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Recombinant varicella vaccines induce neutralizing antibodies and cellular immune responses to SIV and reduce viral loads in immunized rhesus macaques

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Abstract

The development of an effective AIDS vaccine remains one of the highest priorities in HIV research. The live, attenuated varicella-zoster virus (VZV) Oka vaccine, safe and effective for prevention of chickenpox and zoster, also has potential as a recombinant vaccine against other pathogens, including human immunodeficiency virus (HIV). The simian varicella model, utilizing simian varicella virus (SVV), offers an approach to evaluate recombinant varicella vaccine candidates. Recombinant SVV (rSVV) vaccine viruses expressing simian immunodeficiency virus (SIV) env and gag antigens were constructed. The hypothesis tested was that a live, attenuated rSVV-SIV vaccine will induce immune responses against SIV in the rhesus macaques and provide protection against SIV challenge. The results demonstrated that rSVV-SIV vaccination induced low levels of neutralizing antibodies and cellular immune responses to SIV in immunized rhesus macaques and significantly reduced viral loads following intravenous challenge with pathogenic SIVmac251-CX-1.

Keywords

Simian varicella virus; Varicella-zoster virus; Simian immunodeficiency virus

1. Introduction

The AIDS epidemic caused by human immunodeficiency virus (HIV) continues to spread. An estimate of 33.4 million adults and children are currently living with HIV/AIDS with 2.7 million newly acquired cases in 2008 [1]. The availability of multiple antiretroviral drugs has significantly reduced viral loads, HIV transmission, and disease progression, resulting in an extension of life for many HIV infected persons in developed countries. However, the vast majority of HIV infected individuals are in underdeveloped countries worldwide, where therapy is often unavailable.

The current challenge facing this epidemic is a safe and effective AIDS vaccine. Despite decades of intense research, a successful vaccine has still not been produced. The recent

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failure of the phase II STEP trial of recombinant adenovirus type 5-HIV vaccines in humans was a setback to the field, but highlights the critical need for more extensive basic biological research and testing in nonhuman primate models [2]. Novel vaccine strategies are urgently needed to resolve the current HIV-1/AIDS epidemic.

Varicella-zoster virus (VZV) infection causes varicella (chickenpox) in children, and herpes zoster (shingles), primarily in the elderly. The live, attenuated VZV Oka vaccine is safe and effective for prevention of childhood chickenpox and is recommended for children in the U.S. and other countries [3]. This varicella vaccine is even safe and effective in some groups of immunosuppressed patients, including children with HIV infection [4–6]. A related, but distinct VZV shingles vaccine is approved for use in the elderly to prevent herpes zoster and postherpetic neuralgia [7]. The VZV vaccine is also an attractive candidate as a recombinant vaccine vector expressing antigens of other pathogens [8]. However, stringent human host-range restrictions limit development of an experimental animal model for testing candidate varicella vaccines.

The simian varicella model provides an alternative experimental approach to investigate varicella pathogenesis and to evaluate antiviral agents and vaccines [9,10]. Simian varicella virus (SVV) is closely related to VZV and causes a natural, varicella-like disease in nonhuman primates [9,11,12]. Construction of novel recombinant SVV (rSVV) vaccines expressing simian immunodeficiency virus (SIV) antigens and testing in the simian AIDS model offers an experimental approach to evaluate SIV/HIV vaccine candidates. A previous study described construction of rSVV vaccines that expressed SIV antigens and successfully induced immune responses to SIV in immunized African green monkeys [13].

In this report, we extend these findings and show that live, attenuated rSVV-SIV vaccines effectively induce humoral and cellular immune responses to SIV antigens in immunized Rhesus monkeys and significantly reduce SIV viral loads following intravenous (iv) challenge with pathogenic SIVmac251-CX-1.

2. Materials and methods

2.1. Cells and virus

Vero (African green monkey kidney) cells were grown in Eagle's minimal essential medium (EMEM) supplemented with 5% newborn bovine serum, penicillin (5000 U/ml), and streptomycin (5000 U/ml). The Delta herpesvirus strain of SVV was originally isolated from a patas monkey at the Delta Regional Primate Research Center, now the Tulane National Primate Research Center (TNPRC) [14]. SIVmac251-CX-1, a cryopreserved stock at TNPRC, was utilized for iv challenge [15].

