LETTER

Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine

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The acquired immunodeficiency syndrome (AIDS)-causing lentiviruses human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) effectively evade host immunity and, once established, infections with these viruses are only rarely controlled by immunological mechanisms[1–3]. However, the initial establishment of infection in the first few days after mucosal exposure, before viral dissemination and massive replication, may be more vulnerable to immune control[4]. Here we report that SIV vaccines that include rhesus cytomegalovirus (RhCMV) vectors[5] establish indefinitely persistent, high-frequency, SIV-specific effector memory T-cell (T_{EM}) responses at potential sites of SIV replication in rhesus macaques and stringently control highly pathogenic SIV_{MAC239} infection early after mucosal challenge. Thirteen of twenty-four rhesus macaques receiving either RhCMV vectors alone or RhCMV vectors followed by adenovirus 5 (Ad5) vectors (versus 0 of 9 DNA/Ad5-vaccinated rhesus macaques) manifested early complete control of SIV (undetectable plasma virus), and in twelve of these thirteen animals we observed long-term (≥ 1 year) protection. This was characterized by: occasional blips of plasma viraemia that ultimately waned; predominantly undetectable cell-associated viral load in blood and lymph node mononuclear cells; no depletion of effector-site $CD4^+$ memory T cells; no induction or boosting of SIV Env-specific antibodies; and induction and then loss of T-cell responses to an SIV protein (Vif) not included in the RhCMV vectors. Protection correlated with the magnitude of the peak SIV-specific $CD8^+$ T-cell responses in the vaccine phase, and occurred without anamnestic T-cell responses. Remarkably, long-term RhCMV vector-associated SIV control was insensitive to either $CD8^+$ or $CD4^+$ lymphocyte depletion and, at necropsy, cell-associated SIV was only occasionally measurable at the limit of detection with ultrasensitive assays, observations that indicate the possibility of eventual viral clearance. Thus, persistent vectors such as CMV and their associated T_{EM} responses might significantly contribute to an efficacious HIV/AIDS vaccine.

Conventional prime-boost vaccine regimens with non-persistent vectors lead to lymphoid tissue-based memory T-cell responses ('central memory' or T_{CM}), which deliver peak effector responses only after T_{CM} cells have undergone antigen-stimulated expansion, differentiation and trafficking[6]—too late to effectively control pathogens with the rapid replication and spread kinetics and highly developed immune evasion capabilities of the AIDS-causing lentiviruses[2,4,5]. As T-cell effector responses are likely to be much more effective against the smaller, localized and less diverse viral populations present in the first hours and days of mucosally acquired HIV/SIV infection[2,4,7,8], we proposed that a vaccine able to 'pre-position' differentiated effector cells (T_{EM}) at such early replication sites would demonstrate improved efficacy. Such T_{EM} responses are the hallmark of persistent agents[9,10], prompting our development of SIV vectors based on the persistent

β-herpesvirus RhCMV. As recently reported[5] and illustrated in Supplementary Fig. 1, RhCMV/SIV vectors can establish and indefinitely maintain high-frequency SIV-specific, T_{EM}-biased, $CD4^+$ and $CD8^+$ T-cell responses in diverse tissue sites of $RhCMV^+$ rhesus macaques, and in a small efficacy study were associated with early control of intrarectally administered SIV_{MAC239}. To evaluate potential differential effects of persistent vector/T_{EM}-biased versus non-persistent vector/T_{CM}-biased, SIV-specific T-cell responses on the outcome of mucosal SIV_{MAC239} infection, we compared naturally $RhCMV^+$ male rhesus macaques vaccinated with: (1) RhCMV/SIV vectors alone (Group A); (2) RhCMV/SIV vectors followed by replication-defective Ad5 vectors (Group B); and (3) a standard DNA prime/Ad5 vector boost benchmark vaccine (Group C)[11–13] versus unvaccinated control rhesus macaques (Group D; Fig. 1a). RhCMV/SIV vectors efficiently super-infected all Group A and B macaques and elicited robust $CD4^+$ and $CD8^+$ T-cell responses to all vector-encoded SIV proteins (Fig. 1b and Supplementary Figs 2–4). The Ad5 vector boost of Group B macaques, and the DNA/Ad5 regimen given to Group C macaques were also strongly immunogenic (Fig. 1b and Supplementary Figs 3, 4). Although the pattern of development of the SIV-specific T-cell responses differed between these vectors (Supplementary Fig. 3a), the magnitude of the total SIV-specific, $CD4^+$ and $CD8^+$ T-cell responses at the end of the vaccine phase in Groups A, B and C were similar (Fig. 1b and Supplementary Fig. 4). Consistent with previous results[5], RhCMV/SIV-vector-elicited, SIV-specific $CD8^+$ T-cell responses exhibited different epitope targeting than the DNA- and/or Ad5-vector-elicited responses (Supplementary Fig. 3b), and maintained a markedly T_{EM}-biased phenotype over the entire vaccine phase, in contrast to the development of a more T_{CM}-biased response in the DNA/Ad5-vaccinated macaques (Supplementary Fig. 5).

