Control of Viremia and Prevention of AIDS following Immunotherapy of SIV-Infected Macaques with Peptide-Pulsed Blood

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Abstract

Effective immunotherapies for HIV are needed. Drug therapies are life-long with significant toxicities. Dendritic-cell based immunotherapy approaches are promising but impractical for widespread use. A simple immunotherapy, reinfusing fresh autologous blood cells exposed to overlapping SIV peptides for 1 hour ex vivo, was assessed for the control of SIVmac251 replication in 36 pigtail macaques. An initial set of four immunizations was administered under antiretroviral cover and a booster set of three immunizations administered 6 months later. Vaccinated animals were randomized to receive Gag peptides alone or peptides spanning all nine SIV proteins. High-level, SIV-specific CD4 and CD8 T-cell immunity was induced following immunization, both during antiretroviral cover and without. Virus levels were durably ~10-fold lower for 1 year in immunized animals compared to controls, and a significant delay in AIDS-related mortality resulted. Broader immunity resulted following immunizations with peptides spanning all nine SIV proteins, but the responses to Gag were weaker in comparison to animals only immunized with Gag. No difference in viral outcome occurred in animals immunized with all SIV proteins compared to animals immunized against Gag alone. Peptide-pulsed blood cells are an immunogenic and effective immunotherapy in SIV-infected macaques. Our results suggest Gag alone is an effective antigen for T-cell immunotherapy. Fresh blood cells pulsed with overlapping Gag peptides is proceeding into trials in HIV-infected humans.

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Introduction

Several attempts at immunotherapy of HIV using more conventional vaccines have thus far been poorly immunogenic and weakly efficacious in human trials [1,2,3,4]. The use of professional antigen-presenting cells such as dendritic cells to deliver HIV immunotherapies has shown strong immunogenicity in macaques and pilot humans studies but is limited to highly specialized facilities [5,6,7]. A simple intermittent immunotherapy that reduces the need for long-term antiretroviral therapy (ART) would be a quantum advance in treating HIV.

We recently reported the robust T-cell immunogenicity of treating unmanipulated whole blood or peripheral blood mononuclear cells (PBMC) with overlapping peptides of SIV, HIV-1 or hepatitis C virus in outbred pigtail monkeys [8,9]. We termed this simple immunotherapy OPAL (Overlapping Peptide-pulsed Autologous ceLks). This technique is attractive since there is no prolonged ex vivo culture of antigen-presenting cells, robust CD4 and CD8 T-cell responses to both structural and regulatory proteins can be induced, and peptide antigens are simple to manufacture to high purity. This study assessed whether OPAL vaccination improves the outcome of SIV-infected monkeys.

Considerable debate exists regarding the most effective antigens to target for T-cell based therapeutic HIV vaccination. It has been widely believed that broader immunity to multiple proteins would be more efficacious [10,11]. In contrast, recent studies highlight the effectiveness of Gag-specific T cell immunity in comparison to T cell immunity to other antigens. We therefore also assessed whether narrower responses induced only to SIV Gag are as effective as more broadly targeting all 9 SIV proteins.

Materials and Methods

Animals
Juvenile pigtail macaques (Macaca nemestrina) free from Simian retrovirus type D were studied in protocols approved by...
institutional animal ethics committees and cared for in accordance with Australian National Health and Medical Research Council guidelines. All pigtail macaques were typed for MHC class I alleles by reference strand mediated conformational analysis and the presence of Mane-A*10 confirmed by sequence specific primer PCR as described [12,13]. 36 macaques were injected intravenously with 40 tissue culture infectious doses of SIVmac239 [kindly provided by R. Pal, Advanced Biosciences, Kensington, MD] as described previously [14,15] and randomized into 3 groups of 12 animals (OPAL-Gag, OPAL-All, Controls) 3 weeks later. Randomization was stratified for peak SIV viral load at week 2, weight, gender and the MHC I gene Mane-A*10 (which is known to enhance immune control of SIV) [15]. Animals received subcutaneous injections of dual anti-retroviral therapy with tenofovir and emtricitabine (kindly donated by Gilead, Foster City, CA; both 30 mg/kg/animal) for 7 weeks from week 3: daily from weeks 3–5 post-infection and three times per week from weeks 6–10. This dual ART controls viremia in the majority of SIV-infected macaques [16,17,18,19,20].

