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13. ABSTRACT *(Maximum 200 words)*

We report the immune responses of three groups of rhesus macaques vaccinated with recombinant vaccinia viruses (rVVs). Five animals were vaccinated with 10^7 pfu of vHuγ/SIVgen, a Wyeth rVV expressing the human interferon gamma, and the SIV gag, env, and nef genes. Five more macaques received a similar dose of vSIVgen, a Wyeth rVV that expresses the SIV gag, env, and nef genes. Two macaques were inoculated with wild type Wyeth VV as controls. All animals were inoculated twice with 10^7 pfu VV intramuscularly eight weeks apart. The rVV infection in PBMCs was resolved within one week of vaccination. VV infection is resolved differently in PBMCs and LNCs, and co-expression of IFNγ resolved the infection earlier in lymph nodes. All animals except controls exhibited high titers of antibodies to gag, gp160, and nef antigens of SIV. A high anti-gp160 and VV anamnestic response was observed in rVV-vaccinated macaques after the second vaccination. Vaccinated macaques will be boosted with gag/env VLPs produced in recombinant baculoviruses and all macaques will be challenged with pathogenic SIV_{mac251} two weeks after the final boost. We will correlate the outcome with the type (T_H1 or T_H2) of immune responses generated to the SIV antigens co-expressed with IFN-γ. The progress of the work is on schedule as outlined in the proposal.

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TABLE OF CONTENTS

INTRODUCTION

Our long term goal is the development of a safe and efficacious vaccine against infection with the human immunodeficiency virus (HIV). The immediate objective of this study is to develop vaccinia virus (VV) vector vaccines against acquired immunodeficiency syndrome (AIDS) using simian immunodeficiency virus (SIV) as a model in rhesus macaques. On the basis of both biologic and genetic features, SIV infection of macaques is the best animal model for HIV infection and AIDS (1-3). The development of the live VV vaccines is based on results obtained from previous studies in our laboratory and others demonstrating the adjuvant and attenuating effects of lymphokine, interferon-gamma (IFN-γ) on subunit and live attenuated viral vaccines (4-6). These studies have been extended by developing recombinant VVs (rVV) that express IFN-γ and SIV virus-like particles (VLPs) containing SIVgag, env, and nef proteins. We hypothesize that vaccines expressing IFN-γ will induce a Type 1 immune response that would lead to protection against SIV infection. Such a vaccine combines the effectiveness of a live attenuated vaccine with the safety of a subunit immunogen:

1. Antigens expressed by rVV are effective in inducing both cytotoxic T-lymphocyte (CTL) and humoral immune responses;
2. IFN-γ attenuates VV virulence;
3. IFN-γ will favor a Type 1 immune response to SIV antigens;
4. VLPs retain conformational epitopes thus inducing natural immune responses.
5. The expression of nef, a non-structural protein which is expressed early in SIV infection, will induce CTLs early in the infection.
6. If sterilizing immunity is not induced, an immune response to nef may select against SIVnef$^+$ variants, leading to the predominance of SIV$_{\Delta nef}$ viruses.

There is strong evidence to support the critical role of the cell-mediated arm of the immune system in the control of HIV infection (7-9). Also, the preferential development of one T helper cell subset is often apparent at the early stages of an infection, suggesting that the mechanism that drives the direction of the immune response operates soon after exposure to antigen (10). Therefore, we believe that the inclusion of IFN-γ will generate a safe, attenuated vaccine that will lead to the induction of protective immunity mediated by T_H1 cells. Additionally, the priming with SIV VLPs expressed by rVVs and the subsequent boosting with VLPs produced in a baculovirus vector will induce long lasting cell-mediated as well as humoral immunity. There are two reasons for including nef in the vaccine preparation. First, VV-expressing nef will generate nef-specific CTLs in immunized macaques. Second, the immune response to nef will provide a selective advantage for the replication of SIV$_{\Delta nef}$ variants, serving as an attenuated live SIV vaccine (11-12).

Since the last progress report, submitted in June 1996, we have completed the construction, characterization and preparation of high-titer stocks of a number of rVVs that express the IFN-γ gene and/or SIVgag, env, and nef genes. Immunological blots, PCR, and

protein gel electrophoresis have been used to confirm that each of the proteins expressed by these rVVs are authentic. We have also demonstrated that intravenous (I/V) inoculation of rVVs did not induce generalized VV infection in SIV-infected macaques. On the contrary, it was self limiting since we were not able to recover rVVs from plasma or PBMCs. The SIV infection of the intravenously vaccinated macaques did not alter, and there was even a functional recovery of the lymph nodes of 3 out 4 inoculated macaques.