At week 59 after initial vaccination, all rhesus macaques were challenged via the intrarectal route with highly pathogenic SIV_{MAC239} using a repeated, limiting dose protocol[5]. The number of challenges required to achieve measureable infection—plasma viral load > threshold (30 copies ml^{-1})—was not significantly different between Groups A–D (Supplementary Fig. 6), but the subsequent course of infection in these groups was markedly different (Fig. 1c). Of 28 unvaccinated controls (both concurrent and historical), 27 exhibited typical progressive SIV_{MAC239} infection and one exhibited an initially non-progressive infection (transient viraemia) that spontaneously progressed 105 days later. Similarly, all DNA/Ad5-vaccinated macaques (9 of 9) manifested progressive infection, albeit with reduced mean plasma viral load compared to controls (see later). In contrast, 13 of the 24 rhesus macaques that received RhCMV/SIV vectors (6/12 in Group A; 7/12 in Group B) presented with an initial burst of plasma SIV, ranging in magnitude from as few as 60 to as many as 4×10^7 SIV RNA copies ml^{-1}, which was followed by rapid control to undetectable levels (Fig. 1c, d). From 3–18 weeks after infection, all but one of these

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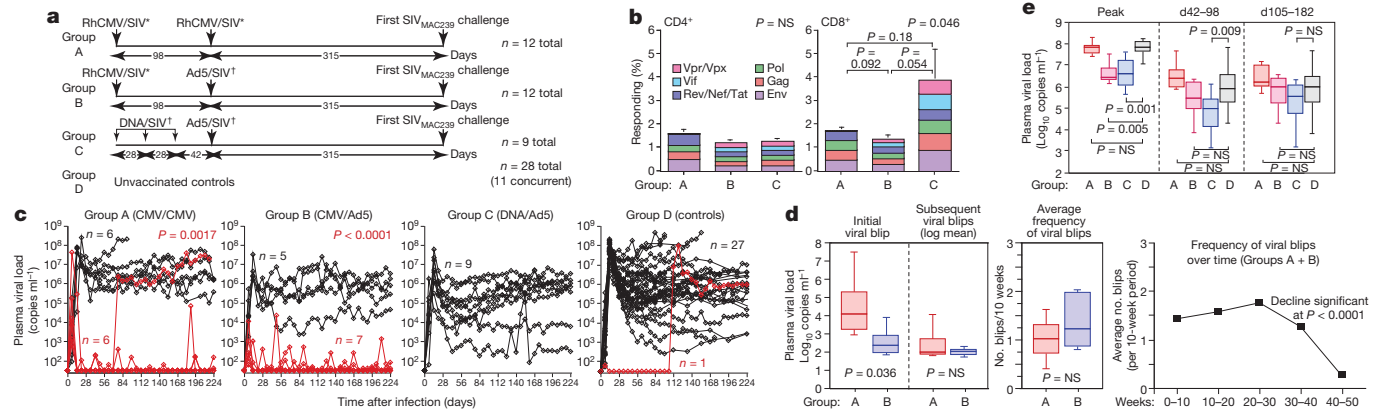
Figure 1 | Immunogenicity and efficacy of RhCMV/SIV vectors.
a, Schematic of the vaccination protocols used in this study. Note that all rhesus macaques used were naturally RhCMV$^+$ at the start of the study. Asterisk indicates Gag + Rev/Nef/Tat + Env + Pol. Dagger indicates pan-SIV proteome. **b**, Comparison of the mean frequency ($\pm$ s.e.m.) of the overall SIV-specific CD4$^+$ and CD8$^+$ T-cell responses and the contribution of the designated SIV proteins to these total responses in the blood memory compartments of Groups A–C rhesus macaques at the end of the vaccine phase. The Kruskal–Wallis test was used to determine the significance of differences in total SIV-specific response frequencies among the three vaccine groups, with the Wilcoxon rank sum test used to perform pair-wise analysis for the CD8$^+$ response. As these latter P values were >0.05, we concluded that overall response frequencies of Groups A, B and C were not significantly different. NS, not significant. P values in top right corners of graphs are for overall Group A versus B versus C. **c**, Outcome of repeated, limiting dose, intrarectal SIV$_{MAC239}$