2.2. Recombinant vaccine design

A cosmid-based SVV genetic recombination system was used to insert the SIVmac239 env and gag genes or respiratory syncytial virus (RSV) G and M2 genes within the nonessential SVV glycoprotein C gene (ORF 14) of the SVV genome [13,16].

2.3. Animals

Nine Indian-origin male rhesus macaques (*Macaca mulatta*), 3–8 years old and weighing 3.8–9.3 kg, were obtained from the TNPRC specific pathogen-free colony and confirmed seronegative for SVV and RSV by antibody neutralization and ELISA assays. Monkeys were screened for MHC-1 class I alleles using a PCR based technique at TNPRC and the MHC Typing Core at University of Wisconsin AIDS Vaccine Research Lab [17]. All animal housing, care, and research were performed in compliance with the *The Guide for the Care*

and Use of Laboratory Animals (National Research Council) and the guidelines at the TNPRC, an institute fully accredited by the Association of the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and in accordance with the Animal Welfare Act guidelines. Protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Tulane University.

2.4. rSVV-SIV immunization and SIV challenge

The animals were divided into two (experimental and control) groups, with five and four animals per group, respectively, randomly assigned by age and weight. Each group included two *Mamu-A*01⁻* and two *Mamu-A*01⁺* animals. The experimental animals received both subcutaneous and intratracheal inoculations of 3×10^4 pfu of rSVV-SIV239gag and 3×10^4 pfu rSVV-SIV239env infected Vero cells. The control groups received similar immunization with rSVV-RSV_G and SVV-RSV_{M2} infected Vero cells. Booster immunizations with the same virus dose were administered on days 35 and 70 post immunization (pi). Humoral immune responses to the SVV vector were determined by serum neutralization assay as previously described [13]. Six months after immunization, all animals were challenged with 100 TCID₅₀ SIVmac251-CX1 by iv inoculation.

2.5. Clinical evaluations

Animals were monitored by physical examinations and monthly clinical hematological and chemical analyses for signs of infection and well-being during the entire study. Parameters of acute SVV virus infection were evaluated as previously described [13,18]. Typical varicella rash was scored on an established scale of 0–4+ with no rash = 0 and severe rash = 4+. SVV viremia was monitored by harvesting peripheral blood mononuclear cells (PBMCs) using Ficoll–Hypaque gradients and co-culture on Vero cell monolayers. Hepatitis was assessed by increase in the liver enzymes, aspartate transaminase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) in blood samples. Following SIV challenge, animals were given physical examinations and blood drawn twice a week for the first month, weekly for the second, biweekly for the third and fourth months, followed by monthly clinical evaluations until the animals became moribund or the study was terminated. All macaques were humanely euthanized according to established TNPRC guidelines. Necropsies were performed with tissues harvested and flash-frozen for further analyses.

2.6. SIV ELISA

SIV antigen, prepared from lysates of purified SIVmac251 virions, was adsorbed to 96-well ELISA plates (0.1 µg/well). Dilutions of monkey serum in triplicate were added to wells for 3 h at 37 °C. After washing, rabbit anti-monkey IgG-alkaline phosphatase was added for 1 h at 25 °C, followed by incubation with substrate (nitrophenol phosphate). An ELISA reader was employed to measure optical density of each reaction.

2.7. SIVmac neutralization assay

Neutralization of an uncloned stock of T-cell line adapted (TCLA) SIVmac251, and molecularly cloned env-pseudotyped virus bearing the env of primary stocks of SIVmac239, and SIVmac251-CX-1 was measured as a reduction in luciferase (Luc) reporter gene expression after a single round of infection in TZM-bl cells as described [19,20]. Neutralization titers are the dilution at which relative luminescence units (RLU) were reduced by 50% compared to virus control wells after subtraction of background RLUs. The assay stock of TCLA-SIVmac251 was produced in H9 cells. Assay stocks of env-pseudotyped viruses were prepared by co-transfection in 293 T-cells with env-defective

backbone plasmid as described [19,20]. The viruses were titrated in TZM-bl cells prior to assay.