Here we report the immune responses of three groups of rhesus macaques vaccinated with rVVs. The first group of five animals was vaccinated with vHuγ/SIVgen, a Wyeth rVV that expresses the human IFN-γ, and the SIV gag, env, and nef genes. The second group of five macaques received a similar dose of vSIVgen, a Wyeth rVV that expresses the SIV gag, env, and nef genes. The third group has two animals that were inoculated with wild type VV (Wyeth strain) as controls. All animals have been inoculated with one ml of sterile JME containing 10^7 plaque forming units (pfu) of the corresponding VV intramuscularly (I/M) twice, once on week 0 and once on week eight, as the first boost. The resolution of VV infection, immune responses to SIV antigens, and lymphokine (IFNγ) activity in plasma have been documented for the first 20 weeks of this experiment. These macaques will be boosted two or three times (based on evaluation of the immune responses) with gag/env virus like particles produced in insect cells. All macaques will be challenged with pathogenic SIV_{mac251} two weeks after the final boost. We will correlate the outcome of challenge studies with the type of immune responses generated to the SIV antigens in the presence or absence of co-expressed IFN-γ.

MATERIALS AND METHODS

1. Preparation of rVV Inoculum: Large-scale preparation of rVVS were propagated in HeLa cells infected at a multiplicity of infection (moi) of 3.0 in spinner cultures. After 48-hour incubation in Jokliks modified Eagles medium (JME) with 5% FBS at 37 °C, the cell pellets were harvested and resuspended in JME. The concentration of infectious virus was titrated by plaque assay. Purification of rVV was performed by sucrose cushion ultracentrifugation. Virus pellets were washed to remove any residual sucrose and resuspended in JME. The purified rVVs were titrated by VV plaque assay.

2. Inoculation of Rhesus Macaques with Recombinant VV Vaccines: Twelve colony-bred, juvenile rhesus macaques (*Macaca mulatta*) seronegative for simian type D retroviruses, simian T-cell leukemia virus, and SIV, are being used in this experiment. They are housed at the California Regional Primate Research Center (CRPRC) in accordance with the American Association for Accreditation of Laboratory Animal Care Guidelines and University of California Animal Care Policies. Two groups of five macaques were vaccinated I/M with 10^7 pfu of rVV and a control group consisting of two macaques was inoculated with a similar dose of wild type VV (Wyeth strain). After 8 weeks, the macaques were boosted with the same preparations, dose, and route. Behavior and clinical condition of the vaccinated animals are routinely recorded by the staff of the CRPRC.

3. Blood Collection: Animals were sedated, and 5 ml of blood was withdrawn in EDTA tubes. Additionally, EDTA tubes were filled with blood for CBC and surface immunofluorescence (13). The blood samples were taken on the day of vaccination, 1, 2, 4, 8, 10, 12, 16, and 20 week post-vaccination (WPV).

4. Lymph Node Biopsy: Using sterile surgical techniques, peripheral lymph nodes (axillary) biopsies were performed on macaques on day 0, 1 WPV, and 2 WPV by the CRPRC veterinary staff. The lymph node were cut in two pieces; one half was processed to obtain cells and nucleic acids, and the other half was immersed in formaldehyde (for immuno-histochemistry and in-situ hybridization).

5. Co-culture of PBMCs and LNCs with BSC 40 for VV Isolation: The presence of infectious VV in PBMCs and LNCs of vaccinated macaques was determined by co-cultivation of rhesus PBMCs and LNCs with BSC 40 cells. One million PBMCs or LNCs were added to BSC 40 cells in 6 well plates and incubated for 48 hours. Non-adherent cells were removed, and the remaining cells were observed for cytopathic effects (CPE). When CPE was not evident, adherent cells were removed from the plate with trypsin, and cell lysates were prepared by three cycles of freeze/thaw and sonication. These cell lysates were used to infect fresh 6 well-plates of

BSC 40 cells that were maintained in 37°C incubator for another 48 hours for recording VV CPE.

6. PCR Amplification of gag and B13R Sequences in PBMCs and LNCs of Vaccinated Macaques: The rVV sequences in the tissues from vaccinated macaques were identified by amplifying the B13R and the recombinant gag regions by PCR. DNA from 1 x 10^6 PBMCs and LNCs was isolated by DNA extraction column (QIAGEN, Inc., Santa Clarita, CA). One tenth of each DNA sample was used in a polymerase chain reaction with forward and reverse oligonucleotide primers complimentary to B13R and gag genes. Negative and positive controls were DNAs from PBMCs/LNCs from unvaccinated macaques and rVV-infected BSC 40 cells, respectively. Each PCR was performed with the 2.5 units of Taq DNA polymerase enzyme from Promega Corporation. After 37 cycles of PCR, one third of the PCR product was loaded on 1% agarose gel that was stained with ethidium bromide.