challenge of Groups A–D. The significance of differences in the fraction of infected rhesus macaques in each group that met controller criteria (see Methods) was determined by Fisher's exact test (closed symbols in Group D are concurrent controls; open, previous controls given the same challenge; red, controllers; black, non-controllers). **d**, Analysis of the magnitude and frequency of plasma viral load 'blips' in Group A and B controllers over the first 50 weeks of infection, with the significance of the differences in blip magnitude and frequency between Groups A and B determined by the Wilcoxon rank sum test, and the significance of the decline in blip frequency of Group A + B macaques after 30 weeks post-infection determined by analysis of variance and linear trend tests. **e**, Comparison of plasma viral loads in Groups A–D rhesus macaques with progressive infection (excluding Group A and B controllers and Group D macaques with protective MHC alleles not represented in Groups A–C) with the significance of differences between Groups A, B and C versus Group D determined by the Wilcoxon rank sum test.

protected rhesus macaques demonstrated one or more repeat episodes of transient viraemia that were always controlled back down to below detection limits (Fig. 1c, d). These periodic viral blips were similar in magnitude in Group A and B controllers, and recurred on average about once every 7 weeks during the first 30 weeks after infection (Fig. 1d). Notably, the frequency of these viral blips declined significantly after week 30 such that by 52 weeks after infection, viral blips were rarely observed (Fig. 1d). No SIV-mediated pathogenesis (loss of effector site CD4$^+$ T cells) was noted in Group A and B controllers (Supplementary Fig. 7), and the vast majority of blood and lymph node mononuclear cell specimens from these macaques were negative for cell-associated SIV RNA and DNA (Supplementary Fig. 8). Six of 12 Group A and 5 of 12 Group B rhesus macaques were not protected in this novel manner, but rather, demonstrated a typical pattern of progressive infection with associated pathogenesis (Fig. 1c, e and Supplementary Fig. 7). The mean peak and plateau phase plasma viral loads of the Group A rhesus macaques with progressive infection were not statistically different from Group D controls (Fig. 1e), indicating that once systemic, progressive infection was established, RhCMV/SIV-vector-elicited responses were unable to control virus replication. The addition of Ad5/SIV vectors in the Group B vaccination regimen was associated with a significantly reduced peak viraemia in Group B macaques with progressive infection compared to Group D controls, but this difference was lost in plateau phase. Consistent with previous reports[11–13], the benchmark DNA/Ad5-vaccinated macaques (Group C) showed significantly reduced log mean peak and early plateau phase (6–14 weeks after infection) plasma viral loads, but for most of these macaques this partial virological control was short-lived, as log mean plasma viral loads in later plateau phase were also not different from Group D controls (Fig. 1c, e). Importantly, the stringent control of SIV infection in protected Group A and B rhesus macaques was not associated with CD8$^+$ T-cell responses restricted by protective MHC alleles (Supplementary Fig. 3b) or with TRIM5 polymorphisms associated with target cell susceptibility to SIV infection (Supplementary Fig. 9).

Taken together, these data indicate that RhCMV/SIV-vector-elicited immune responses mediate a novel pattern of protection in

which mucosally administered SIV$_{MAC239}$ is stringently controlled before the onset of progressive, systemic infection. As shown in Fig. 2a and Supplementary Fig. 10, the peak frequencies of SIV-specific CD8$^+$ (but not CD4$^+$) T cells during the vaccine phase (which occurred shortly after the boost), but not the frequencies immediately pre-challenge, significantly correlated with protection in both Groups A and B. These peak responses reflect the level of overall production of SIV-specific CD8$^+$ T cells by the vaccine, and for a T$_{EM}$-biased response would probably parallel the extent of T$_{EM}$ seeding at effector sites. SIV Env-specific antibody responses are not generated by our RhCMV/SIV vectors[5], and did not develop after SIV infection in Group A controllers (Fig. 2b). Although Ad5/SIV-Env-vector-vaccinated rhesus macaques in Group B developed low-titre SIV Env-specific (tissue-culture-adapted SIV$_{MAC251}$-neutralizing) antibody responses before challenge, these titres did not predict control and were not boosted by controlled infection. In contrast, with the exception of rapid progressors, SIV Env-specific antibody responses developed or were boosted in all macaques with systemic, progressive SIV infection. These findings indicate that antibody responses are unlikely to significantly contribute to the protection observed in Group A and B macaques, and further confirm the stringency of protection in RhCMV/SIV-vector-vaccinated controllers, as SIV replication in these macaques produced insufficient antigen to drive humoral immune responses.