Immunizations

Two animal groups (OPAL-Gag and OPAL-All) were immunized with OPAL immunotherapy using PBMC as previously described [8]. Briefly, peripheral blood mononuclear cells (PBMC) were isolated over Ficoll-paque from 18 ml of blood (anticoagulated with NaHeparain). All isolated PBMC (on average 24 million cells) were suspended in 0.5 ml of normal saline to which either a pool of 125 SIVmac239 Gag peptides or 823 peptides spanning all SIVmac239 proteins (Gag, Pol, Env, Nef, Vif, Tat, Rev, Vpr, Vpx) were added at 10 μg/ml of each peptide within the pool. Peptides were 15mers overlapping by 11 amino acids at >80% purity kindly provided by the NIH AIDS reagent repository program (catalog numbers 6204, 6443, 6883, 6448-50, 6407, 8762, 6205). To pool the peptides, each 1 mg vial of lyophilised 15mer peptide was solubilized in 10 μl of pure DMSO and added together. The concentration of the SIV Gag and All peptide pools was 629 and 72 μg/ml/peptide respectively, although each peptide was pulsed onto cells at 10 μg/ml regardless of vaccine type. The peptide-pulsed PBMC were held for 1 hr in a 37°C waterbath, gently vortexed every 15 minutes and then, without washing, reinfused IV into the autologous animal. Peptide concentrations and timing of incubation were adapted from effective stimulation of T cell responses in vitro. Control macaques did not receive vaccine treatment. This was done since (a) we had not previously observed any significant VL changes with non HIV/SIV peptide sets [8,9] and unpublished data), (b) reinfusion of blood cells pulsed with irrelevant sets of peptides would result in some level of immune activation and drive higher viral loads in controls, artificially magnifying any reductions in the active treatment groups, (c) reinfusion of control peptide pulsed cells might have obscured any unexpected safety problems of the procedure.

Immunology assays

SIV-specific CD4 and CD8 T-cell immune responses were analysed by expression of intracellular IFNγ as previously described [21]. Briefly, 200 μl whole blood was incubated at 57°C with 1 μg/ml/peptide overlapping 13mer SIV peptide pools (described above) or DMSO alone and the co-stimulatory antibodies anti-CD28 and anti-CD49d (BD Biosciences/Pharmin- gen San Diego CA) and Brefeldin A (10 μg/ml, Sigma) for 6 h. Anti-CD3-PE, anti-CD4-FITC and anti-CD8-PerCP (BD, clones SP34, M-T477 and SK1 respectively) antibodies were added for 30 min. Red blood cells were lysed (FACS lysing solution, BD) and the remaining leukocytes permeabilized (FACS Permeabilizing Solution 2, BD) and incubated with anti-human IFNγ-APC antibody (BD, clone B27) prior to fixation and acquisition (LSRII, BD). Acquisition data were analysed using Flowjo version 6.3.2 (Tree Star, Ashland, OR). The percentage of antigen-specific gated lymphocytes expressing IFNγ was assessed in both CD3+CD4+ and CD3+CD8+ lymphocyte subsets. Responses to the immunodominant SIV Gag CD9 T-cell epitope KP9 in Mane-A*10+ animals were assessed by a Mane-A*10/KP9 tetramer as described [13]. Total peripheral CD4 T-cells were measured as a proportion of lymphocytes by flow cytometry on fresh blood.

Virology assays

Plasma SIV RNA was quantitated by real time PCR on 140 μl of plasma at the University of Melbourne (lower limit of quantitation 3.1 log10 copies/ml) at all time-points using a TaqMan probe as previously described [21,22] and, to validate these results with a more sensitive assay, on pelleted virions from 1.0 mL of plasma at the National Cancer Institute (lower limit of quantitation 1.5 log10 copies/ml) as previously described [23].

