A Vaccine Based on a Modified Vaccinia Virus Ankara Vector Expressing Zika Virus Structural Proteins Controls Zika Virus Replication in Mice

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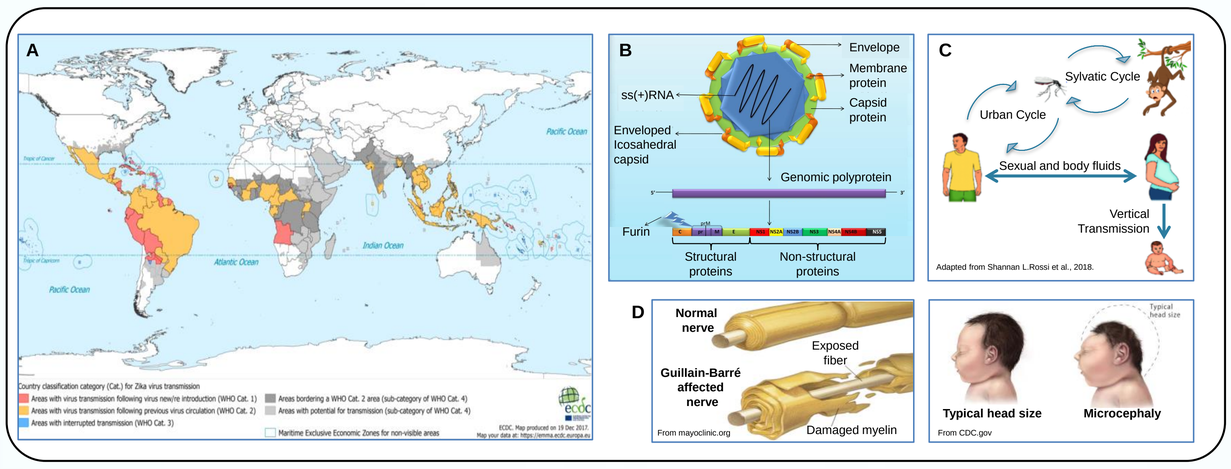
Zika virus (ZIKV) is a re-emerging mosquito-borne flavivirus that affects humans and can cause severe neurological complications, including Guillain-Barré syndrome and microcephaly. Since 2007 there have been three large outbreaks; the last and larger spread in the Americas in 2015. Actually, ZIKV is circulating in the Americas, Southeast Asia, and the Pacific Islands, and represents a potential pandemic threat. Given the rapid ZIKV dissemination and the severe neurological and teratogenic sequelae associated with ZIKV infection, the development of a safe and efficacious vaccine is critical. In this study, we have developed and characterized the immunogenicity and efficacy of a novel ZIKV vaccine based on the highly attenuated poxvirus vector modified vaccinia virus Ankara (MVA) expressing the ZIKV prM and E structural genes (termed MVA-ZIKV). MVA-ZIKV expressed efficiently the ZIKV structural proteins, assembling virus-like particles (VLPs), and was genetically stable upon nine passages in cell culture. Immunization of mice with MVA-ZIKV elicited antibodies that were able to neutralize ZIKV and induced potent and polyfunctional ZIKV-specific $CD8^+$ T cell responses that were mainly of an effector memory phenotype. Interestingly, a single dose of MVA-ZIKV reduced significantly the viremia in susceptible immunocompromised mice challenged with live ZIKV. These findings support the use of MVA-ZIKV as a potential vaccine against ZIKV.