We next investigated the effect of SIV infection on the magnitude of the vaccine-elicited T-cell responses. Notably, Group A rhesus macaques showed an almost complete lack of an anamnestic SIV Gag-specific CD4$^+$ or CD8$^+$ T-cell response to either progressive or controlled SIV infection (Fig. 2c and Supplementary Fig. 11). Group B macaques demonstrated a modest anamnestic response in the setting of control, whereas in the setting of progressive infection they manifested a robust anamnestic response, similar to or only slightly less than that observed in Group C macaques. Thus, despite the facts that Group B macaques manifested circulating SIV-specific CD8$^+$ T-cell responses with the characteristic marked T$_{EM}$ bias of RhCMV/SIV-vector-elicited responses (Supplementary Fig. 5), and the early, abrupt RhCMV/SIV-vector-associated pattern of protection (Fig. 1c), these
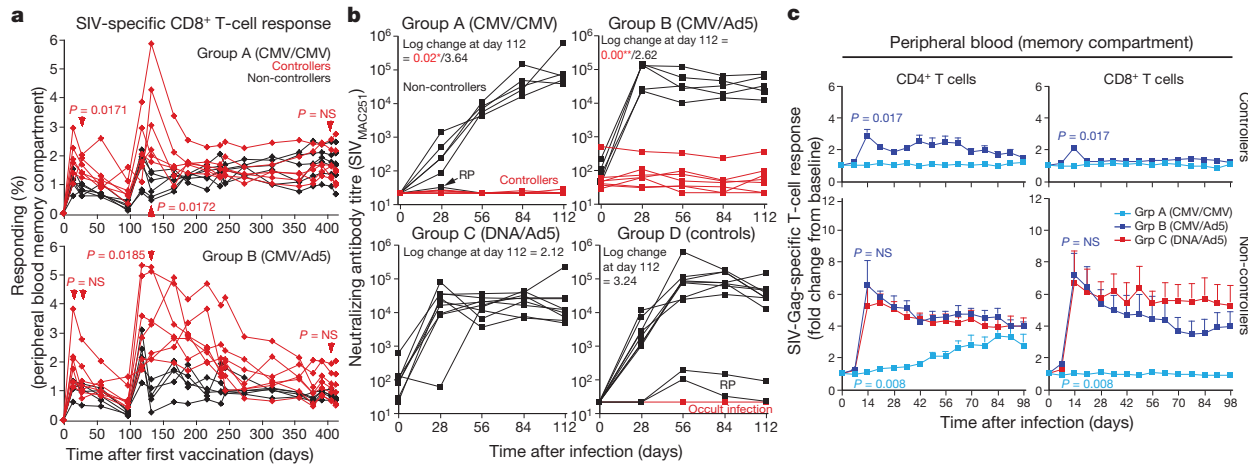
Figure 2 | Immunological correlates of RhCMV/SIV-vector-associated control. a, Analysis of total SIV-specific CD8^{+} T-cell responses (SIV Gag + Rev/Nef/Tat + Pol + Env) in the blood memory compartment during the vaccine phase of Group A and B rhesus macaques with differences in the magnitude of these responses between controllers and non-controllers at the designated time points determined by the Wilcoxon rank sum test. **b,** Comparison of the anti-SIV-Env antibody titres in plasma (as measured by neutralization of tissue-culture-adapted SIV_{MAC251}) before and after infection of controller versus non-controller rhesus macaques among Groups A–C and the concurrent Group D macaques. RP, rapid progressor. Occult infection refers to the initially non-progressive infection (Fig. 1c). The significance of the differences in log change in antibody titre from pre-infection to day 112 post-infection in Group A and B controllers versus Group C macaques was determined by the Wilcoxon rank sum test. *$P < 0.0001$, **$P < 0.005$. **c,** Analysis of the change in the SIV-Gag-specific CD4^{+} and CD8^{+} T-cell response frequency after controlled versus progressive infection in Groups A, B and C with the significance of differences in peak response boosting between the designated groups determined by the Wilcoxon rank sum test. Error bars show mean ± s.e.m. P values are compared to Group A for the controllers (top panels) and to Group C for the non-controllers (bottom panels).