Neutralization of uncloned stocks of the challenge virus, SIVmac251-CX-1, was measured as described [19] using 5.25.EGFP.Luc.M7 (M7-Luc) cells kindly provided by Dr. Nathaniel R. Landau. This is a CEMx174 cell clone that was produced by retroviral vector transduction to express CCR5 (CD4 and CXCR4 are expressed naturally) and transfection to contain Tat-responsive Luc and green fluorescence protein (GFP) reporter genes [21]. Neutralization titers are the serum dilution at which relative luciferase units (RLU) were reduced by 50% compared to virus control wells after subtraction of background RLUs. The assay stocks of SIVmac251-CX-1 were produced in CEMx174 cells and titrated in M7-Luc cells.

2.8. IFN- γ ELISPOT assays

For analysis of SIV-specific T-cell responses, PBMC were stimulated in the presence or absence of SIVmac239 peptide pools of overlapping peptides obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Pools of 20-mer peptides overlapping by 10 amino acids, spanning the entire gp130 sequence of SIVmac239 were used for determining SIVenv specific responses. Three SIVenv pools were generated from these env peptides and tested separately. Similarly a single pool of 15-mer peptides overlapping by 11 amino acids and spanning the SIVmac239 gag region were used to determine SIV gag specific responses.

Interferon gamma (γ -IFN) responses were detected using Mab-Tech ELISPOT kit (Mab-Tech, Inc., Mariemont, USA) following manufacturer instructions. In brief, 96-well plates were pre-coated with anti gamma interferon mAb. After washing and blocking, PBMC were added at 2×10^5 cells/well and incubated for 24 h at 37 °C in 5% CO₂ in the presence or absence of the SIVenv and gag peptide pools. PHA was used as a positive assay control and media plus cells was the negative control. Plates were further washed, incubated with detection antibody and finally developed using BCIP/NBT substrate for the development of spots. Plate counting and analysis was performed by Zellnet (Fort Lee, NJ). The mean number of spots per triplicate wells was calculated and the negative media control mean was subtracted. Results were considered positive if the number of spot forming cells (SFC) for 2×10^5 cells was greater than the average of the negative control wells +2S.D [22]. Sample results were normalized to 1×10^6 cells/sample and values reported. Control animals tested showed ELISPOT values that ranged from 0 to 95 SFU/million cells for SIVenv; and 0–35 SFU/million cells for SIVgag. The slightly increased background response for SIVenv was suggested to be due to either testing of the multiple env peptide pools or possible cross-reactivity with env peptides in vector vaccinated macaques. As such, positive cutoff values were then established as 100 SPC per million cells for env and 40 SFC per million cells for gag.

2.9. T-cell immunophenotyping

T-cell immunophenotyping was performed using CD3-FITC/PerCP (SP34-2), CD4-APC (L200), CD8-PE/PerCP (RPA-T8), all from BD Biosciences. For surface staining, 100 μ l of EDTA whole blood was incubated with titrated mAb for 30 min on ice. Red blood cells were lysed with $1 \times$ FACS lysing buffer (BD Biosciences) using a whole blood lysis technique as previously described [23]. The cells were finally fixed with 1% paraformaldehyde solution (Sigma). At least 20,000 events were collected by gating on lymphocytes, and data were analyzed using FlowJo software, version 8.8.6. (TreeStar Inc., Ashland, OR). Absolute count of different phenotype cell population was calculated based on CBC count and flow cytometry data.

2.10. SIV plasma virus load

The concentration of SIV RNA in plasma was determined by SIV RNA 4.0 bDNA signal amplification nucleic acid probe assay for the direct quantitation of SIVmac using the Siemens System 340 Analyzer (Siemens Diagnostic, Berkeley, CA). Plasma samples, anticoagulated with EDTA, were aliquoted, stored at -80°C and assayed in batches throughout the study. The lower limit of detection was 125 SIV RNA copies/ml of plasma.

For statistical analyses, SIV viral load values were log transformed and data were analyzed using a random effects model clustering on each animal in order to account for the correlation among multiple observations for each primate. Five primates were included in the rSVV-SIV vaccine group and four in the rSVV-RSV control group. Two models were fitted; the first from baseline to 231 days and the second from the point in which the SIV viral load means began to diverge at 11 days to 231 days. Factors considered for the model were vaccine treatment, time, and vaccine treatment by time. All statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC).