Endpoints/statistical analyses

The primary endpoint was the reduction in plasma SIV RNA in OPAL-immunized animals compared to controls by time-weighted area-under-the-curve (TWAUC) for 10 weeks following withdrawal of ART (i.e. samples from weeks 12 to 20). This summary statistical approach is recommended for studies such as these involving serial measurements [24]. We compared both active treatment groups (OPAL-Gag and OPAL-All) to controls separately and together. The primary analysis was restricted to animals that controlled viremia on the ART at week 10 (VL<3.1 log10 copies/ml), since control of VL is an important predictor of the ability of animals to respond to immunotherapies [8,25]. A pre-planned secondary virologic endpoint was studying all live animals adjusting for both VL at the end of ART (week 10) and Mane-A*10 status. Group comparisons used two-sample t-tests for continuous data, and Fisher’s exact test for binary data. Survival analyses utilised Cox-regression analyses.

Power calculation

Prior to initiating the study, we estimated the standard deviation of the return of VL after treatment interruption would be approximately 0.8 log10 copies of SIV RNA/mL plasma [5,16,17,18,19,20]. In this intensive study we estimated that 2 of the 12 monkeys within a group may have confounding problems such as incomplete response to ART or death from acute SIV
infection. A 10 control vs 10 active treatment comparison yields 80% power (p=0.05) to detect a 1.0 log$_{10}$ difference in TWAUC VL over the first 10 weeks. An estimated comparison of 10 control vs all 20 actively treated animals (OPAL-Gag plus OPAL-All) gave 80% power to detect differences of 0.87 log$_{10}$ copies/ml VL reduction.

Study conduct

This study was conducted according to a pre-written protocol using Good Laboratory Practice Standards from the Australian Therapeutic Goods Administration as a guide. Protocol deviations were minor and did not affect the results of the study. Partial data audits during the study did not raise any concerns about the study conduct.

Results

OPAL Vaccinations

OPAL immunotherapy was studied in SIV-infected pigtail macaques receiving ART. Pigtail macaques have at least an equivalently pathogenic course of SIV infection as alternate rhesus macaque models [14,26]. Thirty-six macaques were infected with SIVmac251 and 3 weeks later treatment with the antiretrovirals tenofovir and emtricitabine for 7 weeks was initiated. The animals were randomly allocated to 3 groups stratified by peak plasma SIV viral load (VL), Mane-A*10 status (an MHC class I gene that improves VL in SIV-infected pigtail macaques [15]), weight and gender. Macaques were immunized 4 times under the cover of antiretroviral therapy (weeks 4, 6, 8, 10) with autologous fresh PBMC mixed for 1 hour ex vivo with 10 μg/ml peptide of either 125 overlapping SIV Gag 15mer peptides only (OPAL-Gag), 823 SIV 15mer peptides spanning all 9 SIV proteins (OPAL-All) or unimmunized. The macaques were initially followed for 26 weeks after ceasing ART on week 10.

All 36 macaques became infected following SIV$_{mac251}$ exposure and had a mean peak VL of 7.1 log$_{10}$ copies/ml (Table S1). Prior to vaccination, 4 animals died during acute SIV infection with diarrhoea, dehydration, lethargy, anorexia and weight loss. The vaccinations were well tolerated, with no differences in mean diarrhoea, dehydration, lethargy, anorexia and weight loss. The to vaccination, 4 animals died during acute SIV infection with diarrhoea, dehydration, lethargy, anorexia and weight loss. The vaccinations were well tolerated, with no differences in mean diarrhoea, dehydration, lethargy, anorexia and weight loss. The vaccinations were well tolerated, with no differences in mean diarrhoea, dehydration, lethargy, anorexia and weight loss.

