Delipidated Retroviruses as Potential Autologous Therapeutic Vaccines—A Pilot Experiment

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This pilot experiment in a simian immunodeficiency virus (SIV) chronic infection model aimed at extending our previous findings that vaccination with delipidated SIV resulted in more potent and diversified antiviral responses (1). Macaques chronically infected with SIVmac239 treated with antiretroviral therapy (ART) were vaccinated with autologous delipidated virus via consecutive lymph node targeted immunizations-1, 1 and 10 μg of virus spaced monthly. Results showed all animals had lasting viral load reduction approaching 1 log compared to set-point, and disease delay. Delipidation may enhance processing/presentation of viral antigen eliciting potent antiviral control even at such late infection stage. Exp Biol Med 233:732–740, 2008

Key words: therapeutic vaccination; SIV; delipidation

Introduction

Increasingly potent and effective spectrum of antiretroviral drugs (HAART) are currently being used to prevent onset of AIDS in HIV infected patients. Although side-effects of HAART have generally become more manageable, the toxicity of this therapy remains a significant factor in patient's non-compliance, leading to selective emergence of drug resistant isolates (2–4). Furthermore, cessation of HAART leads to rapid viral load rebound and loss of CD4 cells (5, 6). In addition, it is important to note that even prolonged HAART is unable to restore full immune competency. Therefore, the need for alternative therapeutic strategies that can reduce viral loads by itself or in concert with HAART remains a priority.

Therapeutic vaccines for human immunodeficiency virus (HIV) face the enormous challenge of inducing cellular and humoral immune responses in the face of high viral loads in blood and in tissues. The major obstacle for a therapeutic vaccine is not the quantitative lack of antigen to stimulate the immune system, rather the constant presence of high levels of antigen appears to alter the quality of the immune response (7–9). Thus, one of the goals of therapeutic immunization is to either alter the quality of the response or redirect it towards relevant protective epitopes (10). The use of vaccination during HAART has shown some promise as an approach to boosting immune responses. Examples of such success are an ALVAC/IL-2 based strategy which succeeded in prolonging viremia rebound following cessation of HAART when compared to non immunized controls (11). The benefit from such therapeutic immunization has, however, appeared limited at best. To date, the most successful approach for a therapeutic vaccine has been an ex vivo immunization strategy involving the use of SIV pulsed autologous rhesus macaque dendritic cells (DC) which were infused back to the test animals (12) and a repeat of this approach in a human cohort (13). The pre-clinical study utilized rhesus macaques which were at the early chronic infection stage (day 60 post infection) of SIVmac251 infection. While the treatments resulted in significant viral load reduction, the model utilized relatively healthy animals which were infected for only 2 months prior to vaccination. Such an early time frame involves animals which were likely to have a relatively preserved immune system.

An alternative approach has been loading autologous dendritic cells with peptides representing the predominant viral sequences present within the patient (14). However, this immunization strategy had no measurable effect on the viral load set-point, although modest peptide specific

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immune responses were observed in some of the patients. Of note, this approach utilized peptide sequences encompassing only the gag, reverse transcriptase, and protease genes. Thus it is reasonable to assume that the inclusion of other viral gene sequences such as the env gene could have led to a more effective viral load reducing immune response.

We have developed a novel therapeutic HIV vaccine strategy that utilizes delipidated autologous HIV as a vaccine. As a validation of this strategy, we have previously shown that delipidated SIV significantly enhanced immune responses in a prime-boost non-infectious murine model (1). As a follow up, we tested this strategy in a pathogenic infectious model with the hypothesis that vaccination with low doses of autologous, delipidated virus, when injected into the inguinal lymph nodes without the help of an adjuvant in SIV infected rhesus macaques, would result in enhanced control of viral replication upon cessation of antiretroviral PMPA ((R)-9-[2-(phosphonomethoxy)propyl]adenine) chemotherapy. We utilized an animal cohort of four SIV chronically infected rhesus macaques (>1 year post infection), with high sustained viremia and low CD4 counts, in a pilot experiment. All four animals were treated with PMPA and while under PMPA therapy each animal was immunized with autologous delipidated SIV. Such autologous vaccination led to an increase in the virus specific immune response. Upon cessation of PMPA, the plasma viral load rebounded initially back to the previous set-point. However, shortly thereafter, a consistent and sustained decline of the plasma viral loads was observed. The survival data of these three SIV infected and immunized monkeys when compared to a set of 9 historical control macaques that were infected with the same SIV stock and went through the same cycle of transient PMPA therapy, suggested a marked improvement in the survival time of the vaccinated monkeys as compared with the historical controls.