3. Results

3.1. Immunization and SIV challenge of rhesus macaques

rSVVs expressing env and gag genes of SIVmac239 or RSV G (glycoprotein) and RSV M2 (matrix) genes under the control of the human cytomegalovirus (CMV) immediate early promoter/enhancer were constructed using the SVV cosmid recombination system as described [13].

Five SVV seronegative male rhesus macaques were immunized subcutaneously and intratracheally with 3×10^4 pfu of rSVV-SIV_{env} and 3×10^4 pfu of rSVV-SIV_{gag} infected Vero cells (experimental group). Four additional monkeys were immunized in a like manner with rSVV-RSV_G and rSVV-RSV_{M2} infected Vero cells (control group). Following primary immunization, slightly elevated body temperature and mildly enlarged lymph nodes were documented in 8 of 10 experimental and 6 of 10 control animals. Typical clinical signs of SVV infection of generalized rash and elevated liver enzymes (AST, ALT, LDH) were not observed, confirming a previous finding that the rSVV-SIV vaccines are attenuated in nonhuman primates, possibly due to insertional inactivation of the SVV glycoprotein C gene, a possible varicella virulence gene [24,25]. The molecular basis of the VZV Oka vaccine is unknown, but defective gC expression has been reported [26,27].

Two vaccine booster immunizations were given, one at 35 days and the second at 70 days post prime immunization. Following the first boost, two control (EK04, EP23) and two experimental animals (DR47, FD34) presented with mild to moderate rash, and slightly enlarged peripheral lymph nodes whereas LDH increase were observed in two control (DB31, EP23) and three experimental animals (DE36, DH08, FH96). The second boost was well tolerated with no rash or significant clinical findings associated with the SVV vector. Following this immunization protocol all animals were confirmed to be SVV seropositive by serum neutralization assay (data not shown).

Six months following primary immunization, all animals were challenged by iv inoculation with 100 TCID₅₀ SIVmac251-CX1, a cryopreserved stock routinely used in previous studies [15]. The animals were monitored for 231 days post challenge for signs of clinical SIV infection.

3.2. Antibody and cellular immune responses to SIV following immunization and challenge

Humoral antibody responses to SIV antigens, as determined by ELISA, were initially low, but detectable, at the time of SIV challenge. Following SIV challenge, the experimental animals immunized with rSVV-SIVenv/gag generated a rapid anamnestic immune response to SIVmac with 2–8 fold increases in SIV antibody titer by day 14 post challenge and higher titers at day 28 (Fig. 1). In contrast, the control animals, immunized with rSVV-RSV_{G/M2}, did not have an elevated SIV antibody response until day 28 following SIV challenge, indicative of a primary antibody response to the challenge virus. The results indicated that the rSVV-SIV vaccine induced SIV-specific humoral responses in the immunized animals.

IFN- γ ELISPOT analyses of longitudinally collected PBMCs from each of the animals were tested for specific cellular responses against SIV env and SIV gag. Three of the five experimental rSVV-SIV immunized animals showed responses to the SIVenv peptides (Fig. 2A). Animal, DH08, showed a striking increase of SIV env specific IFN γ producing cells of 125, 275, and 130 spot forming cells (SPC) per 10^6 cells, on days 34, 77 and 84 post immunization, respectively. DE36 produced 155 SFC per million cells, on day 84, 14 days following the second boost, and finally, FH96 had a spike to 210 SFC per million just prior to SIV challenge. The remaining two experimental animals and all control animals had levels at or below the 100 SPC per million cell cutoff and were considered negative.

Animals were also tested for specific cellular responses against SIVgag and, while lower in magnitude to that against SIVenv, showed two experimental animals with positive responses (Fig. 2B). One animal, DH08, showed an increase of SIV gag specific IFN γ producing cells of 100 cells per 10^6 cells on the day of challenge. A second animal, DR47 produced 48 cells per 10^6 cells at day 77 following immunization. The other results, those of DE36, FD34, and FH96, and those of the control animals, had values at or below 40 cells per 10^6 cells, below cutoff and were considered negative.