7. Lymphokine Activity in Plasma of Rhesus Macaques: Levels of interferon in macaques plasma was determined by a standard antiviral assay **(14)**. Briefly, plasma samples were diluted in duplicate in a 96 well plate, A549 cells (human lung cells) were added to each well, and the plate was incubated at 37°C. After a 24-hour incubation, 1 x 10^4 plaque forming units (pfu) of encephalomyocarditis virus was added to each well, and the plates incubated for 24 hours at 37°C. The plates were read under the microscope and stained. The units of interferon were expressed as the reciprocal of the last dilution that provided 50% or more protection against virus infection (CPE).

8. Immune Responses to VV, gp160, gag, and nef Antigens:

(a) Anti-VV Antibody Titers by Plaque Reduction Assay: Antibodies induced by the vaccine vector were measured by VV plaque reduction assay on BSC 40 cells **(15)**. Four-fold serial dilutions of plasma (in duplicate) were mixed with a predetermined amount of VV. The virus antibody mixture was incubated for 1 hour at 37°C and then used to infect BSC 40 cells to detect virus infectivity. Antibody titers are expressed as the reciprocal of the highest sample dilution where the number of plaques is reduced by at least 50%

(b) Anti-gp160, -gag, and -nef by Western Blot Analysis: Plasma samples obtained two weeks post-primary vaccination usually have very low levels of anti-SIV antibodies. To detect these low level antibodies in plasma 2 WPV, we transferred the capture antigens (gp160, gag, nef) to Immobilon-P (PVDF) membranes for western blot analysis **(16)**. One blot was prepared and adsorbed with a 1:100 dilution of each serum sample in 3% blotto (non-fat dry milk in Tris.HCl, NaCl buffer). Anti-monkey Ig conjugated to horseradish peroxidase (Cappel Inc., Alexandria, VA) was used as the second antibody (diluted to 1:2,000 in 3% blotto). The color was developed by adding 1% AEC in acetate buffer (pH 5.5) and hydrogen peroxide.

c. Antibody to gp160, -gag, and -nef measured by ELISA: These ELISAs contained the following capture antigens expressed in baculovirus vectors: (1) SIVmac p55gag, (2) SIVmac gp160 env, and (3) SIVmac nef (16-17). Anti-monkey Ig conjugated to horseradish peroxidase (Cappel Inc., Alexandria, VA) was used as second antibody for these ELISAs. Two-fold serial dilutions of plasma were assayed in duplicate and mean values were calculated. Positive controls were plasma samples from macaques infected with uncloned SIVmac251. Negative controls were plasma samples from macaques never exposed to SIV. Antibody titers were expressed as the reciprocal of the highest sample dilution where optical density (OD) was at least double the negative control OD.

9. Lymphocyte phenotyping. PBMCs from macaques were stained with anti-human monoclonal antibodies to CD4 (phycoerithrin-conjugated OKT4, Ortho Diagnostic Systems Inc., Raritan, NJ) or to CD8 (Leu 2a-FITC, Becton Dickinson Immunocytometry Systems, San Jose, CA)) as instructed by the manufacturers, and immunofluorescence was measured with a dual-laser flow cytometer (FACSCAN, Becton Dickinson).

RESULTS AND DISCUSSION

1. Intramuscular Vaccination of Juvenile Rhesus Macaques with rVV is Safe:

This study used a total of twelve juvenile rhesus macaques placed in three groups as outlined in Table I. The first group consisted of five animals which were vaccinated with vHuγ/SIVgen, a Wyeth recombinant that expresses the human interferon gamma, and the SIV gag, env, and nef genes. The second group had five macaques that received a similar dose of vSIVgen, a Wyeth recombinant that expresses the SIV gag, env, and nef genes. The third group had two control animals that received wild type Wyeth VV (vWY). Animals were inoculated I/M with one ml of sterile JME containing 10^7 pfu of the corresponding VV. After 8 weeks, the animals were re-vaccinated with the respective viruses and same dose and route (Table I)

Table: I.   *Vaccination of juvenile rhesus macaques with recombinant vaccinia viruses*