The primary endpoint comparison of VL between combined OPAL-All and OPAL-Gag treatment groups in the 10 weeks after ART withdrawal was 0.5 log$_{10}$ copies/ml lower than controls (p = 0.084, Fig 4, Table 1). Each vaccination group (OPAL-All and OPAL-Gag) had very similar reductions in VL. By 6 months after ART withdrawal, the mean difference in VL between control and OPAL-immunized groups was 0.93 log$_{10}$ copies/ml (p = 0.028, Table 1).

As a secondary endpoint, we also analysed all 32 remaining animals by adjusting for VL control on ART and Mane-A*10 status. There was a significant difference in VL between controls and vaccinated macaques with these analyses at both 10 and 26 weeks off ART (p = 0.050, 0.016 respectively, Table 1).

To confirm the virologic findings using a sensitive independent VL assay, frozen plasma (1 ml) from study week 32 was shipped to the National Cancer Institute (NCI) in Maryland, USA. Drs M Piatak and J Lifson kindly analysed the samples for SIV RNA blindly using an assay with a limit of quantitation of 1.5 log$_{10}$ copies/ml (Table S1) [23]. The University of Melbourne and NCI assays were tightly correlated (r = 0.97, p=0.001) and showed an almost identical mean reduction in viremia in vaccinees compared to controls at this time (0.82 vs 0.88 log$_{10}$ copies/ml respectively).

Durability of OPAL immunotherapy

To further assess the durability of SIV control and prevention of disease with OPAL immunotherapy, we re-boosted all 32 animals in the same randomized groups 3 times with the identical procedure (at week 36, 39, 42) without ART cover and followed the animals for an additional 6 months. Despite the lack of ART cover, SIV-specific T cell immunity was dramatically enhanced in immunized animals 2 weeks after the last vaccination, similarly to the primary vaccination (Figs 1, 2). The T cell responses to Gag were again highest in the OPAL-Gag group with broader responses in the OPAL All group. The pattern of enhancement of T cell immunity was similar for the first and second vaccination sets (Figs 1, 2).

We again sampled plasma for viral load every 3–6 weeks. To account for the death of animals from AIDS, we used a "last
**Figure 1. T-cell immunogenicity of OPAL vaccination.** SIV Gag-specific CD4 (A) and CD8 (B) T-cells expressing IFNγ were studied over time by intracellular cytokine staining. Mean ± standard error of vaccine groups compared to control unvaccinated animals (circles) is shown. The primary OPAL vaccinations of macaques (arrows, weeks 4, 6, 8 and 10 after SIVmac251 infection) consisted of autologous PBMC pulsed with either overlapping SIV Gag 15mer peptides (OPAL-Gag, triangles) or peptides spanning all 9 SIV proteins (OPAL-All, squares). Initial vaccinations were given under the cover of antiretroviral treatment (ART). Animals were re-boosted with OPAL immunotherapy in the same randomised groups, without ART, at weeks 39, 42 and 42.

At week 12, two weeks after the last vaccination, CD4 (C) and CD8 (D) T-cell responses to pools of overlapping peptides spanning SIV Gag, Env, Pol or combined Regulatory/Accessory proteins (Nef, Tat, Rev, Vif, Vpx, Vpr [Reg]) were assessed in all animals by intracellular cytokine staining. In addition, responses to a SIV Gag CD8 T-cell epitope KP9, were assessed by a Mfe-A10/KP9 tetramer. Mean ± standard error of vaccine groups is shown along with 2-sided t-test p values of <0.10. (E) SIV Gag specific CD8 T-cell responses correlated inversely with CD8 T-cell responses to the summation of non-Gag (Env+Pol+Regulatory protein) responses across all 21 live OPAL-immunized animals. The animals with >50% CD8 T-cell responses to the combined pool had total responses of 50.4% and 54.5%, primarily to Env (50.1% and 54.2% respectively). Spearman rank correlation is shown.

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Significant viral control was maintained throughout the follow-up period of just over 1 year off ART (Fig 4A, Table 1). In animals which controlled VL on ART, there was a mean 0.98 log10 copies/ml difference between controls and vaccinees 54 weeks after coming off ART (p = 0.019 for time-weighted analysis).