3.3. Neutralizing antibody responses to SIV following immunization and challenge

The neutralizing antibody response of immunized animals to SIV was tested against three established SIVmac strains with known neutralization sensitivities, including SIVmac239 CS.23, a highly neutralization resistant strain; SIVmac251-TCLA (T-cell line adapted), an uncloned, highly neutralization sensitive strain; and finally, SIVmac251-CX-1, a moderately neutralization sensitive strain and the challenge virus for this study (Table 1). As expected, neither rSVV-SIV nor rSVV-RSV immunized animals generated antibodies that were able to neutralize the resistant SIV-mac239.CS.23 strain during the immunization phase or after SIV challenge. Likewise, following immunization, neither the rSVV-SIV nor rSVV-RSV vaccinated animals generated a neutralizing antibody response to the moderately sensitive challenge virus, SIVmac251-CX-1. However, during the immunization phase, 4 of the 5 rSVV-SIV vaccinated animals (DE36, DR47, FD34, FH96) generated detectable neutralizing antibody titers against the highly sensitive SIVmac251-TCLA with ID₅₀ neutralization titers on day of challenge (doc) ranging from 43 to 105 (Table 1) and peak levels ranging from 52 to 244 (not shown), whereas rSVV-RSV immunized control animals did not generate a neutralizing antibody titer to SIVmac251-TCLA.

Following SIV challenge, rSVV-SIV immunized animals generated a rapid anamnestic neutralizing antibody response by day 28 when tested against the sensitive SIVmac251-TCLA strain with ID₅₀ titers spiking up to 365,651 in FD34 and >437,400 in the other four animals at day 28 (Table 1). By day 84 post challenge, FD34 levels increased to >437,400, DR47 and FH96 remained at >437,400 and DE36 and DH08 had levels reduced to 258,122 and 90,675, respectively (not shown). In contrast, rSVV-RSV control animals only developed positive ID₅₀ neutralizing antibody titers in the low range of 555–6796 at day 28

(Table 1) and titers ranging from 297 to 80,373 by day 84 (not shown), as expected for a primary SIV infection. Importantly, neutralizing antibodies against the moderately sensitive challenge strain, SIVmac251-CX1 were demonstrated in 3 of the 5 experimental animals (DH08, FD34, FH96) with titers of 26, 91, and 110, while all control animals were completely negative for neutralizing antibodies (Table 1). The results demonstrate that rSVV-SIV immunization induces a low level neutralizing antibody response against SIV.

3.4. Viral loads in immunized animals following challenge with SIVmac251-CX-1

Animals were monitored for plasma viremia by SIVmac bDNA assay. By 1 week post challenge, all animals showed detectable SIV-mac levels that increased to peak levels by day 14 and declined to set points by day 56 (Fig. 3A and Table 1). SIVmac viral load values were log-transformed and mean values for each vaccine treatment group were determined. Both the peak and set points showed a much greater trend towards lower viral loads in the rSVV-SIV immunized experimental animals ($n = 5$), compared with the rSVV-RSV immunized control animals ($n = 4$) (Fig. 3B). Lower mean viral loads were clearly demonstrated at day 14 peak viremia ($\log^{6.8 \pm 0.1}$ vs $\log^{7.6 \pm 0.2}$) and at day 56 viral set point ($\log^{5.3 \pm 0.2}$ vs $\log^{6.3 \pm 0.4}$) (Fig. 3B). This trend continued until day 231 with the final mean $\log^{4.7 \pm 0.2}$ for rSVV-SIV immunized animals significantly reduced more than 1.5 logs from the mean $\log^{6.3 \pm 0.9}$ for rSVV-RSV control animals, and significantly reduced when calculated from day 11 through day 231 ($p = 0.004$, Fig. 3B and Table 2).

Known Mamu-A*01+ animals were present in each group to observe any considerable control from these alleles (Table 1). However, values of each Mamu-A*01+ animal were within the range of the other animals during the entire longitudinal follow-up in their respective groups (Fig. 3A) and did not show significant control due to their MHC-A01+ genotype.