macaques seemed to maintain a distinct Ad5-vector-elicited, SIV-specific T_{CM} population capable of anamnestic expansion upon either controlled or progressive SIV infection. Importantly, Group A and B controllers robustly responded to infection with *de novo* (Group A) or boosted (Group B) CD4^{+} and CD8^{+} T-cell responses to SIV Vif, an antigen not included in the RhCMV/SIV vectors used in this study (Supplementary Fig. 12), confirming both the presence of SIV infection in these macaques, and the normal ability of their naive T-cell (Group A) and T_{CM} (Group B) compartments to respond to the infection. These results indicate that not only does RhCMV/SIV-vector-associated viral control occur in the absence of an overt anamnestic response, but that the SIV-specific T_{EM} populations generated by RhCMV/SIV vectors alone seem unable to significantly expand after infection, regardless of whether antigen levels are limiting (controlled infection) or abundant (progressive infection). This lack of anamnestic expansion may account for the inability of Group A macaques (in contrast to Group B macaques) to manifest any suppression of viral replication once a systemic, progressive infection was established.

The decline in the frequency of SIV RNA blips in the plasma of RhCMV/SIV-vector-vaccinated controllers over time suggests progressive loss of SIV-infected cells, either by immune clearance, virolysis or other attritive mechanisms. To explore the extent of residual infection in long-term RhCMV/SIV-vaccinated controllers, we used monoclonal antibodies to deplete CD4^{+} or CD8^{+} lymphocytes from two Group A and two Group B controllers, in comparison to a Group C (DNA/Ad5) and a Group D (unvaccinated) macaque with partial virological control, for each treatment. Administration of the anti-CD4 huOKT4A monoclonal antibody depleted CD4^{+} T cells, but did not increase plasma viraemia in either Group C and D partial controllers or Group A and B complete controllers (Supplementary Fig. 13). In keeping with previous studies[14,15], CD8^{+} lymphocyte depletion with the cM-T807 monoclonal antibody did result in a pronounced increase in plasma viral load in Group C and D rhesus macaques with partial control, associated with a robust expansion of SIV Vif-specific CD4^{+} T cells in effector sites (Fig. 3a). In contrast, CD8^{+} lymphocyte depletion failed to increase plasma viraemia in RhCMV/SIV-vector-vaccinated controllers,
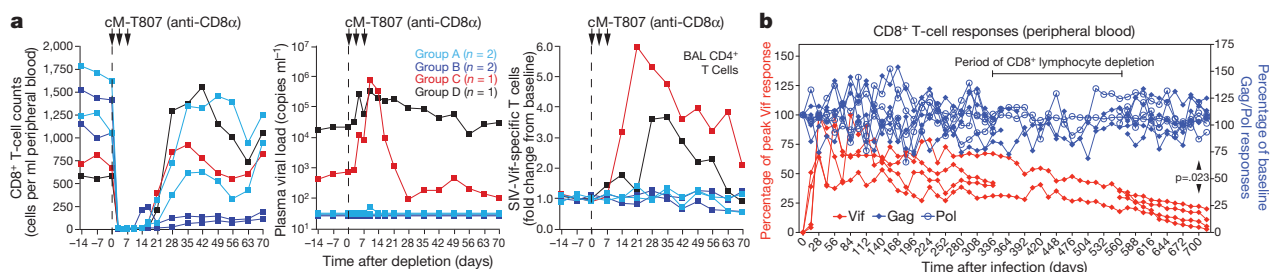
Figure 3 | Immunological characterization of long-term control associated with RhCMV/SIV vector vaccination. a, Analysis of the effect of depletion of CD8^{+} lymphocytes with cM-T807 monoclonal antibody on viral replication and boosting of SIV Vif-specific T-cell responses (in the non-depleted CD4^{+} subset) in four long-term RhCMV/SIV-vector-vaccinated controllers (two Group A and two Group B rhesus macaques) versus two conventional controllers (one Group C, DNA/Ad5-vaccinated controller; one Group D spontaneous controller). BAL, broncho-alveolar lavage lymphocytes. **b,** Analysis of the frequencies of blood CD8^{+} T cells specific for SIV proteins that were (Gag, Pol) or were not (Vif) included in the CMV/SIV vectors in the four Group A 'controllers' for which long-term data are available. The response frequencies were normalized to the

response frequencies immediately before SIV infection for the Gag- and Pol-specific responses, and to the peak frequencies following SIV infection for the Vif-specific responses. The four rhesus macaques used in this long-term response analysis include those subjected to transient CD4^{+} or CD8^{+} lymphocyte depletion (two each). As antigen-specific CD8^{+} T-cell responses cannot be reliably determined during the period of overall CD8^{+} lymphocyte depletion, these periods are shown as gaps for two affected rhesus macaques. The significance of differences in the maintenance of response frequencies of Gag- and Pol- versus Vif-specific CD8^{+} T cells in these rhesus macaques was determined by Wilcoxon rank sum analysis.