Twelve of the remaining 32 animals developed incipient AIDS and were euthanised during the extended follow up. All 6 animals...
Figure 3. SIV-specific antibody responses. All 32 live macaques had serial measurements of SIV-specific antibodies utilizing HIV-2 Western Blot strips. (A) Representative examples of the evolution of the Western Blot profiles of a macaque within each vaccine group. A positive control sample from HIV-2 infected (+) and uninfected (−) human subjects are shown at the right of the panel. (B) Mean ± standard error densitometry measurements of Gag (anti-p26) responses and (C) Env (anti-gp36) responses over time.

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that did not control viremia on ART required euthanasia. Of the 6 euthanised animals which did control viremia on ART, 5 were in the control group and one in the OPAL-Gag group. OPAL immunotherapy resulted in a survival benefit, analysing either the 26 animals that controlled viremia on ART \( (p = 0.053, \text{Fig } 4B, \text{Table 1}) \) or all 32 animals, adjusted for Mane-A*10 status and control of viremia on ART \( (p = 0.02, \text{Table 1}) \).

**Discussion**

In summary, OPAL immunotherapy, either using overlapping Gag SIV peptides or peptides spanning the whole SIV proteome was highly immunogenic and resulted in significantly lower viral loads and a survival benefit compared to unvaccinated controls. The virologic efficacy in OPAL-immunized macaques was durable for 12 months after ART cessation. Our findings on OPAL immunotherapy were observed despite the virulent SIV\(_{mac251}\)-pigtail model studied \([14]\) and provide strong proof-of-principle for the promise of this immunotherapy technique.

The OPAL immunotherapy approach is simpler than many other cellular immunotherapies, particularly the use of dendritic cells. The use of DNA, CTLA-4 blockade and viral vector based approaches are also now showing some promise in macaque studies \([17,27]\), although such approaches have not yet been translated into human studies. This study added peptides to PBMC, however we have shown an even simpler technique, adding peptides to whole blood is also highly immunogenic, a technique that will be more widely applicable \([8]\) and unpublished studies).

This is one of the largest therapeutic SIV vaccine studies yet reported. Although it may have been ideal to have studied...
Table 1. Statistical analyses of VL and Survival.

| Animals analyzed | Comparison | n | 2 sided t-test Reduction in VL 6 months off ART (p-value) | 2 sided t-test Reduction in VL 1 year off ART (p-value) | Survival 1 year off ART (p-value) |
|------------------|-----------|---|-----------------------------------------------------|-----------------------------------------------------|----------------------------------|
| All animals   | Combined vaccine vs controls | 16 | 0.09 (0.56) | 0.03 (0.67) | 0.04 |
| 0.066 | 0.69 | 0.22 |
| OPAL-Gag vs controls | 8 vs 10 | 0.14 (0.70) | 0.60 (0.29) | 0.01 | 0.01 |
| NA | 0.02 | 0.02 |
| OPAL-Gag+M vs controls | 8 vs 10 | 0.06 (0.50) | 0.01 (0.67) | 0.06 | 0.06 |
| 0.20 | 0.20 | 0.20 |
| OPAL All vs controls | 21 vs 11 | 0.26 (0.63) | 0.01 (0.67) | 0.06 | 0.06 |
| 0.01 | 0.01 | 0.01 |
| OPAL Gag vs controls | 11 vs 11 | 0.06 (0.63) | 0.01 (0.67) | 0.06 | 0.06 |
| 0.22 | 0.22 | 0.22 |
| VS All arms & OPAL All vs controls | 11 vs 11 | 0.07 (0.63) | 0.01 (0.67) | 0.06 | 0.06 |
| 0.05 | 0.05 | 0.05 |

Reduction in VL values reductions are log10 copies/ml compared to controls. Values shown reflect time-weighted area-under-the-curve VL between vaccinated animals and controls after coming off ART, and absolute mean reduction at the end of the period in parentheses.