INTRODUCTION

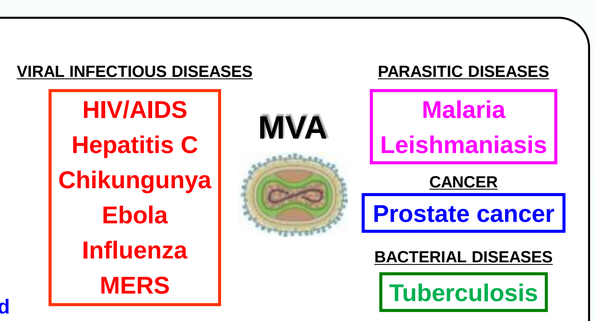


(A) ZIKV was discovered in Uganda in 1947, but was confined for the first 60 years to an equatorial zone across Africa and Asia. However, in 2007 a ZIKV outbreak emerged in Yap Island, in the Western Pacific Ocean, and between 2013 to 2014 a second larger outbreak spread eastward to French Polynesia and other Pacific Islands that finally reached Latin America in 2015, and disseminated further to North America in 2016; as a consequence, the World Health Organization (WHO) declared the Public Health Emergency of International Concern in February 2016. Actually, ZIKV is circulating in the Americas, Southeast Asia, and the Pacific Islands, and represents a potential pandemic threat.
(B) Zika virus (ZIKV) is a mosquito-borne virus from the family *Flaviviridae* and the genus *Flavivirus*. The viral particle has 50 nm in diameter and contains an inner nucleocapsid composed of a linear plus-strand genomic RNA and multiple copies of the viral capsid (C) protein and an outer host cell-derived lipid bilayer bearing 180 copies each of two proteins: the viral membrane [(M), a cleavage product of the premembrane (prM)] protein and the envelope (E) protein.
(C) ZIKV is transmitted to humans primarily through the bite of infected mosquitoes from genus Aedes. Furthermore, ZIKV can also be transmitted from mother to child during pregnancy or spread through sexual contact, breastfeeding, or blood transfusion. The multiple modes of ZIKV transmission make it difficult to develop control strategies against the pathogen.
(D) In most cases ZIKV infection causes no symptoms or only a mild self-limiting illness, but recent epidemiological studies derived from outbreaks in 2007 and 2015 to 2016 linked ZIKV infection to a rising number of concerning severe neurological diseases, including Guillain-Barré syndrome and microcephaly.

Poxviruses as vaccines (Recombinant viral vectors):

- Genome flexibility. Insertion of foreign DNA (25Kb).
- Easy to generate highly stable recombinant viruses.
- High levels of expression of heterologous antigens.
- Induction of both humoral and T cellular immune responses.
- Low cost of production.
- Ease of manufacture and administration.
- Stability of freeze-dried vaccine.

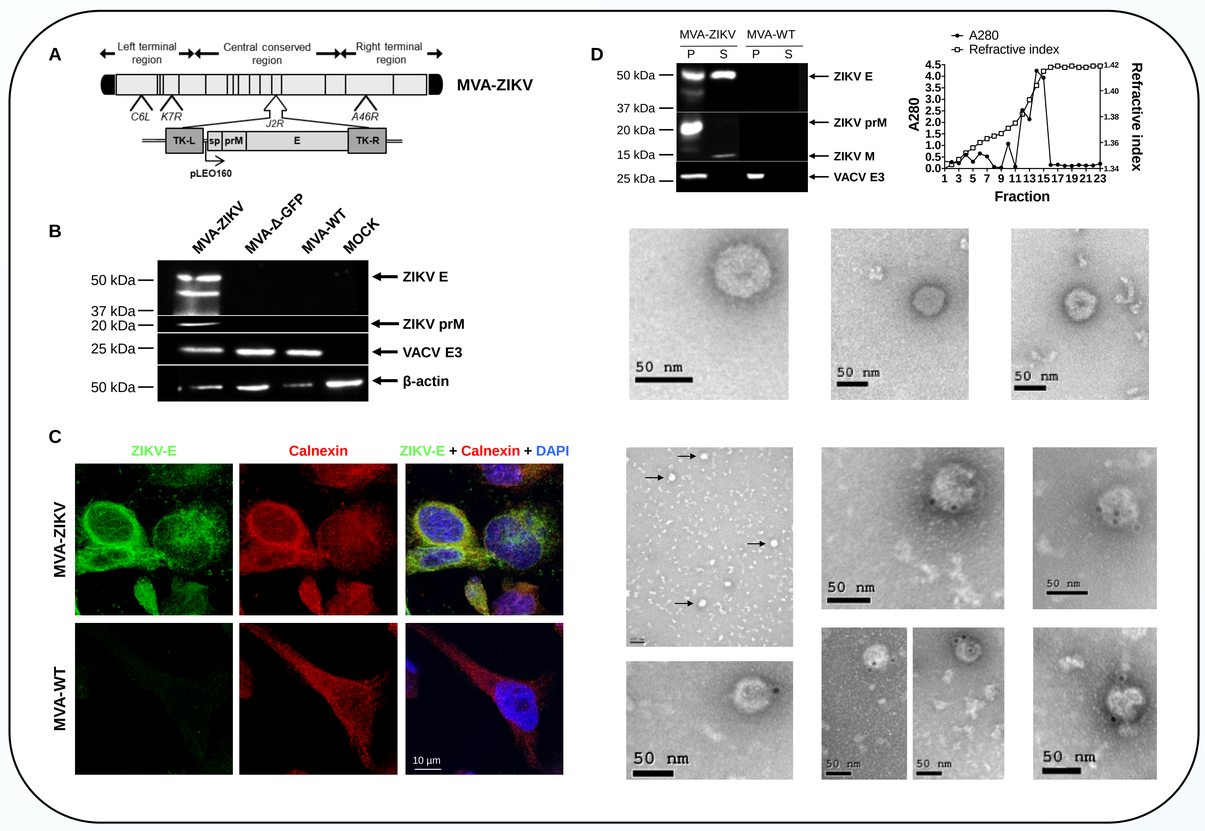
Highly attenuated poxvirus vectors, such as MVA, are safe and efficacious vectors used as vaccines against several diseases.