and the SIV Vif-specific CD4$^+$ T-cell responses in these macaques were unchanged after depletion, suggesting the absence of even a transient increase in viral replication not detectable by plasma viral load measurements. These studies extend our previous data on the insensitivity of RhCMV/SIV-vector-associated control to CD8$^+$ lymphocyte depletion[5] to rhesus macaques that manifested a higher initial viraemia as well as a period of subsequent, intermittent plasma viral load blips.

As CD8$^+$ T-cell depletion with cM-T807 monoclonal antibody is typically not complete in tissues (Supplementary Fig. 14), lack of viral rebound after such treatment of RhCMV/SIV-vector-vaccinated controllers may simply reflect the potent antiviral function of such residual SIV-specific CD8$^+$ T$_{EM}$ cells or, possibly, the compensatory activity of antiviral CD4$^+$ T$_{EM}$ cells. On the other hand, these observations also raise the possibility that the frequency of SIV-infected and potentially infectious cells in long-term RhCMV/SIV-vector-vaccinated controllers might have been reduced over time to levels that made detectable viral rebound unlikely. In this regard, we found that in Group A controllers, both CD8$^+$ and CD4$^+$ T-cell responses to SIV Vif, an antigen that was not included in the RhCMV/SIV vectors and therefore only available from SIV-infected cells, progressively waned over time to an average of <10% of their peak response immediately after (controlled) infection (Fig. 3b and Supplementary Fig. 15). This observation indicates that the numbers of productively infected cells present in these long-term controller macaques are very few, below the threshold necessary to support the initially high-frequency Vif-specific responses. To further examine the extent of residual infection in long-term RhCMV/SIV-vaccinated controllers, we rigorously quantified SIV RNA and DNA at necropsy in four such macaques ($\geq$week 52 after infection; lacking plasma viral load blips for $\geq$10 weeks before necropsy) in comparison to an uninfected macaque, two macaques with SIV infections that were well controlled by standard criteria, and an additional macaque with poorly controlled, progressive SIV infection. As shown in Fig. 4, extensive analysis of lymphoid tissues and immune effector sites of the