Group #	Animal	1° Vaccination	2° Vaccination	Dose/Route
1	MMU 27436	vHuγ/SIVgen	vHuγ/SIVgen	10^7 pfu/IM
	MMU 27672	vHuγ/SIVgen	vHuγ/SIVgen	10^7 pfu/IM
	MMU 27991	vHuγ/SIVgen	vHuγ/SIVgen	10^7 pfu/IM
	MMU 28067	vHuγ/SIVgen	vHuγ/SIVgen	10^7 pfu/IM
	MMU 28243	vHuγ/SIVgen	vHuγ/SIVgen	10^7 pfu/IM
2	MMU 28281	vSIVgen	vSIVgen	10^7 pfu/IM
	MMU 28358	vSIVgen	vSIVgen	10^7 pfu/IM
	MMU 28401	vSIVgen	vSIVgen	10^7 pfu/IM
	MMU 28472	vSIVgen	vSIVgen	10^7 pfu/IM
	MMU 28703	vSIVgen	vSIVgen	10^7 pfu/IM
3	MMU 29116	vWY	vWY	10^7 pfu/IM
	MMU 29117	vWY	vWY	10^7 pfu/IM

We decided to vaccinate the rhesus macaques with rVV by I/M inoculations rather than I/V. The intramuscular inoculations were performed for the following reasons: 1) There is a slight probability of systemic VV infection complication of I/V inoculation of rVV especially in individuals with a compromised immune system, although I/V inoculation of VV in SIV-infected macaques did cause any complications as reported last year. 2) In a comparative study in cattle, I/M inoculations of rVV expressing the surface glycoproteins of rinderpest virus were superior to intradermal (I/D) vaccinations in inducing immune responses to the antigens and providing

sterilizing immunity to 1,000X lethal challenge with rinderpest virus **(unpublished data)**. 3) Moreover, I/M vaccination of VV was used in some human vaccinee populations during the smallpox eradication program.

Eating patterns, elimination, and behavior of all macaques were noted to be normal on a daily basis. No local or systemic reactions to the vaccine were recorded. Once or twice a month, a physical examination was performed to measure body weight. No indication of lymphadenopathy and splenomegaly was noticed by palpitation. Peripheral blood was collected immediately prior to vaccination; differential blood counts and chemistries (CBC) were found in normal range except a slight rise in the lukocytes in response to VV infection. None of the animal exhibited thrombocytopenia and anemia consequent to the vaccination. All of these procedures and diagnostic tests were performed by veterinary staff at the CRPRC at U. C. Davis.

2. Resolution of VV infection in PBMCs and LNCs Rhesus Macaques:

One major concern in the use of live VV as a vaccine is the safety to vaccinees and contact populations of humans and animals. In this study, we followed the co-culture assays and polymerase chain reaction to determine the fate of rVV in PBMCs and lymph node cells (LNCs) of vaccinated macaques. PBMCs from all twelve vaccinated macaques were co-cultured with BSC 40 cells, a cell line highly susceptible to VV infection. No extensive CPE or individual virus plaques were observed on BSC 40 cell monolayer. Similar studies with LNCs are in progress. We could not isolate the rVV or vWY from PBMCs collected as early as one week post-primary and two weeks post-secondary vaccination in all twelve macaque.

We resorted to a more sensitive technique, PCR to identify the presence of VV in PBMCs and LNCs from vaccinated macaque since VV is a highly cell associated virus. We were able to identify the presence of rVV nucleotide sequences one week after primary vaccination.

The gag and B13R regions of the rVV genome were amplified from total DNA extracted from PBMCs and LNCs. In our laboratory, the gag region is commonly used to identify SIV in virus, cell, and tissue preparations from cultured cells and animal tissues. The B13R open reading frame is present in the VV *Hind* IIIB genomic region close to the right inverted terminal repeat. This immune-modulating gene codes for a serine protease inhibitor (serpin) homolog **(18)**. Our laboratory has recently cloned and characterized the B13R gene of VV as part of an NIH-sponsored project. The oligonucleotide B13R primers have been used successfully yielding DNA bands of the expected size (1.1 kbp), whose identity was confirmed by cloning, restriction endonuclease analysis, and/or sequencing. Consequently, these primers were chosen to amplify the B13R region of the VV genome present in the PBMCs and LNCs of macaques vaccinated with rVVs.

In PBMCs from 1 WPV, one animal (MMU 28067) was positive for the presence of the gag gene; all others were negative. None of the samples were positive for B13R sequences (Figs 1 & 4). This suggests that VV infection in PBMCs was resolved one week post-inoculation in rhesus macaques. The gag oligonucleotide primers are more sensitive than B13R primers in identifying the rVV DNA sequences in tissues. When LNCs were assayed for the presence of

these two gene sequences, 4 of 5 animals vaccinated with vSIVgen and none of the animals vaccinated with vHuγ/SIVgen were positive for gag sequences 1 WPV(Fig. 2). These results suggest that VV infection is resolved more rapidly in PBMCs than in LNCs, possibly by different mechanisms. Moreover, the expression of IFNγ by the rVV appeared to aid in earlier resolution of the infection in lymph nodes of macaques.