The highly attenuated poxvirus modified vaccinia virus Ankara (MVA) has been extensively used in numerous preclinical and clinical trials as a vaccine vector against several infectious diseases, being a cost-effective, safe and efficacious vector. In addition, recombinant MVA vaccines express high levels of the heterologous antigens, and are potently immunogenic inducing antigen-specific humoral and T cellular immune responses. Therefore, MVA should be a good vector to develop a vaccine against ZIKV that would induce potent ZIKV-specific humoral and T cellular responses able to prevent ZIKV infection and dissemination.

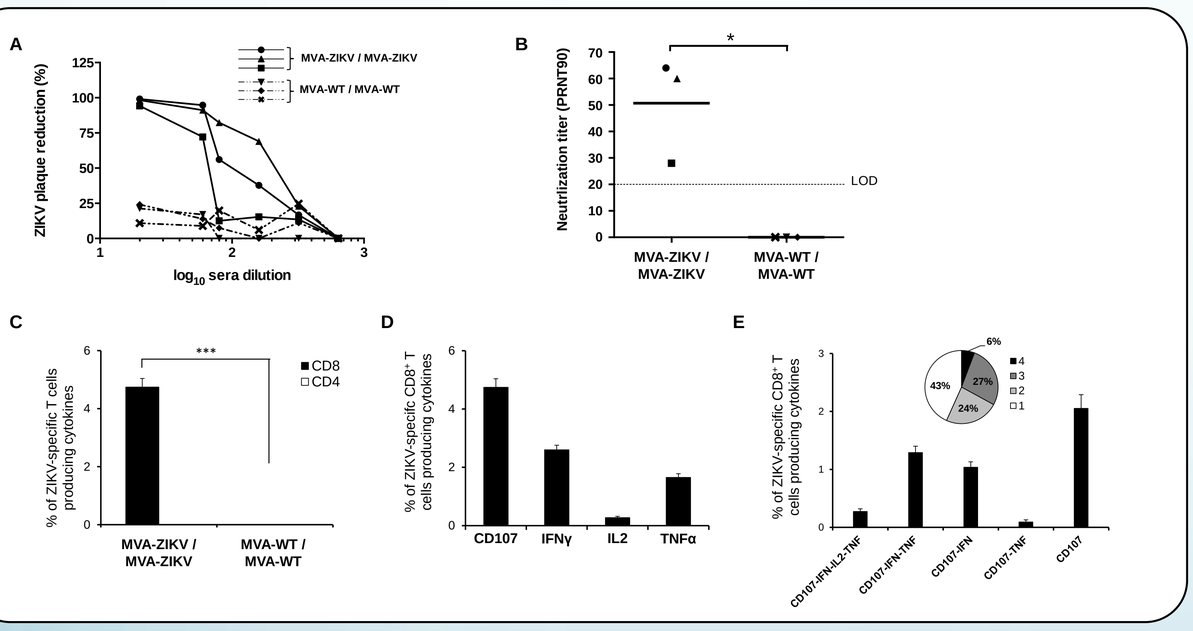
RESULTS

Figure 1 — Generation and *in vitro* characterization of MVA-ZIKV



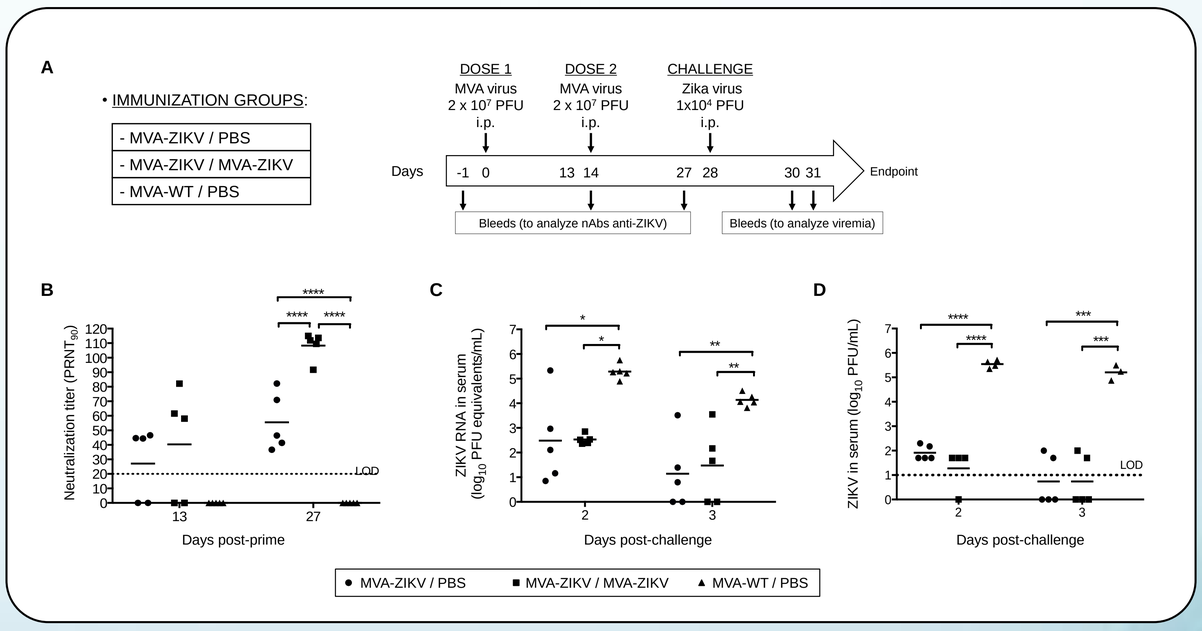
(A) Scheme of the MVA-ZIKV genome map. ZIKV signal peptide (sp) following by the ZIKV prM-E structural genes (isolate Z1106033) driven by the novel VACV synthetic pLEO160 promoter are inserted within the VACV TK viral locus (J2R). Deleted VACV C6L, K7R, and A46R genes are indicated. **(B)** Expression of ZIKV prM and E proteins. DF-1 cells were mock infected or infected at 5 PFU/cell with MVA-ZIKV, MVA-Δ-GFP, or MVA-WT. At 24 hpi, cells were lysed, fractionated by 8% SDS-PAGE, and analyzed by Western blotting. **(C)** Immunofluorescence analysis of the expression of ZIKV E protein by MVA-ZIKV. Detection of ZIKV E protein in the ER. HeLa cells were infected at 0.5 PFU/cell with MVA-ZIKV or MVA-WT for 24 h. Then, permeabilized cells were labeled with an anti-ZIKV E monoclonal