Materials and Methods

Experimental and Vaccination Protocol. A cohort of four Indian rhesus macaques (had served as control animals in other pre-clinical trials) in the chronic phase of SIVmac239 infection was included in the present study. At the time of study inception, opportunistic infection and/or AIDS defining clinical events had been documented in all four animals leading to diarrhea and weight loss. The protocol utilized is summarized in Figure 1. Briefly, SIV was harvested in vitro from the CD8 depleted PBMC from each of the three macaques. Of note, only autologous CD8 depleted PBMC were used to derive each monkey's own SIV batch to avoid the selective outgrowth of minor non-representative viral isolates in vitro. Once approximately 50 μg of total viral protein was obtained, virus was purified by two consecutive ultracentrifugation steps. The delipidation procedure was performed as previously described (1). Three vaccine doses were aliquoted; two doses containing 1 μg and one dose containing 10 μg of viral protein. In efforts to facilitate the generation of virus specific immune response against the vaccine, all three animals were treated with PMPA administered daily starting 30 days prior to, and during the period of vaccination to lower viral loads. The vaccination protocol consisted of three boosts of 1 μg, 1 μg and 10 μg spaced monthly and delivered into inguinal lymph nodes. Thirty days after the last vaccination of 10 μg, PMPA was withdrawn and virological and immunological parameters were measured.

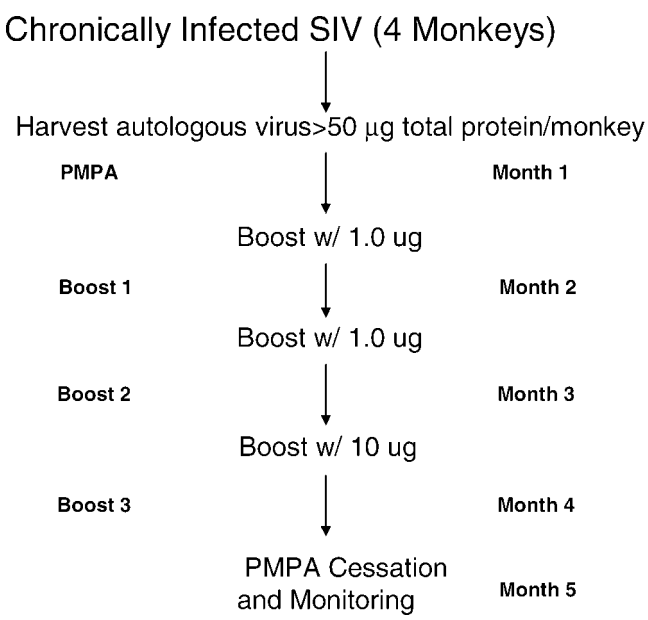

Figure 1. Schema of the Vaccination Protocol.

Intracellular IFN-γ Response Evaluation of Cell Mediated Responses. The standard protocol consisted of a 12 hr re-stimulation of 1×10^6 PBMC with pools of peptides spanning the entire SIV Gag and SIV Env proteins, each pool containing 7–9 peptides. Positive control samples consisted of PBMC stimulated with the mitogens PMA/ionomycin and PHA; negative controls consisted of cells incubated in media alone or with the irrelevant Ovalbumin specific peptide SYNFEKL. After a 2 hr stimulation with the antigen, Brefeldin A was added for the remaining time of re-stimulation. Each of the PBMC samples was then stained for CD3, CD4, and CD8 and for intracellular interferon-gamma (IFN-γ). Frequencies of IFN-γ positive $CD4^+$ and $CD8^+$ T cells were analyzed using a FACS Calibur (Beckton Dickinson, Mountain View, CA).

Serology. *SIV EIA.* ELISA micro plates were adsorbed with 4 μg/ml purified SIVmac251 Env gp120 or Gag p55/well overnight in standard bicarbonate coating buffer, pH 9.6 at 4°C. Following a blocking step with PBS containing 1% bovine serum albumin and 0.05% Tween 20, sequential two-fold dilutions of the sera to be tested was added to duplicate wells of the plate. Both a pre-determined

positive and negative control samples were included with each assay and the plate incubated at 37°C for 2 hr. After washing the unbound antibodies, the plates were incubated for 1 hr at 37°C with an horseradish peroxidase-anti human (cross reactive with rhesus macaque) IgG conjugate (Sigma, St. Louis, MO), and later developed with tetra-methylene blue (TMB) substrate (Sigma) at room temperature. The reaction was stopped by the addition of 2N H_2SO_4 and the plates were read at a 450 nm wavelength using an ELISA reader (Molecular Devices, Sunnyvale, CA).

SIV Western Blots. For Western blot analysis, commercially available SIV western blot kits (Zeptometrix, Buffalo, NY) were utilized. The monkey sera to be assayed were diluted 1:100 in PBS and developed according to the manufacturer's instructions.

Autologous Virus Preparation. *CD8 Depletion.* Peripheral blood mononuclear cells (PBMC) were purified using Ficoll-Hypaque (Pharmacia) gradients and the cells at the interface re-suspended to 10^7/ml with PBS/2% FCS. The PBMC were depleted of CD8+ T cells using magnetic beads coated with anti-CD8 antibodies (Pharmacia, San Diego, CA) at a concentration of 100 µl of the beads/ml of cells. Cells and beads were incubated for 30 min at 4°C with gentle rotation. Cells were then brought to a final volume of 5 ml and placed on a rotator for an additional 5 min. The test tube containing the PBMC was placed in a magnetic field, and the unbound PBMC in the supernatant fluid was harvested. An aliquot of these unbound PBMC was tested by flow cytometry and shown to de depleted of >90% CD8+ T cells.