RhCMV/SIV-vector-vaccinated controllers with ultra-sensitive nested, quantitative reverse transcription polymerase chain reaction (RT–PCR) and polymerase chain reaction (PCR) assays (10 reactions per tissue specimen) demonstrated that cell-associated SIV RNA and DNA were undetectable (0/10 reactions positive) in 72% and 80% of specimens, respectively. In those tissues where viral sequences were detected, the levels were extremely low (approximately one copy per 10^7–10^8 cell equivalents). Notably, the majority of specimens with detectable SIV DNA or RNA (77% and 73%, respectively) were from outside the rectal mucosa. Cell-associated SIV RNA and DNA were not detected in any tissues from an SIV-negative macaque, but were readily detected in all tissues of macaques with conventionally controlled SIV infection. Overall, tissue levels in these conventional controllers averaged >3 logs higher than the measureable values of RhCMV/SIV-vector-vaccinated controllers ($P < 0.0001$ by the Wilcoxon rank sum test). Levels of cell-associated SIV were higher still in a macaque with poorly controlled infection. We also assayed lymphoid tissue cells from these macaques for the presence of inducible, replication-competent SIV by co-culture (Supplementary Table 1). All co-cultures (up to 20 replicates per specimen) from RhCMV/SIV-vector-vaccinated controllers were negative for recoverable SIV, whereas replication-competent SIV was readily detected in co-cultures of tissue cells from the conventional controllers. The paucity of SIV nucleic acid and the lack of recoverable SIV in RhCMV/SIV-vector-vaccinated controller macaques are in sharp contrast to the levels of HIV or SIV found in either humans or macaques receiving highly active antiretroviral therapy or in elite controllers[16–21], and suggest an unprecedented level of SIV control and even the possibility of progressive clearance of the SIV infection over time. Importantly, despite little or no SIV replication in the RhCMV/SIV-vector-vaccinated controllers, peripheral blood T cells specific for SIV proteins included in the RhCMV/SIV vectors (for example, Gag and Pol) were stably maintained at high frequency for 700 days after infection (CD8$^+$ T-cell responses with 94 $\pm$ 0.5% T$_{EM}$ phenotype); in
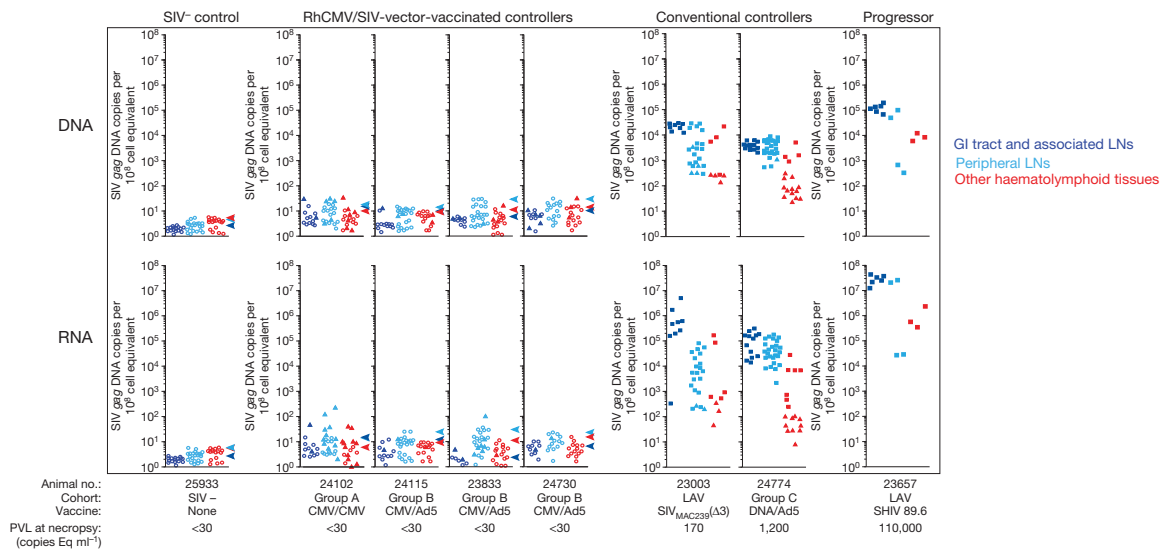
Figure 4 | Measurement of SIV RNA and DNA in long-term RhCMV/SIV-vector-vaccinated controllers. Nested quantitative PCR and quantitative RT–PCR analysis of SIV DNA and RNA, respectively, on tissue obtained at necropsy from an uninfected rhesus macaque, four long-term (>52 weeks) RhCMV/SIV-vector-vaccinated controller rhesus macaques (one Group A; three Group B), two conventional controller rhesus macaques (a live attenuated SIV (LAV)-vaccinated macaque that resisted wild-type SIV$_{MAC239}$ challenge 33 and 10 weeks before necropsy, a Group C, DNA/Ad5-vaccine-protected macaque at 55 weeks post-infection), and a rhesus macaque with poorly controlled SIV infection (a LAV-vaccinated macaque 24 weeks after wild-type SIV$_{MAC239}$ challenge). Filled square plot symbols indicate DNA or RNA copy numbers based on directly measured values for samples giving 10/10 replicate reactions positive. Filled triangles indicate results for samples giving at least one, but less

than ten, replicate reactions positive, with copy number imputed by Poisson distribution. Open circles indicate specimens that gave 0/10 replicates positive with the symbol's position in the plots at the threshold value corresponding to a Poisson distribution imputed copy number corresponding to 1/10 replicates positive. PVL, plasma viral load. All values are normalized for nucleic acid input. Arrowheads indicate the highest threshold value for negative samples (0/10 replicates positive) for all of the tissues analysed for that macaque. Gastrointestinal (GI) tract and associated lymph nodes (LNs) include colon/rectum, ileum, jejunum, superior/medial/inferior mesenteric and ileocaecal lymph nodes. Peripheral LNs include axillary, submandibular, inguinal, iliosacral and tracheobronchial lymph nodes. Other haematolymphoid tissues include liver, spleen, bone marrow, tonsil and thymus.

contrast to the SIV-infection-elicited Vif-specific responses; Fig. 3b and Supplementary Fig. 15). Thus, persistent RhCMV/SIV vectors provide for long-term maintenance of high-frequency SIV-specific T_{EM} responses, which would otherwise wane with stringent virological control, thereby ensuring continuous, high-level surveillance for SIV-infected cells, even when only rare infected cells are present.