Two weeks after the second vaccination (first boost) with rVVs, PBMCs from all 10 macaques were negative for the virus as determined by amplification of gag region. These samples are being processed for B13R region amplifications by PCR. From limited results available, the rVV infection was completely resolved in these primed macaques within two weeks after the boost (Fig 3).

3. Immune responses of Rhesus Macaques to VV:

All vaccinated animals were positive for anti-VV antibodies as determined by a plaque reduction assay (Table II). The anti-VV antibody titers ranged from <4-64 after primary immunization. At 8 WPV, the mean anti-VV titer was 18.4 ± 26 in vHuγ/SIVgen-vaccinated macaques compared to 35.2 ± 28 in vSIVgen-vaccinated macaques (Table II). This may be due to the lower replication of vHuγ/SIVgen and an indication of attenuation of VV by the expression of IFN-γ during primary infection. However, after the second vaccination with rVV, the mean anti-VV titer in vHuγ/SIVgen-vaccinated macaques was approximately twice that in vSIVgen-vaccinated macaques (Table II). This increase may be due to the immune enhancing activity of the IFN-γ. In our laboratory, we have seen immune enhancement by IFN-γ in cattle and mice (4). The antibody responses to gp160 may confirm the immune enhancing activity of IFN-γ when we complete the end point titers for antibody to gag, gp160, and nef which is in progress. Upon revaccination with rVV, there appears to have been a replication of the rVV in the macaques because a 5-20 fold increase in anti-VV responses was observed 2-8 weeks after the second inoculation (Table II).

Table: II.   *Anti-VV antibody titers in plasma of rhesus macaques vaccinated with rVV by virus plaque reduction assay*

Animal #	Day 0 1° Vaccine	1 WPV	2WPV	4 WPV	8 WPV 2° Vaccine	10 WPV	12 WPV	16 WPV
MMU27436	<4	<4	<4	4	4	200	100	200
MMU27672	<4	<4	4	8	4	1600	400	400
MMU27991	<4	<4	4	4	4	400	200	400
MMU28067	<4	<4	4	8	64	400	400	800
MMU28243	<4	<4	8	8	16	800	400	100
MEAN ± SD					18.4 ± 26			380 ± 268
MMU28281	<4	<4	8	4	8	200	800	200
MMU28358	<4	8	4	4	64	128	400	100
MMU28401	<4	<4	<4	32	64	400	800	100
MMU28472	<4	<4	8	16	8	400	400	100
MMU28703	<4	4	16	64	32	1600	800	400
MEAN ± SD					35.2 ± 28			180 ± 130
MMU29116	<4	<4	4	4	<4	800	400	200
MMU29117	<4	<4	16	64	32	400	800	800
MEAN ± SD					ND			500 ± 424

WPV, weeks post-vaccination; SD, standard deviation; ND, not done.

4. Immune responses of Rhesus Macaques to SIV Antigens (gag, gp160, nef):

All macaques in first and second group responded to primary vaccination by producing the antibodies to SIV antigens. We expected very low titers of anti-gag, -gp160, and -nef antibodies during the first two weeks after the primary vaccination with rVV, thus western blots were used to detect antibodies against the respective antigens. Only two animals (MMU27672, MMU27436) had anti-gp160 and anti-gag antibodies by 2 WPV. On the 8th WPV, macaques received the first boost with the same dose and route of respective rVVs. All the animals except

-13-

the control animals had high titers of antibodies to SIV gag, gp160, and nef antigens (Table III). We will analyze the antibody data after having endpoint titers from all the animals. The raw data suggest a considerable anti-gp160 anamnestic response in all rVV-vaccinated macaques, although there may not be significant differences in the anti-gag and anti-nef titers before and after the first boost (Table III).

Table: III.   *Anti-gag, -gp160, and -nef antibody titers in plasma of rhesus macaques vaccinated with rVV by ELISA*

Animal #	Anti-gag titers		Anti-gp160 titers		Anti-nef titers	
	8 WPV	16 WPV	8 WPV	16 WPV	8 WPV	16 WPV
MMU27436	1024	1024	512	>1024	>1024	512
MMU27672	>1024	>1024	>1024	>1024	>1024	>1024
MMU27991	>1024	1024	256	>1024	256	512
MMU28067	1024	1024	>1024	>1024	256	512
MMU28243	1024	1024	>1024	>1024	256	1024