PBMC Activation. Cells depleted of CD8 cells were then re-suspended in 10 ml of complete media with 5 µg/ml concanavalin A (ConA) and incubated at 37°C, 5% CO_2 for 3 days. Cells were then washed 1× with PBS and re-suspended to 10^6/ml in RPMI-1640 containing 10% FCS and 30 U rIL-2/ml. Viral harvest was begun 7 days post ConA activation. Supernatants were filtered through a 0.45µm filters and stored at −70°C. Viral capsid SIV p27 was quantitated using a commercial ELISA (Coulter, Fullerton, CA).

Viral Purification. When a sufficient amount of virus was harvested from an individual monkey, the virus containing supernatant fluids were pooled and ultra-centrifuged media in a 50.2 Ti rotor (Beckman Coulter, Fullerton, CA) at 40,000 *g* at 4°C for 2 hr. The pellets were re-suspended in 8.0 ml of ice-cold PBS and transferred to a 80 Ti rotor and centrifuged again spun at 38,000 *g* for 2 hr at 4°C. Finally, the pellet was re-suspended in 1 ml of ice-cold PBS and an aliquot tested for levels of total protein (Biorad) and SIV Gag p27 (Coulter).

DIPE Solvent Treatment. Purified SIVmac239 total protein (corresponding to 40 µg SIV p27 Gag protein) were diluted in phosphate buffered saline (PBS) containing 1% di-isopropyl ether (DIPE) (VWR, West Chester, PA) and the volume adjusted to 1 ml in Eppendorf microfuge tubes. Solvent treatment of the viral antigen preparation was performed for 20 min at room temperature, using end-over-end rotation. After centrifugation at 2000 *g* for 2 min, the solvent was removed by passage through an activated charcoal column. The virus was reconstituted to 1 ml using injection grade sterile water. Each solvent treated virus preparation was then aliquoted in 1 ml volumes containing either 1 or 10 µg of total SIV protein sufficient for the planned booster immunization and stored at −70°C until use.

Anti-Retroviral Therapy (PMPA). After sufficient virus for the study had been cultured from the PBMC of each of the infected animal, these progressor animals were started on antiviral chemotherapy using the NRTI PMPA (courtesy of Dr. M. Miller, Gilead Sciences, Foster City, CA, 20 mg/kg daily subQ) for a period of 15 weeks. It was reasoned that PMPA therapy would result in low to undetectable viral replication allowing for partial immune recovery and decrease the antigen burden to a level such that an immune response to the DIPE-treated material could be elicited and detected. The goal was to enhance and/or redirect the magnitude and diversity of antiviral responses towards viral variants that have evolved in a given host using auto-vaccination, while PMPA therapy was expected to protect newly activated antiviral CD4 T cells from viral destruction or impairment. After completion of the three projected immunizations (Fig. 1), the administration of PMPA chemotherapy was stopped. This allowed for the evaluation of the ability of the autologous vaccination with delipidated virus to enhance immune responses by monitoring the kinetics of viral rebound and for defining post therapy viral set-points.

Vaccination. Animals were inoculated bilaterally directly into the inguinal lymph nodes with 0.5 ml per injection site (total of 1 ml) for each immunization (15, 16). The vaccination timeline is illustrated in Figure 1. Briefly, animals were inoculated in the inguinal lymph node region with one dose/month of 1 µg, 1 µg, and 10 µg of delipidated SIV, respectively. One month after the third dose of 10 µg was administered, PMPA therapy was stopped, and viral loads, immunological parameters, clinical status and survival were monitored.

Analysis of Plasma Viral Loads. Determination of plasma SIV vRNA was performed by the Emory virology CFAR core, using real-time RT-PCR. The limit of detection of this assay was 10 vRNA copies/ml (17).

Cell-Mediated Immune Responses by Intracellular Cytokine Staining. Individual peptides spanning the entire coding region of SIVmac239 Env and Gag were either obtained from the AIDS Reagent Reference Program, or prepared by the Microchemical Facility at Emory University. The SIV Env peptides which number a total of 72 individual peptides were 25 mers each with 13 amino acids overlapping and the SIV Gag peptides, which number 62 individual peptides, were 20 mers each with 12 amino acids overlapping. A total of 17 peptide pools for Env and 16 for Gag were prepared in a grid fashion and each peptide pool used at a final concentration of 104 µg/well. Each well

contained the appropriate peptide pool in a 50 μl volume, 2×10^6 cells/well in a 100 μl volume and 1 μg/ml of unconjugated antibodies to CD49d and CD28 in a 50 μl volume. The plates were incubated for 2 hr at 37°C at 5% CO_2. Then 50 μl of Brefeldin A (Sigma, St. Louis, MO) at a stock of 20 μg/ml was added to each well, and the plates incubated overnight. The next day, 150 μl of media was carefully removed and replaced with 50 μl of media containing anti-CD4 PerCP and anti-CD8 FITC antibodies and incubated in the dark at 4°C for 30 min. Cells were washed and subjected to Perm wash/fix (BD Biosciences) for 20 min at 4°C. Cells were washed and Perm Wash solution was added and plates centrifuged again for 10 min. All wells then received 100 μl of anti-IFN-γ APC antibodies (Pharmingen), incubated at 4°C for 30 min. Plates were then washed and re-suspended with 200 μl of FACS buffer (BD Biosciences), and samples analyzed for the frequency of CD4 or CD8+ T cells that synthesized IFN-γ using a FACS Caliber (Beckman, San Diego, CA) and appropriate Cell Quest software.