None of the OPAL-All vaccinated animals that had VL undetectable on ART died compared to 5 of 10 controls – this comparison did not permit an estimate of significance of this comparison.

Control of viremia was similar for the OPAL-Gag and OPAL-All groups. Gag-specific CD4 and CD8 T-cell responses in OPAL-Gag animals 5.1- and 3.5-fold greater than those in the OPAL-All animals, despite an identical dose of Gag overlapping peptides. This suggests antigenic competition between peptides from Gag and the other SIV proteins. Inducing immunodominant non-Gag T-cell responses by multi-protein HIV vaccines may limit the development of Gag-specific T-cell responses [21]. A large human cohort study demonstrated Gag-specific T-cell responses were the most effective in controlling HIV viremia [32]. Useful subdominant T cell responses may be particularly susceptible to dominant non-Gag T cell responses [33,34]. The utility, if any, of inducing T-cell responses to non-Gag proteins (i.e. excluding Gag peptides from the vaccine antigens) can be addressed in future studies of this flexible vaccine technology. Therapeutic HIV vaccines may not need to aim for maximally broad multi-protein HIV-specific immunity.

OPAL immunotherapy with Gag peptides is proceeding into initial trials in HIV-infected humans. Additional peptides can readily be added into standard consensus strains mixes to cover common strain or subtype variations between strains with this technology [35]. Additional technologies such as toggling variable amino acids peptides may provide further T cell immunogenicity with this general technology [36]. Immunotherapy with peptides delivered onto fresh blood may have potential applicability for other chronic viral diseases such as hepatitis C virus infection and some cancers such as melanoma [37].

A ~1.0 log10 reduction in VL would result in a substantial delay in progressive HIV disease in humans and allow a reasonable time period without the requirement to reintroduce ART [29] if these findings are confirmed in human trials. Both the control and vaccinated macaques were treated with ART early in this study (3 weeks after infection), which alone can be associated with a transiently improved outcome in humans [30]. None-the-less, a massive loss of CD4+ T cells in the gut occurs within 2 weeks of infection [31]. Although it may be challenging to identify humans within 3 weeks of infection, this is when HIV-1 subjects typically present with acute infection. The durable control of viremia exhibited by the vaccinated animals is interesting and consistent with other recent macaque studies [27], suggesting the need for re-immunization may not be substantial. We cannot attribute the durable control of viremia to the second set of immunizations; there was only a marginal, non-significant, increase in the difference in VL between OPAL vaccinees and controls before and after the second immunization series. Further studies are required to address the timing and benefit of ART cover during boosting immunizations with OPAL immunotherapy.
References
1. Markowitz M, Jin X, Hurley A, Simon V, Ramratnam B, et al. (2002) Discontinuation of antiretroviral therapy commenced early during the course of human immunodeficiency virus type 1 infection, with or without adjunctive vaccination. J Infect Dis 186: 634–643.

2. Tubiana R, Cercaux G, Gravel M, Gourlain K, Dalban C, et al. (2005) Therapeutic immunization with a human immunodeficiency virus (HIV) type 1 recombinant canarypox vaccine in chronically HIV-infected patients. The Vaccaer Study (ANRS 964). Vaccine 23: 4292–4301.

3. Kahn JO, Chen RG, Mayer K, Murray H, Lagakos S (2000) Evaluation of HIV-1 immunogens, an immunologic modifier, administered to patients infected with HIV having 300 to 549 copies/ml CD4 cell counts: A randomized controlled trial. JAMA 284: 2193–2202.

4. Katalma C, Wincker N, Costaglio D, Tubiana R, Murphy R, et al. (2007) A Phase II, Randomized, Placebo-controlled Study to Evaluate the Immunogenicity of 2 Schedules of an Homologous Prime-boost with the ALVAC-HIV vCP1452 in Chronically HIV-infected Patients (MANON 62). 14th Conference on Retroviruses and Opportunistic infections, February 23-26, 2007, Los Angeles Abstract 406.