MMU28281	1024	1024	512	>1024	256	512
MMU28358	1024	1024	512	>1024	256	1024
MMU28401	1024	1024	512	>1024	512	1024
MMU28472	1024	1024	512	>1024	>1024	1024
MMU28703	>1024	>1024	>1024	>1024	512	>1024

MMU29116	<8	<8	<8	<8	<8	<8
MMU29117	<8	<8	<8	<8	<8	<8

WPV, weeks post-vaccination

5. Lymphokine Responses of Rhesus Macaques Vaccinated with the rVV:

Vaccinated macaques exhibited low IFN-γ antiviral activity (<10-100 IU/ml) in their

plasma during first 8 weeks post-vaccination (WPV). ELISA will also be done to confirm the presence of IFN-γ in the plasma of these animals. This test was done to detect any IFN-γ from the rVVs as well as to monitor the immune responses to vaccination. We have previously noted that an increase in antiviral activity in the plasma of SIV-infected macaques sometimes precedes final illness from SAIDS, so establishing the basic levels of antiviral activity in plasma will be useful for evaluation of this activity after challenge with virulent SIV.

Table: III.   ***Anti-viral activity (IU/ml) in plasma of rhesus macaques vaccinated with rVV by ELISA***

Animal #	DAY 0 1° Vaccine	1 WPV	8 WPV (Boost)	10 WPV
MMU27436	10	10	32	32
MMU27672	<10	<10	32	32
MMU27991	<10	32	32	<10
MMU28067	10	32	10	32
MMU28243	32	32	32	32
MMU28281	32	32	32	32
MMU28358	<10	32	32	32
MMU28401	<10	32	<10	32
MMU28472	<10	32	<10	32
MMU28703	<10	32	<10	32
MMU29116	100	<10	32	32
MMU29117	<10	32	100	32

WPV, weeks post-vaccination.

CONCLUSIONS

We can make the following conclusions from our current results:

1. All animals were inoculated I/M with one ml of sterile, cold JMEM containing 10^7 pfu of the corresponding VV. We decided to vaccinate the rhesus macaques with rVV by I/M inoculations rather than I/V since, in a comparative study in cattle, I/M inoculations of rVV expressing the surface glycoproteins of rinderpest virus outperformed the traditional I/D route in eliciting immune responses to the antigens and provided sterilizing immunity to higher challenge doses of virulent rinderpest virus. Moreover, the I/M route vaccination of VV was used safely and effectively in some human vaccinees during the smallpox eradication program. Additionally, there is a slight probability of systemic VV infection complication of I/V inoculation of rVV especially in individuals with a compromised immune system, although I/V inoculation of VV in SIV-infected macaques did cause any complications as reported last year.

2. Although the primary antibody response to rVVs expressing IFN-γ was lower than that to rVVs not expressing this lymphokine, the anamnestic response to VV after the second vaccination suggests that: 1) The first vaccination did not induce a sterilizing immunity to VV and 2) Expression of IFN-γ increased the antibody response to VV and gp160, suggesting that IFN-γ has a detectable immune enhancing activity on the anamnestic antibody response to some antigens.

3. All macaques in first and second group responded to primary vaccination by producing the antibodies to SIV antigens. By 8 WPV, all animals except controls had high titers of antibodies to the gag, gp160, and nef antigens of the SIV. After the second vaccination, animals had higher anamnestic responses to gp160 than to nef and gag antigens. These results indicate that the rVVs and the I/M route used in this study have induced high anti-SIV responses in macaques. We expect that the proposed future vaccinations with VLPs will enhance these responses further.

4. No infectious VV was detected by coculture of PBMCs and LNCs with the highly susceptible cell line BSC 40. This suggests that the amount of virus circulating in the animal by one week after primary and two weeks after secondary vaccination is very low. This result implies that the chance of accidental infection by contact is very low. The PCR results suggest that there is a very low level of virus still present in the animals after vaccination and that: 1) The virus expressing IFN-γ is cleared more rapidly than virus not expressing the lymphokine, increasing the attenuation and safety of the vaccine. 2) The virus is cleared more slowly in the LNCs than in PBMCs. This suggests that the theories about virus retention in lymph nodes as a method of maintaining the immune response to antigens may have some validity and perhaps the mechanisms of virus clearance differ in these two parts of the body.

6. In conclusion, the vaccine regimen proposed in the original project has induced good antibody responses to SIV antigens to date. We will be analyzing the cell-mediated immune responses in these macaques next year. Vaccinated macaques will be boosted with gag/env VLPs produced in recombinant baculoviruses and all macaques will be challenged with pathogenic SIV_{mac251} two weeks after the final boost. We will correlate the outcome with the type (T_H1 or T_H2) of immune responses generated to the SIV antigens co-expressed with IFN-γ. The progress of the work is on schedule as outlined in the proposal.