SIV p55 and gp120 Enzyme Linked Immunosorbent Assay (ELISA). Recombinant SIV p55 (Protein Sciences Corp., Meriden, CT) and SIV gp120 (Advanced Bioscience Labs, Kensington, MD) were diluted to 5 μg/ml in ELISA buffer (50 mM Tris, pH 9.5) and 100 μl were coated/well overnight at room temperature in a 96-well plate. The plates were blocked with a 3% BSA-PBS solution for 2 hr at 37°C. After washing the plates, a serial dilution of the serum to be analyzed was added in a diluent buffer of 5% normal goat serum-PBS, and the plates incubated at room temperature for 60 min. After washing, 100 μl of a 1:5000 dilution of anti-monkey HRP conjugate (Sigma) antibody was added and the plates incubated for 45 min at room temperature in the dark. Wells were developed after washing, by adding 200 μl TMB Substrate (Sigma), and plates incubated in the dark for 30 min. The development was stopped by adding 50 μl 1N H_2SO_4. Plates were read in a plate reader (Molecular Devices, Sunnyvale, CA) at O.D. 450 nm.

Results

Viral Loads, CD4 Counts Pre- and Post-Vaccination. The chronically infected macaques selected for this study all exhibited stable viral load set-points $>10^4$ vRNA copies /ml of plasma and had been infected with SIVmac239 for over 12 months. The animals had all undergone a 28-day PMPA therapy starting at 69 days post infection (pi) (Fig. 2A) in the frame of a previous study with viral load set-points resumed shortly after cessation of the therapy, indicating that antiretroviral therapy in and by itself was unable to induce long-term viral control even when performed during early chronic infection. At the time of the second PMPA therapy, all four animals had already experienced bouts of bacterial diarrhea typical of opportunistic infection in SIV infected monkeys. Initiation of PMPA

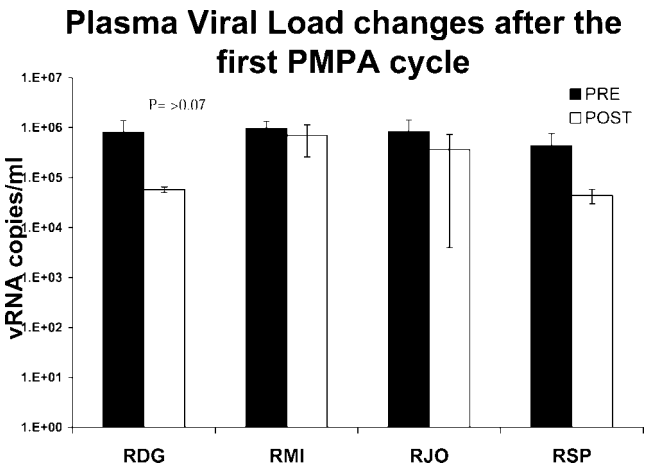

Figure 2A. Viral load kinetics in the four monkeys prior to enrollment into the present study, including a 28-day PMPA therapy. Data represents three timepoints pre-PMPA vs. three timepoints post-PMPA. Statistical analysis performed using the Student's *t* test.

therapy successfully controlled the plasma viral load to below the limits of detection in all monkeys except RSP (Fig. 2B). All four monkeys were then immunized with autologous delipidated virus and the PMPA therapy stopped. While a rebound of viral loads to the original set-points was documented in all four animals following PMPA cessation, these viral loads thereafter exhibited a general decrease 2–3 months after the rebound and this decrease was sustained over time. However, shortly after viral load decrease, monkey RSP developed bacterial infection and diarrhea and was euthanized. When viral loads obtained pre-vaccination were compared to 7 timepoints post PMPA cessation, a statistically significant reduction in viral load ($P < 0.001$) was observed in three out of four macaques (Fig. 2C). This decrease in viral load set-point was in sharp contrast to the return to stable viral load set-points following the initial early PMPA therapy (Fig. 2A) suggesting marked improvement of antiviral control in these animals in spite of the late timepoint pi. Furthermore, three out of four animals exhibited weight gains after cessation of PMPA and an absence of AIDS associated opportunistic infection. Absolute CD4 T cell counts were low at the time of PMPA initiation (<500 cells/μl) commensurate with the stage of infection in these animals. PMPA therapy induced a surge in CD4 T cell counts in all animals although the size of this surge appeared proportional to the absolute counts at therapy initiation. Except for monkey RDG, the absolute numbers of CD4+ T cells returned to pre-therapy levels in the remaining animals (Fig. 3A, 3B). While there was a trend to declining CD4 counts as disease progressed, the change was not significant. There were no significant detectable changes in CD8 counts during the study period in this cohort (data not shown).