5. Lu W, Wu X, Lu Y, Guo W, Andrieu JM (2003) Therapeutic dendritic-cell vaccine for simian AIDS. Nat Med 9: 27–32.

6. Lu W, Araes LC, Ferreira WT, Andrieu JM (2004) Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. Nat Med 10: 1359–1363.

7. Connolly NC, Whiteside TL, Wilson C, Kondragunta V, Rinaldo CR, et al. (2007) Therapeutic Immunization with HIV-1 Peptide-Loaded Dendritic Cells is Safe and Immunogenic in HIV-1-infected Individuals. Clin Vaccine Immunol.

8. Chea S, Dale CJ, De Rose K, Ramshaw IA, Kent SJ (2005) Enhanced cellular immunity in macaques following a novel peptide vaccination. J Virol 79: 3748–3757.

9. Stratov I, Dale CJ, Chea S, Mccluskey J, Kent SJ (2005) Induction of T-cell immunity to antiretroviral drug-resistant human immunodeficiency virus type 1. J Virol 79: 7729–7737.

10. Amarra RR, Smith JM, Staprans SI, Montefiori DC, Villinger F, et al. (2002) Critical Role for Env as well as Gag-Pol in Control of a Simian-Human Immunodeficiency Virus 89.6P Challenge by a DNA Prime/Recombinant Modified Vaccinia Ankara Virus Vaccine. J Virol 76: 6130–6146.

11. Polacino PS, Stallard V, Klaeckley J, Pennathur S, Montefiori DC, et al. (1999) Role of immune responses against the envelope and the core antigens of simian immunodeficiency virus SIVmne in protection against homologous cloned and uncloved virus challenge in Macaques. J Virol 73: 8201–8215.

12. Pratt BF, O’Connor DH, Lafont BA, Mankowski JL, Fernandez CS, et al. (2006) MHV class I allele frequencies in pigtail macaques of diverse origin. Immunogenetics 58: 995–1001.

13. Smith NZ, Fernandez CS, Chung A, Dale CJ, De Rose R, et al. (2005) The pigtail macaque MHC class I allele Mane-A*10 presents an immunodominant SIV Gag epitope: identification, tetramer development and implications of immune escape and reversion. J Med Primatol 34: 292–293.

14. Batten CJ, Rose RD, Wilson KM, Agy MB, Chea S, et al. (2006) Comparative Evaluation of Simian, Simian-Human, and Human Immunodeficiency Virus Infections in the Pigmacta Macaque (Macaca nemestrina) Model. AIDS Res Hum Retroutroviruses 22: 580–588.

15. Smith NZ, Dale CJ, De Rose R, Stratov I, Fernandez CS, et al. (2005) Analysis of Pigmacta Macaque Major Histocompatibility Complex Class I Molecules Presenting Immunodominant Simian Immunodeficiency Virus Epitopes. J Virol 79: 644–653.

16. Lori F, Lewis MG, Xu J, Varga G, Zinn DE Jr., et al. (2001) Control of viral rebound through structured treatment interruptions during early infection. Science 290: 1391–1393.

17. Lisziewicz J, Trojcio J, Xu J, Whitman L, Ryder A, et al. (2005) Control of viral rebound through therapeutic immunization with DermaVir. AIDS 19: 53–54.

18. Villinger F, Brice GT, Mayne AE, Bostik P, Moni K, et al. (2002) Adoptive transfer of simian immunodeficiency virus (SIV) naïve autologous CD4+ T cells to macaques chronically infected with SIV is sufficient to induce long-term nonprogresor status. Blood 99: 590–599.

19. Shen A, Zink MC, Mankowski JL, Chadwick K, Margolick JB, et al. (2003) Resting CD4+ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian immunodeficiency virus-Macaca nemestrina model of human immunodeficiency virus type 1-infected patients on highly active antiretroviral therapy. J Virol 77: 4938–4949.