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Amplification of gag sequences of rVV from PBMCs
of macaques vaccinated with vHuγ/SIVgen, vSIVgen, and VV
by polymerase chain reaction
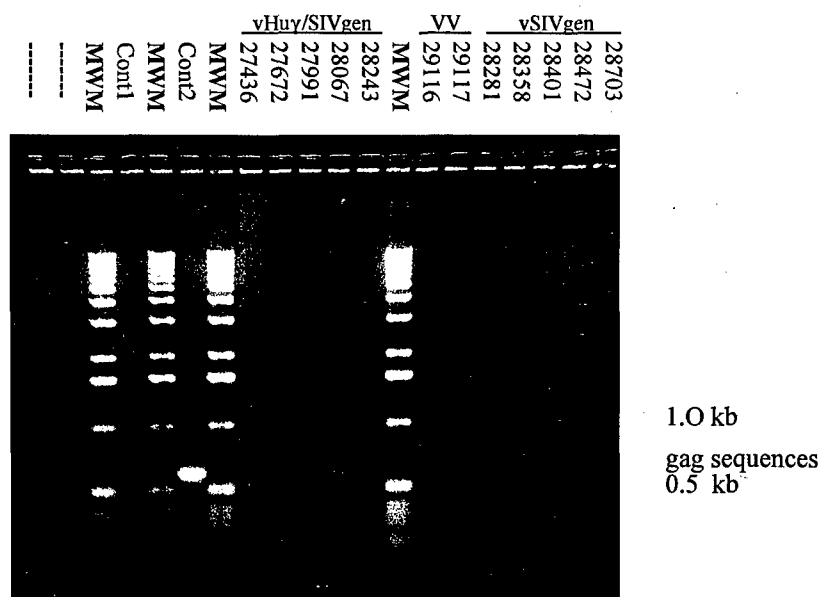## (1 week post-primary vaccination)

Fig. 1: DNA from 1 x 10⁶ PBMCs was isolated by DNA extraction column (QIAGEN, Inc., Santa Clarita, CA). One tenth of each DNA sample was used in a polymerase chain reaction (PCR) with oligonucleotide primers complimentary to the gag region of rVV. One third of the PCR product was then loaded on a 1% agarose gel that was stained with ethidium bromide. Negative and positive controls were used and are labeled as Cont1 and Cont2 respectively.

Amplification of gag sequences of rVV from LNCs
of macaques vaccinated with vHuγ/SIVgen, vSIVgen, and VV
by polymerase chain reaction
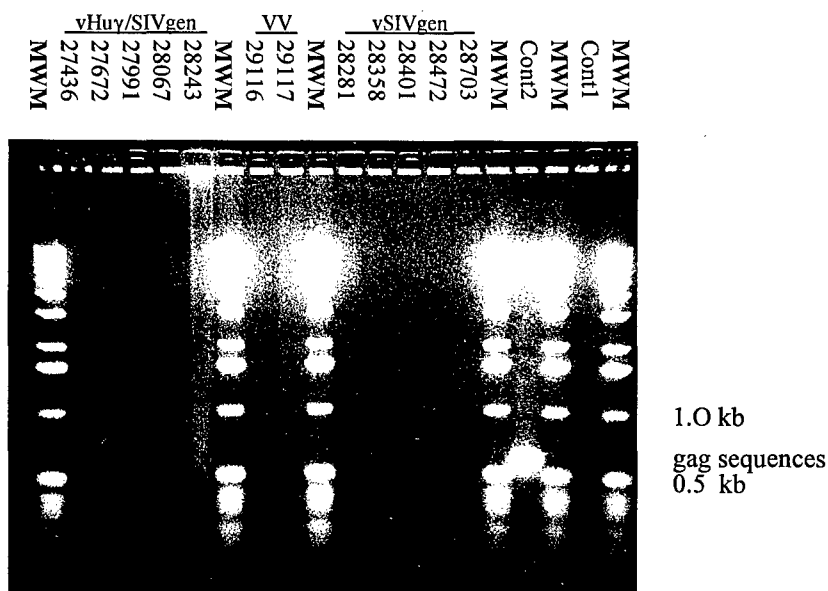## (1 week post-primary vaccination)

Fig. 2: DNA from 1×10^6 LNCs was isolated by DNA extraction column (QIAGEN, Inc., Santa Clarita, CA). One tenth of each DNA sample was used in a polymerase chain reaction (PCR) with oligonucleotide primers complimentary to the gag region of rVV. One third of the PCR product was then loaded on a 1% agarose gel that was stained with ethidium bromide. Negative and positive controls were used and are labeled as Cont1 and Cont2 respectively.