Increased Cell Mediated Immune Responses in Vaccinated Macaques. The cell mediated immune responses were measured by the frequencies of CD4 and

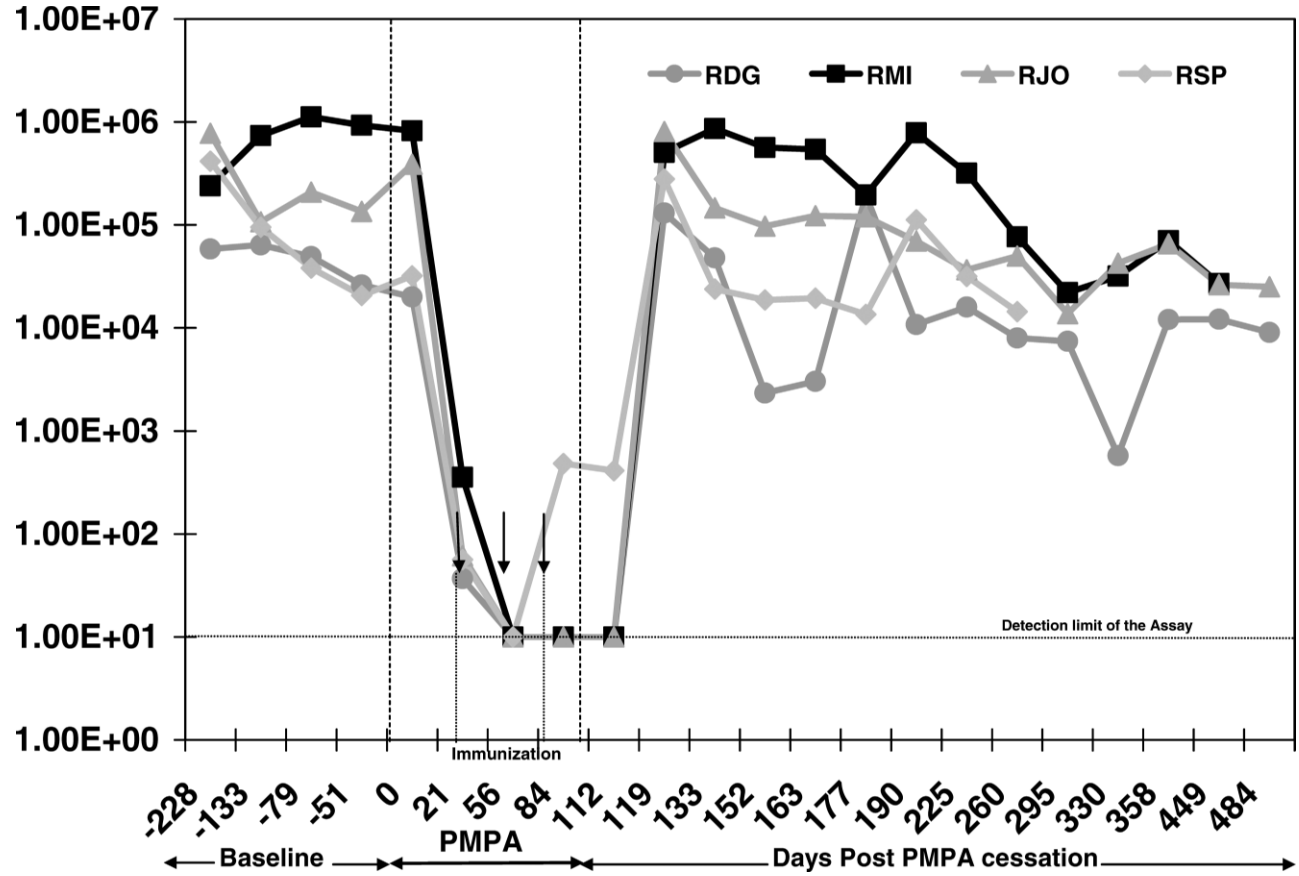

Figure 2B. Viral load kinetics during the Study for the four monkeys. After completion of the immunization schedule, PMPA was withdrawn and viral loads were monitored for post-PMPA changes. The limit of detection for this RT-PCR assay was 10 vRNA copies/mL.

CD8 T cells producing IFN-γ following re-stimulation with overlapping SIV Gag and Env peptide pools. A statistically significant enhancement of the breadth of CD8 responses to Env ($P = 0.01$) and Gag ($P = 0.005$) peptide pools was observed (Fig. 4A). Although there was an enhancement of the overall numbers of peptide pool responses in CD4 cells, the difference was not significant. A general mapping of the protein regions corresponding to the peptide pools inducing a response following immunization and PMPA cessation is illustrated in Figure 4B. Thus, a novel set of peptides specific response was noted in the post-vaccination PBMC samples as compared to the pre-vaccination data (Fig. 4B).

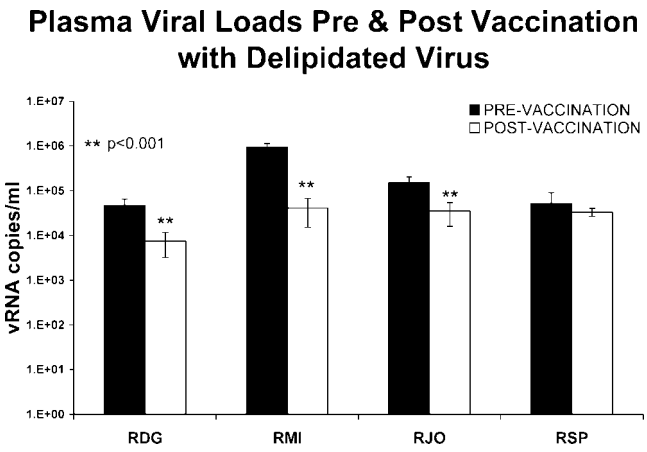

Figure 2C. Viral Load statistical analysis pre- and post-vaccination. Each bar represents the mean value of 3 measurements before PMPA therapy inception vs. 7 consecutive measurements post-vaccination. Statistical analysis performed using the Student's *t* test.

