fig. 5. Four weeks post-vaccination, mice were challenged with a lethal dose (50 LD_{50}) of ECTV subcutaneously and monitored for 14 days. Viral load was determined by plaque assay from organs collected either at euthanasia (PBS mock-vaccinated group) or at the end of the 14-day monitoring period. Data presented as mean PFU/organ: 10^2-10^4 PFU (++); 10^4-10^6 PFU (+++); no plaques detected (-).

Supplemental Methods

E2-specific ASC ELISPOT

ELISPOT plates (MSIPS4510; Millipore) pre-wetted with 35% ethanol for ≤ 1min were washed with sterile water and coated overnight at 4°C with 1μg per well of E2 in PBS. The plates were washed with PBS and blocked with RPMI-1640 supplemented with 10% FBS and 50μM 2-mercaptoethanol for 1 hr at 37 °C. Two-fold serial dilution of cells were added to the plates and incubated for 24 hrs at 37 C, 5% CO_2. Subsequently, plates were washed with PBS-T and incubated with biotinylated anti-IgG detection Ab (1μg/ml in PBS-T; Mabtech) for 2h at RT. Following washes, the plates were incubated with Streptavidin-Alkaline phosphate (1:1000; Mabtech) for 1.5 hrs at RT. E2-specific ASCs were visualised using BCIP/NBT-plus substrate (100μl per well; Mabtech). Spots were counted using an AID ELISPOT classic reader (Autoimmun Diagnostika).

IFN-γ ELISPOT

ELISPOT plates (MSIPS4510; Millipore) pre-wetted with 35% ethanol for ≤ 1min were washed with sterile water and coated overnight at 4°C with 10μg per well of anti- IFN-γ Ab (clone AN-18) in PBS. The plates were washed with PBS and blocked with RPMI-1640 supplemented with 10% FBS and 50μM 2-mercaptoethanol for 1 hr at 37°C. Two-fold serial dilution of cells were added to the plates and stimulated with 10μM of H2-Kb-restricted CHIKV E1-specific (HSMTNAVTI), E2-specific (IILYYYELY) and capsid-specific (ACLVGDKVM) peptides for 20 hrs at 37°C, 5% CO2. Subsequently, plates were washed with PBS-T and incubated with biotinylated anti-mouse IFN-γ Ab (clone R4-6A2; 1μg/ml in PBS-T; Mabtech) for 2h at RT. Following washes, the plates were incubated with Streptavidin-Alkaline phosphate (1:1000; Mabtech) for 1.5 hrs at RT. IFN-γ secreting cells were visualised using BCIP/NBT-plus substrate (100μl per well; Mabtech). Spots were counted using an AID ELISPOT classic reader (Autoimmun Diagnostika).

Cytotoxic T-lymphocyte (CTL) assay

Ex vivo VACV and ECTV specific CTL responses were measured in SCV-CHIK vaccinated mice using ^{51}Chromium-labelled virus infected P815 target cells as described elsewhere[1]. The percent specific lysis was determined using the equation: [(Sample ^{51}Cr release − Spontaneous ^{51}Cr release)/(Maximum ^{51}Cr release − Spontaneous ^{51}Cr release)] ×100. Percent specific lysis of uninfected target cells was subtracted from infected target cells to calculate the virus specific CTL activity.

Supplemental References

1. Shen, X, Wong, SB, Buck, CB, Zhang, J, and Siliciano, RF (2002). Direct priming and cross-priming contribute differentially to the induction of CD8+ CTL following exposure to vaccinia virus via different routes. *J Immunol* **169**: 4222-4229