**Amplification of gag sequences of rVV from PBMCs
of macaques vaccinated with vHuγ/SIVgen, vSIVgen, and VV
by polymerase chain reaction
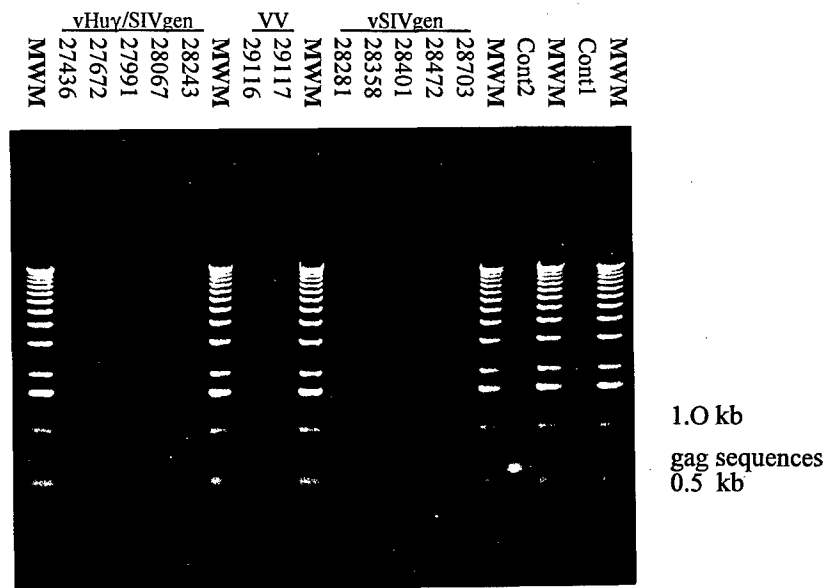(2 weeks post-secondary vaccination)**

1.0 kb

gag sequences
0.5 kb

Fig. 3: DNA from 1 x 10⁶ PBMCs was isolated by DNA extraction column (QIAGEN, Inc., Santa Clarita, CA). One tenth of each DNA sample was used in a polymerase chain reaction (PCR) with oligonucleotide primers complimentary to the gag region of rVV. One third of the PCR product was then loaded on a 1% agarose gel that was stained with ethidium bromide. Negative and positive controls were used and are labeled as Cont1 and Cont2 respectively.

Amplification of B13R sequences of rVV from PBMCs
of macaques vaccinated with vHuγ/SIVgen, vSIVgen, and VV
by polymerase chain reaction
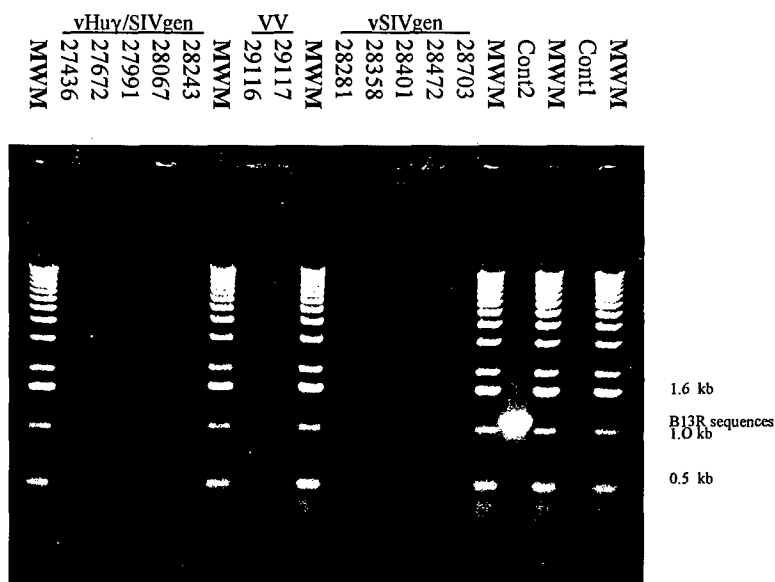## (1 week post-primary vaccination)

Fig. 4: DNA from 1×10^6 PBMCs was isolated by DNA extraction column (QIAGEN, Inc., Santa Clarita, CA). One tenth of each DNA sample was used in a polymerase chain reaction (PCR) with oligonucleotide primers complimentary to the B13R sequences of rVV. One third of the PCR product was then loaded on a 1% agarose gel that was stained with ethidium bromide. Negative and positive controls were used and are labeled as Cont1 and Cont2 respectively.

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