SIV p55 Gag and Env Antibody Responses. With the exception SIV Gag specific antibody responses in monkey RMI, the antibody O.D. (highest dilution for serum tested was 1:31,250) to SIV antigens transiently decreased or were maintained during the PMPA/immunization procedure. Following PMPA discontinuation, a general increase in Env antibody O.D. (highest dilution for serum tested was 1:31,250) was noted in all four macaques suggesting a surge in humoral responses also corresponding to the resurgence of viral load (Fig. 5A, 5B). This may be due to increased viral replication leading to higher amounts of viral proteins and perhaps enhanced helper cell function.

Survival of Vaccinated Macaques Significantly Enhanced. The survival of the vaccinated macaques was compared to a cohort of 9 chronically infected historical controls that were infected with the same SIV stock, with similar viral loads as our cohort. The Kaplan-Meier plot (Fig. 6) show a significant enhancement of survival in the vaccinated/treated group ($P < 0.006$) post infection.

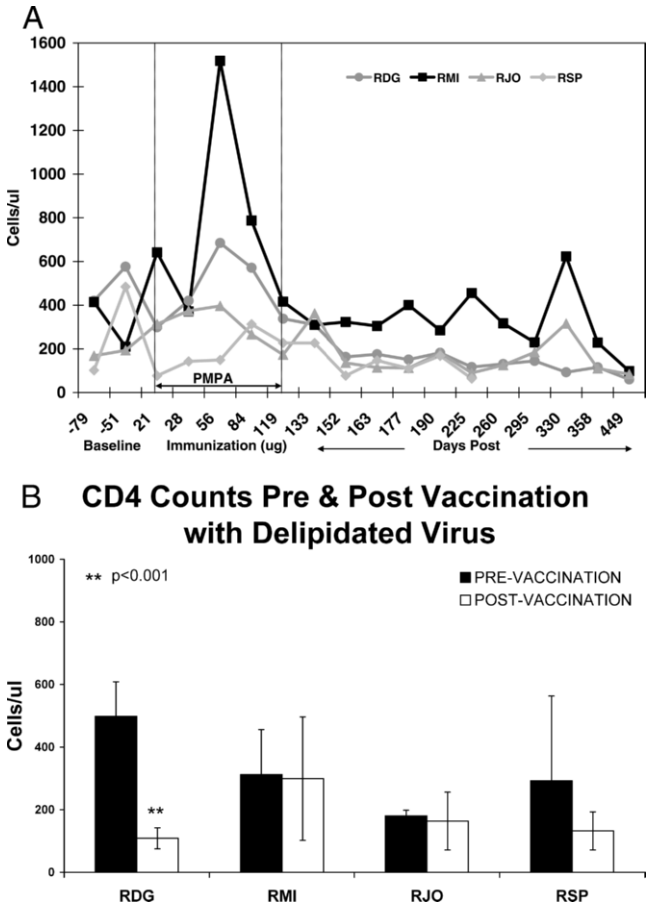

Figure 3. A: CD4 counts for the four animals during the study. After completion of the immunization schedule, PMPA was withdrawn and CD4 counts were monitored for post-PMPA changes. B: Statistical analysis (performed using the Student's *t* test) of the CD4 counts pre- and post-vaccination. Each bar represents the mean value of 3 measurements before PMPA therapy inception vs. 7 consecutive measurements post-vaccination (days).

Considering the chronic high viral loads prior to vaccination and low CD4 T cell counts in these animals, such clinical improvement was thus considered significant.

Discussion

In this proof-of-concept pilot experiment, the potential of delipidated retroviruses was investigated as a therapeutic vaccine strategy. Although the strategy was primarily tested for its safety, of interest was our finding that such vaccination in these SIV chronically infected macaques' enhanced survival, and reduced viral loads. In contrast, data obtained from the same animals treated with PMPA early post infection (Fig. 2A) or from a cohort of historical controls infected with the same virus dose and stock (18) showed sustained high viral load and shortened survival upon ART cessation. The vaccinated macaques had a transient increase of immune responses, while the antibody responses did not seem to show significant changes during the course of the study. Overall, two out of four macaques maintained their CD4 counts, which were <500 cells/μl prior to vaccination.