mouse antibody and an anti-calnexin antibody. Anti-ZIKV E was detected with a mouse secondary antibody conjugated with the fluorochrome Alexa Fluor 488 (green). Anti-calnexin was detected with a rabbit secondary antibody conjugated with Alexa Fluor 594 (red). Cell nuclei were stained using DAPI (blue). The degree of co-localization of E and calnexin proteins is shown on the right by the yellow color. Scale bar: 10 µm. **(D)** Virus-like particles production. (Upper panel, left) Western blot analysis of the ZIKV proteins detected in cells extracts (P) or in supernatants (S) concentrated through a 20% sucrose cushion and derived from HeLa cells infected with MVA-ZIKV or MVA-WT. Arrows on the right indicate the positions of the ZIKV prM, M and E proteins. The sizes of standards (in kDa) are indicated on the left. (Upper panel, right) Amount of protein and sucrose density in fractions obtained after ultracentrifugation of MVA-ZIKV-concentrated supernatants loaded into a 20–60% w/v sucrose gradient. The amount of protein in each fraction was determined by spectrophotometry measuring the absorbance at 280 nm (A280). The sucrose density in each fraction was determined by refractometry. (Middle and lower panels) Detection by electron microscopy of VLPs produced by MVA-ZIKV. Negative-stained (Middle panel) or immunogold-stained (Lower panel) transmission electron microscopy images of purified ZIKV VLPs contained in the fraction 14 of the MVA-ZIKV gradient. Arrows indicate the VLPs detected in a lower magnification image of the immunogold-stained assay.