To determine significance of vaccine efficacy over this time period, a random effects model (SAS version 9.1) was used. Values from both the experimental animals and the control animals were included in these analyses. Table 2 shows the F -statistics and p -value for virus load differences between (1) the two vaccine groups (Experimental vs Control), (2) time points for a Time trend, and (3) the Vaccine groups by time; for either of the two time periods: days 0–231 or days 11–231. rSVV-SIV immunized animals had lower viral loads compared to rSVV-RSV immunized animals groups over the 231 days of follow-up (Fig. 3, $p < 0.005$). There was no difference in SIV viral loads in either group from baseline to day 11 and the greatest divergence occurred after day 11. Vaccine group data analyzed by time from baseline to day 231 showed significant reduction of experimental animals from the control animals (Table 2, $p < 0.03$). However, when the data set included only day 11 to day 231, the difference between experimental and control groups was even more pronounced ($p < 0.004$).

3.5. CD4+ T-cell counts in immunized monkeys following viral challenge

Animals were monitored for changes of CD4+ T-lymphocyte subsets following immunization and challenge. Fig. 4 displays the expected decline of CD4+ T-cells in both experimental and control animals, following SIV challenge. Absolute values of CD4+ T-cells at the time of challenge ranged from 1000 to 2500 cells/ml. At day 14 post SIV challenge, CD4+ values in 8 of 9 animals dropped to a range of 600–1500 cells/ml for both groups of animals, showing no overall difference. One single control animal, EK04, showed levels on day 14 that increased to 2800 cells/ml, declined on day 28 to 1736 cells/ml, showed a trend of CD4+ expansion with a peak of 4500 cells/ml at 147 days, and a final level of 3300 cells/ml at 231 days post SIV challenge. A second control animal, DB31, showed a single spike to 3300 cells/ml at day 28 and then declined to 1153 cells/ml at day

56. Finally at 231 days post SIV challenge, the remaining animals had decreased similarly to levels ranging from 300 to 1500 cells/ml.

4. Discussion

This work describes the first immunization/challenge testing of a rSVV-SIV vaccine in rhesus macaques employing an iv challenge with a pathogenic, uncloned stock of SIVmac251. A previous study demonstrated that these live, attenuated rSVV vaccines expressing SIV env and gag, are safe and immunogenic, producing both humoral and cellular immune responses when tested in African green monkeys [13]. The results of this latest vaccine challenge study in rhesus macaques demonstrates production of neutralizing antibodies and cellular immune responses to SIV, and importantly, reduction of viral loads following SIV challenge, indicating efficacy of these novel rSVV-SIV vaccine candidates. Since the experiment was terminated at day 231 post challenge, before development of clinical signs of simian AIDS, vaccine efficacy on survival was not assessed.

Live, attenuated varicella vaccines offer the advantage of limited viral replication in immunized hosts, thus inducing a broad spectrum of cellular and humoral immune responses, as shown with the VZV Oka vaccine virus, as well as other live viral vectors [28–31]. Thus, recombinant varicella vaccines may induce effective immune responses to the SVV/VZV vector, as well as to the expressed recombinant antigens.

In this study, rSVV-SIV vaccination induced a relatively low antibody response to SIV during the immunization phase as indicated by SIV ELISA and serum neutralizing antibody titers. However, effective vaccine priming against SIV antigens during immunization was demonstrated by a rapid, anemestic response in serum antibodies in the rSVV-SIV immunized animals following SIV challenge compared with a low rise in antibody titers in the rSVV-RSV control group, as indicated by ELISA. This anemestic response was confirmed by a multi-log increase in SIV neutralizing antibody titers following SIV challenge in the rSVV-SIV immunized animals. SIV neutralizing antibody responses were also demonstrated by augmented titers against the SIVmac251-CX-1 challenge virus as well as the more neutralization sensitive SIVmac251-TCLA strain. As such, these responses against TCLA-SIVmac251 are comparable to responses documented in other vectored vaccine studies ALVAC [32], NYVAC [33] and AAV [34], but somewhat weaker than the responses observed with live, attenuated SIVmac [35–37].

In addition, rSVV-SIV vaccination induced cell-mediated immune responses in all experimental animals against SIV env and in one experimental animal against SIV gag, during prime-boost immunizations, compared with low level cellular immune responses in control animals, as indicated by INF- γ ELISPOT assay. These latter ELISPOT results show the rSVV-SIVenv to be more effective at generating antigen specific immune responses than the rSVV-SIVgag and may suggest modification of this construct for later studies. Collectively however, these results clearly demonstrate that the rSVV-SIV vaccine effectively primed the immune response against SIV antigens resulting in augmented immune responses following SIV challenge.