A

	CD4 ENVELOPE		CD8 ENVELOPE		CD4 GAG		CD8 GAG	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
RDg	7	**11**	8	**15**	9	**15**	6	**12**
RJo	5	**9**	10	**15**	6	**10**	5	**8**
RMi	7	**7**	11	**13**	12	**14**	8	**12**
RSp	1	**4**	14	**17**	15	**15**	7	**11**
Geo Mean	4	7	11	15	10	13	6	11
TTEST	0.1148		0.0147		0.1175		0.0050	

B

	ENVELOPE						GAG					
	CD4			CD8			CD4			CD8		
Region	C1	C2	Gp41	C1	C2	Gp41	P9	P17	P27	P9	P17	P27
RDG	X	X	X				X	X			X	
RMI	X	X	X		X	X	X	X		X	X	X
RJO	X	X	X	X	X	X		X	X			
RSP			X	X	X	X	X		X	X	X	X

Figure 4. A: Vaccination elicited significant increases (measured by Student's *t* test) in the number of CD8 peptide pools responses, as highlighted in bold. While vaccination induced an increase in the number of peptide pools responding in CD4 cells, the increase was not statistically significant. B: Vaccination elicited novel additional epitope responses, compared to pre-vaccination epitope responses. The analyses represent ICS analyses performed twice before PMPA inception vs. thrice post immunization and PMPA cessation.

During vaccination, there was a transient increase in the cell counts, but this increase was not sustained post PMPA cessation. The survival of the vaccinated macaques was compared to a cohort of 9 chronically infected historical controls that were infected with the same SIV stock, with similar viral loads as our cohort. Our initial analysis shows an enhancement of survival in the vaccinated/treated group post infection ($P < 0.006$, Fig. 6). Considering the chronic high viral loads prior to vaccination and low CD4 T cell counts in these animals, such clinical improvement was thus considered significant.

Several studies have expressed the importance of controlling viral loads for survival. In a recent publication, a predicted 1 log viral load reduction due to a non-sterilizing vaccination was shown to reduce mortality in the first 20 years after vaccination (19). In another study, mathematical computation using natural history data from over 300 HIV-1 sero-converters was utilized to show that a progressive increase in time to AIDS was associated with incremental decreases in viral loads. Thus, a reduction of viral load of 0.5 logs increased the time to AIDS progression by about 3 years, while a 0.75 log reduction of viral loads increased the time to AIDS progression by 6 years, and a 1.0 log reduction increased the time to AIDS progression by about 8 years (20).

The immune and genetic determinants of individual viral load set-points remain to be elucidated. Of interest is the fact that such set-points appear generally stable in any given individual. Thus, resetting such value to a lower level