Figure 2 — MVA-ZIKV is highly immunogenic in immunocompetent mice



Balb/c mice were immunized with MVA-ZIKV/MVA-ZIKV or MVA-WT/MVA-WT and 10 days after the last immunization ZIKV-specific humoral (A, B) and T cellular (C-E) immune responses were analyzed. **(A)** Percentage of ZIKV plaque reduction, determined by a PRNT assay, from each individual serum sample at different serial dilutions. **(B)** ZIKV-neutralizing antibody titers represented as the reciprocal of the serum dilution that inhibited plaque formation by 90% (PRNT90), relative to samples incubated with negative control sera. Dashed line indicates the limit of detection (LOD) of the neutralization assay. The statistically significant difference between both groups is indicated (*, $P < 0.05$). **(C)** Overall magnitude of ZIKV-specific $CD4^+$ and $CD8^+$ T cells. The values represent the sums of the percentages of T cells producing CD107a and/or IFN-γ and/or TNF-α and/or IL-2 against the ZIKV E protein peptide pool. **(D)** Pattern of ZIKV-specific $CD8^+$ T cell immune responses in MVA-ZIKV-vaccinated mice. Frequencies were calculated by reporting the number of $CD8^+$ T cells producing CD107a or IFN-γ or TNF-α or IL-2. **(E)** Polyfunctional profile of ZIKV-specific $CD8^+$ T cell immune responses in MVA-ZIKV-vaccinated mice. Those T cell populations with a positive response are shown on the x axis, while the percentages of $CD8^+$ T cells producing CD107a and/or IFN-γ and/or TNF-α and/or IL-2 against the ZIKV E peptide pool are shown on the y axis. Responses are grouped and coded on the basis of the number of functions (4, 3, 2, or 1). The pie charts summarize the data, with each slice corresponding to the proportion of ZIKV-specific $CD8^+$ T cells exhibiting one, two, three, or four functions within the total population of ZIKV-specific $CD8^+$ T cells.

Figure 3 — MVA-ZIKV controls viral replication in a challenged mouse model



(A) Immunization scheme. Groups of IFNAR-/- mice (n=10 mice/group) were immunized with 2 x 10^7 PFUs of MVA-WT (one dose, at day 0) or MVA-ZIKV (one or two doses, at days 0 and 14, respectively) by the i.p. route. Twenty eight days after the first immunization, mice were challenged with 10^4 PFUs of ZIKV (PA259459, strain Panama) via the i.p. route. **(B)** ZIKV-neutralizing antibody titers (PRNT90). Titers of neutralizing antibodies against ZIKV PA259459 strain were analyzed at 13 and 27 days post-prime immunization in sera (n=5) of animals immunized with one dose of MVA-WT or one or two doses of MVA-ZIKV. Titers were determined by a PRNT assay and are expressed as the reciprocal of the serum dilution that inhibited plaque formation by 90% (PRNT90), relative to samples incubated with negative control sera (from day 1 pre-prime). Dashed line indicates the limit of detection (LOD) of the neutralization assay. The statistically significant difference between the groups is indicated (***, $P < 0.001$). **(C)** ZIKV RNA viremia after challenge. Blood samples were collected at days 2 (n=5) or 3 (n=5) post-challenge, and ZIKV RNA viremia was analyzed by quantitative real-time PCR (PFU equivalents/ml). Graph shows mean with each point representing an individual mouse. P values indicate significantly higher responses between the different groups (*, $P < 0.05$; **, $P < 0.005$). **(D)** ZIKV infectious virus after challenge. Blood samples were collected at days 2 (n=5) or 3 (n=5) post-challenge, and ZIKV infectious virus was analyzed by a plaque assay (PFUs/ml). Graph shows mean with each point representing an individual mouse. P values indicate significantly higher responses between the different groups (***, $P < 0.001$, ****, $P < 0.0001$).

CONCLUSIONS

- We have developed a novel and promising ZIKV vaccine candidate (MVA-ZIKV) that produced VLPs.
- MVA-ZIKV is highly immunogenic in mice inducing ZIKV-specific neutralizing antibodies and potent $CD8^+$ T cell immune responses.
- MVA-ZIKV is strongly effective in reducing ZIKV viremia after a challenge with ZIKV in immunocompromised mice.

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