The rSVV-SIV induced antibody and cellular immune responses in rhesus macaques during the immunization phase were not as potent as the immune responses stimulated by immunization of this same vaccine in African green monkeys in a prior rSVV-SIV immunogenicity study [13]. Although both species are susceptible to SVV infection [18,38] the SVV virus used was from a single virus stock propagated in African green monkey kidney (Vero, BSC-1, CV-1) cell lines. Therefore, the observed disparity in immune responses may be attributed to species-specific factors. Future studies will adapt the rSVV

viruses by propagation in rhesus macaques cells as an approach to improve immunogenicity in rhesus monkeys. As such, in spite of the relatively lower immune response following immunization, these data show rSVV-SIV immunization markedly stimulated effective memory immune responses following SIV challenge in rhesus monkeys.

A vital endpoint assessment of any simian AIDS vaccine is the ability to significantly reduce viremia in immunized animals following SIV challenge. Assessment of plasma viral load (PVL) as a measurement of virus replication is important, as viremia during the peak, acute, and most reliably, the chronic stages of SIV infection correlates directly with disease progression and outcome [39]. In the current study, rSVV-SIV immunized rhesus macaques were challenged with SIVmac and all animals became infected. However, by as early as day 14 post challenge, there was a marked reduction in SIV bDNA PVL in the rSVV-SIV vaccinated animals compared with the rSVV-RSV control animals. The geometric mean PLV in the control animals during the chronic phase remained >6 logs virus for the duration of the study. However, the rSVV-SIV immunized experimental animals exhibited a steady decline in viremia between the acute and chronic phase of infection with final PVLs reduced greater than 1.5 logs compared to rSVV-RSV immunized control animals.

Assessments of memory CD4⁺ T-cells populations following SIV challenge were conducted to monitor SIV infection and disease progression. In both HIV and SIV terminal infections, a progressive depletion of central memory CD4⁺ T-cells from the peripheral blood occurs [40,41]. In the current study, on the day of SIV challenge, peripheral blood CD4⁺ T-lymphocytes from all immunized animals, experimental and control, ranged from 1000 to 2500 cells per ml with no significant difference between the rSVV-SIV and rSVV-RSV vaccinated groups. Following SIV challenge through 231 days, the majority of animals in both groups decreased similarly to levels ranging from 300 to 1500 cells/ml with one control animal, EK04 showing a rise in CD4⁺ cells to 3300 cells/ml. These findings are in contrast to a previous study in which immunization of rhesus monkeys with a recombinant VZV vaccine expressing SIV env (rVZV-SIVenv) and SIVmac251 challenge correlated with enhanced disease progression rather than immune protection [42]. In that study, after SIV challenge, significant early increase in CD4⁺ T-cells proliferation and elevated SIV plasma loads were observed in all rVZV-SIVenv immunized monkeys, but not in control animals, either naive or VZV alone immunized animals. The authors speculated that the limited capacity of human VZV to replicate in rhesus cells precluded efficient cellular processing required for effective induction of class I-restricted CD-8 T-cell responses and allowed proliferation of virus susceptible CD4⁺ target cells. As such, rhesus monkeys do not appear to be appropriate for preclinical testing of human VZV vaccines. Based upon our findings, evaluation of rSVV vaccines in rhesus monkeys provides an excellent alternative approach for evaluation of recombinant varicella vaccines.

Recombinant herpesvirus vaccines expressing SIV antigens offer a novel approach for AIDS prevention. The ability of herpesviruses, such as HSV, VZV, and SVV, to persist in the host in a latent state and to periodically reactivate, often subclinically, thus boosting long-lasting immune responses, offers a potential advantage as a recombinant vaccine against HIV/SIV infection. HSV-1 recombinant viruses expressing SIV gp120 and nef antigens induced strong, persistent antibody and cell-mediated immune responses to HSV-1 and SIV antigens in immunized rhesus macaques [43]. Two of seven immunized monkeys were solidly protected and another had reduced viral load following challenge with SIVmac239 at 22 weeks following the final rHSV-SIV vaccination. A rVZV vaccine expressing the HIV-1 env gene elicited humoral and cellular immune responses to HIV gp120 and VZV antigens in guinea pigs, although HIV challenge was not possible in this model [44]. In summary, our studies demonstrate that the simian varicella model and evaluation of rSVV-SIV vaccines in

rhesus monkeys offers an effective approach for preclinical testing of potential recombinant varicella AIDS vaccines with subsequent transfer into rVZV for human clinical trials.