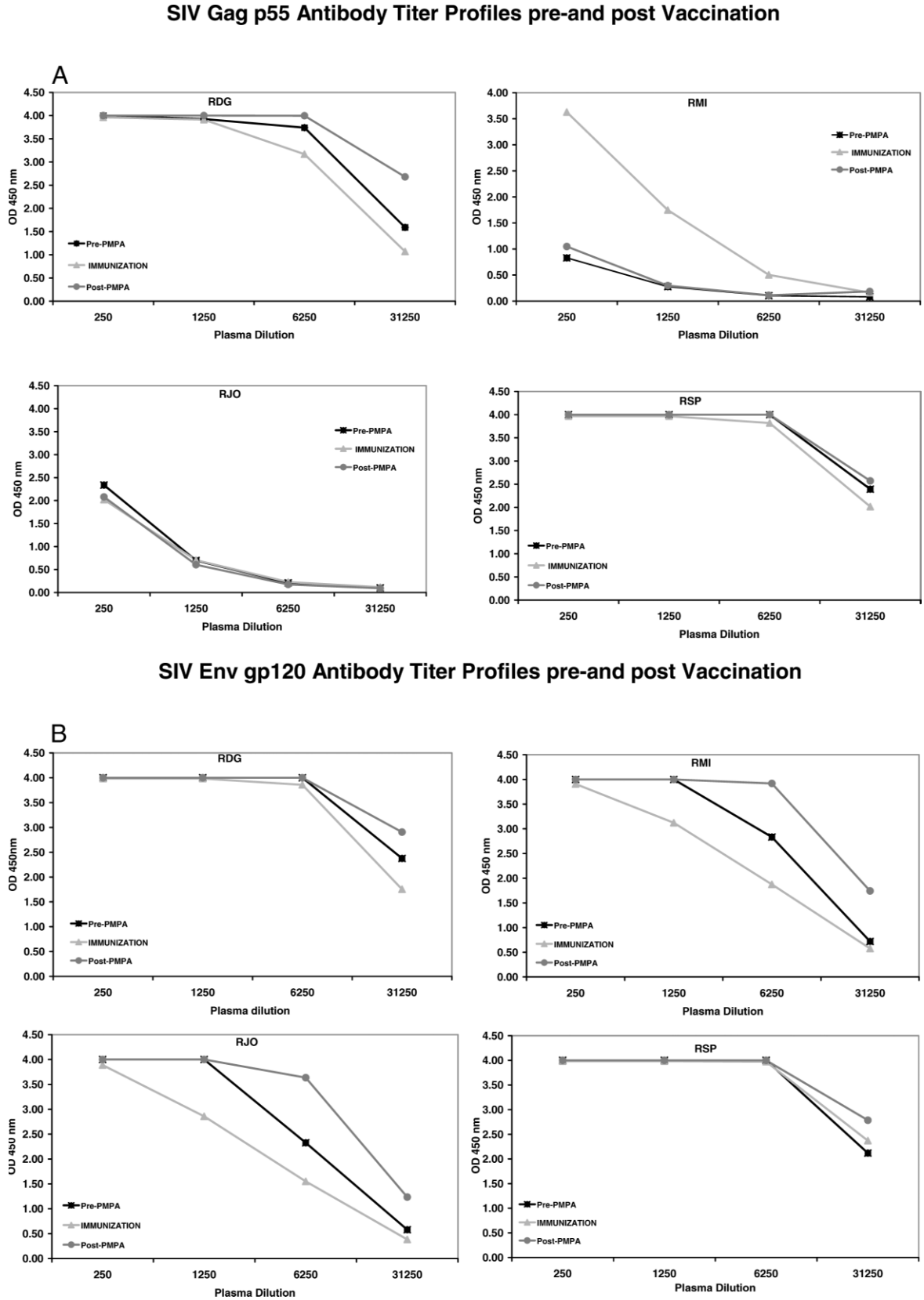

Figure 5. A: Antibody O.D. to SIV Gag during various stages of the study for the four animals. The immunization sample corresponds to 1 week post 10 μg delipidated SIV administration. The post-immunization timepoint is 20 weeks post PMPA cessation. B: Antibody O.D. to SIV gp120 during various stages of the study for the four animals. The immunization sample corresponds to 1 week post 10 μg delipidated SIV administration. The post-immunization timepoint is 20 weeks post PMPA cessation.

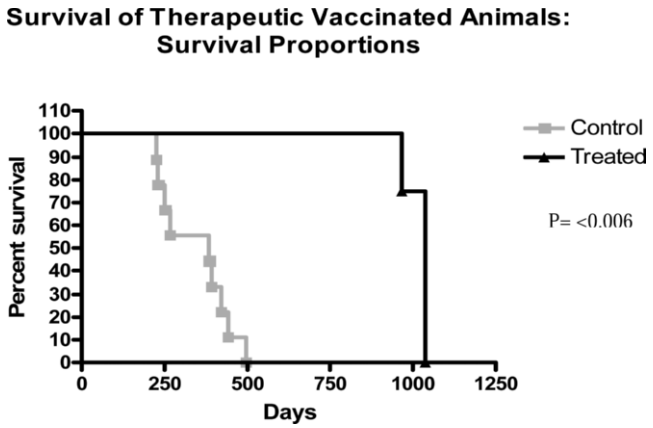

Figure 6. Kaplan-Meier Survival Plot indicating significant survival enhancement in the treated group.

has proven difficult in the SIVmac251 infected macaque even at earlier timepoints post infection or using prophylactic immunization (21).

The reasons for the encouraging data obtained in this pilot study are not fully understood. However, while current vaccine strategies focus on including either consensus sequences, center-of-the-tree sequences or several representative isolate sequences from one or more clades, our approach is uniquely tailored to the predominant isolates replicating in any given host. Thus, if the original antigenic sin plays a role in the lack of more effective control in chronic SIV infection, the proposed strategy may provide the immune system a temporal refocusing of immune responses aimed at the most relevant viral variants replicating before and likely after PMPA therapy. This concept is clearly supported by the observed surge in both the magnitude and breadth of cellular responses to the virus as well as humoral responses, even though the role of the latter appeared modest at best.

The second potentially important feature of this strategy relies on the alteration of immunogenicity brought about by the delipidation of virions. Although the precise mechanisms of such altered antigenicity remain to be identified, our preliminary immunization data in the Balb/c mouse clearly supports this concept, whereby delipidated SIV induced markedly different and higher SIV specific responses than untreated or even AT-2 treated virus (1). Unlike monkeys, mice "view" SIV as a nominal antigen instead of an infectious virus inducing immunodeficiency, and thus, we posit that delipidation results in enhanced antigen processing/presentation and/or T cell recognition. The precise delineation of such mechanism is currently in progress.

Although the data generated in this pilot experiment does not allow for the differentiation between the contributions of the use of autologous SIV antigens vs. delipidation, the effect of therapeutic immunizations in the context of SIV infection even when administered under ART has thus far been modest and generally not seen at a late stage of infection. However, the effects of continuous viral replication on immune exhaustion have been amply demonstrated in HIV-1 infected patients, especially the loss of IL-2/IFN-γ, and IL-2 secreting T cells within the central memory T cell subset (22). Vaccination under ART has been thought to facilitate the generation of heterogeneity in T cell responses (23).

While the experiment reported herein tested the effects of autologous therapeutic vaccinations on long-term chronically infected and relatively immuno-compromised rhesus macaques, a new study is currently being carried out aimed at testing the hypothesis that autologous therapeutic vaccination during early chronic infection would have more pronounced benefits. This view is supported by the current approach being utilized for HIV vaccination which involves beginning intervention during early chronic HIV infection. The rationale for this approach is based on the fact that the CD4 and CD8 repertoire have not yet undergone extensive exhaustion and deletions during this time period, and we hypothesize that such an approach might provide an even more profound clinical benefit to the infected host with immune responses that have not been as extensively compromised.

While the data clearly showed benefit in improving cell mediated antiviral responses predominantly in the CD8 compartment, antibody responses may have been improved also, e.g. in terms of affinity and perhaps neutralizing antibody titers rather than binding titers, two parameters that were not tested. However, the SIVmac239 isolate is notoriously difficult to neutralize and induces only low levels of neutralizing antibodies in vivo. Thus, we submit that the investigation of the effect of therapeutic immunization using delipidated virus on humoral responses will require further study.

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