20. Hei Z, Venzon D, Poudyal M, Tsi WP, Giuliani L, et al. (2006) Viremia control following antiretroviral treatment and therapeutic immunization during primary SIVmac251 infection of macaques. Nat Med 6: 1140–1146.

21. De Rose R, Barnett CJ, Smith MZ, Fernandez CS, Poust V, et al. (2007) Comparative Efficacy of Subtype AE Simian-Human Immunodeficiency Virus Priming and Boosting Vaccines in Pigmacta Macaques. J Virol 81: 292–300.

22. Dale CJ, Liu XS, De Rose R, Purcell DF, Anderson J, et al. (2002) Chimeric human papilloma virus-simian/human immunodeficiency virus virus-like-particle vaccines: immunogenicity and protective efficacy in macaques. Virology 301: 176–187.

23. Cline AN, Bes JW, Piatak M Jr., Lisson JD (2005) Highly sensitive SIV plasma viral load assay: practical considerations, realistic performance expectations, and application to reverse engineering of vaccines for AIDS. J Med Primatol 34: 303–312.

24. Matthews JN, Altman DG, Campbell MJ, Royston P (1999) Analysis of serial measurements in medical research. Can J 300: 230–235.

25. Miller DH, Rajakumar PA, Xu MS, McMahon CW, Shipley T, et al. (2006) DNA immunization in combination with effective antiretroviral drug therapy controls viral rebound and prevents simian AIDS after treatment is discontinued. Virology 348: 200–215.

26. Reimann KA, Parker RA, Seaman MS, Beaudry K, Beddall M, et al. (2005) Pathogenicity of simian-human immunodeficiency virus SHIV89.6P and SIVmac is attenuated in cynomolgus macaques and associated with early T-lymphocyte responses. J Virol 79: 8878–8885.

27. von Gergelt AS, Rosati M, Alcena C, Valentin A, Roth P, et al. (2007) Long-lasting decrease in viremia in macaques chronically infected with simian immunodeficiency virus SIVmac251 after therapeutic DNA immunization. J Virol 81: 1972–1979.

28. Rosenberg ES, Billinge JY, Caliendo AM, Boswell SL, Sax PE, et al. (1997) Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 278: 1447–1450.

29. O'Brien WA, Hartigan PM, Marin D, Einhardt J, Hill A, et al. (1996) Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. N Engl J Med 334: 426–431.

30. Kaufmann DE, Lichterfeld M, Aftfeld M, Addo MM, Johnston MN, et al. (2004) Limited Durability of Viral Control following Treated Acute HIV Infection. PLoS Med 1: e6. doi:10.1371/journal.pmed.0010036.

31. Martinez-Palpat J, Dosiek DC, Hill R, Nakamura Y, Martin M, et al. (2005) Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature 434: 1093–1097.

32. Kiepiela P, Ngumbela K, Thobakgale C, Ramduth D, Honeyborne I, et al. (2007) CD4(+) T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med 13: 46–53.

33. Frahm N, Kiepiela P, Adams S, Linde CH, Hewitt HS, et al. (2006) Control of human immunodeficiency virus replication by cytotoxic T lymphocytes targeting subdominant epitopes. Nat Immunol 7: 173–179.

34. Friedrich TC, Valentine LE, Yant LJ, Rakasz EG, Piatak SM, et al. (2007) Subdominant CD4(+) T-cell responses are involved in durable control of AIDS virus replication. J Virol.

35. Fischer W, Perkins S, Theiler J, Bhattacharya T, Yusi K, et al. (2003) Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. Nat Med 9: 100–106.

36. Frahm N, Kaufmann DE, Yusi K, Muldoon M, Ksirim C, et al. (2007) Increased sequence diversity coverage improves detection of HIV-specific T cell responses. J Immunol 179: 6566–6575.

37. Davis JD, Chen W, Jackson H, Parente P, Shackleton M, et al. (2004) Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4(+) and CD8(+) T cell responses in humans. Proc Natl Acad Sci U S A 101: 10697–10702.