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Fig. 1. Serum antibody response to SIVmac251 antigen immunized in rSVV-SIV (black symbols) and rSVV-RSV (open symbols) immunized and SIV challenged animals as determined by ELISA. Optical density (O.D.) values of reactions (1:50 dilutions) after background subtraction was determined at 405 nm.

Fig. 2.

SIVmac env specific T-cellular responses (A) and SIVmac gag specific T cellular responses (B) in immunized monkeys in days (d) following immunization, on day of challenge (doc), and days post challenge (dpc). ELISPOT assays determined numbers of SIV-specific INF- γ cytotoxic cells present in PBMC following specific stimulation with SIVmac env peptide pools or SIVmac gag peptide pools, in days following immunization. Results were analyzed by Zellnet (Fort Lee, NJ), background control subtracted, and final counts normalized to 1×10^6 PBMC for reporting. The cutoff values established for SIVenv and SIVgag was 100 SFC/per million cells and 40 SPC per million cells, respectively, and are shown with hatched lines.

Fig. 3.

(A) SIV plasma viral load as determined by bDNA analysis (Seimens) following SIV iv challenge in rSVV-SIV (gray) and control rSVV-RSV (black) groups of immunized animals over the course of 231 days post challenge. (B) Mean log SIV values in rSVV-SIV experimental (gray) and rSVV-RSV control (black) groups.

Fig. 4. Absolute numbers of peripheral blood CD4⁺ CD3⁺ T-cells in immunized animals following SIV challenge. No significant differences between the rSVV-SIV immunized (gray dashed lines) and rSVV-RSV immunized control groups (black solid lines) were observed.

Table 1

Summary of immunological and virological results.

Vaccine	Macaque	ELISPOT	Anti-SIVmac251 neutralizing ab titer (peak)						Virus load (bDNA)			CD4					
			SIVmac239CS.23			SIVmac251-CX-1 ^a			SIVmac251-TCAL								
			Peak values	ENV	GAG	Post vaccine	Post challenge	Post vaccine	Post challenge	Post vaccine	Post challenge		Peak	Trough	Plateau		
																Absolute cell# (day 56)	
Experimental	SVV-SIVenv	DE36	155	22		<20	<20	<20	<20	52	>437,400	>437,400	5,792,900	219,797	35,544	1076	
	SVV-SIVgag	DH08	275	100		<20	<20	<20	26	<20	>437,400	>437,400	27,682,600	486,158	246,860	1312	
		DR47 ^c	75	48		<20	<20	<20	<20	86	>437,400	>437,400	10,570,600	477,196	107,894	885	
		FD34 ^c	84	7		<20	<20	<20	110	43	365,651	365,651	22,512,400	1,090,700	894,321	1549	
		FH96	210	25		<20	<20	<20	91	105	>437,400	>437,400	3,539,400	148,130	211,085	1356	
Control	SVV-RSV-GM2	DB31 ^c	95	35		<20	<20	<20	<20	<20	1518	1518	40,998,200	3,903,200	2,664,300	1153	
		DN76 ^c	61	10		<20	<20	<20	<20	<20	6537	6537	13,418,400	508,718	253,692	844	
		EK04	83	22		<20	<20	<20	<20	<20	6796	6796	75,888,600	1,724,300	1,734,800	2906	
		EP23	90	0		<20	<20	<20	<20	<20	555	555	87,199,200	9,515,700	14,981,500	822	

^achallenge SIV virus stock.

^bPeak value per animal following vaccination.

^cMamu-A01+.

Table 2

Statistical analysis of SIV viral loads.

	From baseline to day 231		From day 11 to day 231	
	<i>F</i> -Statistic	<i>p</i> -Value	<i>F</i> -Statistic	<i>p</i> -Value
Vaccine group (experimental vs control)	16.67	0.005	13.61	0.008
Time trend	73.92	0.0001	8.34	0.001
Vaccine group by time	1.82	0.03	2.47	0.004