In summary, the 16 long-term RhCMV/SIV-vector-vaccinated controllers described in this and our previous study[5] unequivocally demonstrate a previously undescribed form of immune-mediated control of highly pathogenic SIV in which mucosally acquired infection is arrested before irreversible establishment of disseminated, progressive infection. Although stringently controlled, residual SIV infection is still present for weeks to months in most of these controllers, but wanes over time until eventually it is barely detectable by the most sensitive molecular virological and immunological criteria. The available data strongly indicate that this unique control is related to the high-frequency $CD8^+$, and possibly $CD4^+$, T_{EM}-biased, SIV-specific T-cell responses that are elicited and indefinitely maintained by the persistent RhCMV/SIV vectors, are situated in both mucosal portals of entry and potential sites of distant viral spread, and can protect without anamnestic expansion (see Supplementary Discussion). The ability of RhCMV/SIV vectors to indefinitely maintain SIV-specific T_{EM} responses in these sites, independent of the level of SIV replication, provides for continuous surveillance for SIV-infected cells, preventing relapse and, perhaps, ultimately clearing residual infection. Thus, CMV vectors provide a powerful new approach for HIV/AIDS vaccine development that could be used alone or in combination with complementary vaccine strategies that exploit different HIV immune vulnerabilities.

METHODS SUMMARY

Sixty-seven purpose-bred male rhesus macaques (*Macaca mulatta*) of Indian genetic descent were used in this study. RhCMV/SIV vectors were given subcutaneously at a dose of 5×10^6 plaque-forming units per vector. DNA and Ad5 vectors were given intramuscularly at doses of 1.6 mg per vector and 2×10^{10} particle units per vector, respectively. Rhesus macaques were challenged intrarectally with SIV_{MAC239} using a repeated (weekly) limiting dose protocol[5]. After the onset of infection (plasma viral load $\geq$30 SIV RNA copy equivalents (Eq) per ml), macaques were followed weekly until onset of AIDS or a minimum of 224 days for progressive infection and 365 days for controlled infection. SIV- and RhCMV-specific $CD4^+$ and $CD8^+$ T-cell responses were measured in mononuclear cell preparations from blood and tissues by flow cytometric intracellular cytokine analysis[5]. SIV Env-specific antibodies were determined by neutralization of tissue-culture-adapted SIV_{MAC251} using a luciferase reporter gene assay[22]. Levels of SIV RNA and DNA in plasma and from isolated cell preparations were quantified by standard quantitative real-time PCR and RT–PCR assays[23,24]. Tissue-associated SIV RNA and DNA at necropsy were quantified by an ultra-sensitive nested, quantitative real-time PCR and RT–PCR approach (see Methods). The presence of inducible, replication-competent SIV in mononuclear cell preparations was detected by co-cultivation with CEMx174 cells, as previously described[16].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

1. Grovit-Ferbas, K., Pappas, T. & O'Brien, W. A. in *Persistent Viral Infections* (eds Ahmed, R. & Chen, A. I.) 3–45 (John Wiley & Sons, 1999).
2. Haase, A. T. Perils at mucosal front lines for HIV and SIV and their hosts. *Nature Rev. Immunol.* **5**, 783–792 (2005).
3. Goulder, P. J. & Watkins, D. I. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nature Rev. Immunol.* **8**, 619–630 (2008).
4. Haase, A. T. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature* **464**, 217–223 (2010).
5. Hansen, S. G. *et al.* Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nature Med.* **15**, 293–299 (2009).
6. Robinson, H. L. & Amara, R. R. T cell vaccines for microbial infections. *Nature Med.* **11**, S25–S32 (2005).
7. Keele, B. F. *et al.* Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc. Natl Acad. Sci. USA* **105**, 7552–7557 (2008).
8. Chun, T. W. *et al.* Early establishment of a pool of latently infected, resting $CD4^+$ T cells during primary HIV-1 infection. *Proc. Natl Acad. Sci. USA* **95**, 8869–8873 (1998).
9. Bachmann, M. F., Kundig, T. M., Hengartner, H. & Zinkernagel, R. M. Protection against immunopathological consequences of a viral infection by activated but not resting cytotoxic T cells: T cell memory without "memory T cells"? *Proc. Natl Acad. Sci. USA* **94**, 640–645 (1997).
10. Ochsenbein, A. F. *et al.* A comparison of T cell memory against the same antigen induced by virus versus intracellular bacteria. *Proc. Natl Acad. Sci. USA* **96**, 9293–9298 (1999).
11. Casimiro, D. R. *et al.* Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. *J. Virol.* **79**, 15547–15555 (2005).
12. Letvin, N. L. *et al.* Preserved $CD4^+$ central memory T cells and survival in vaccinated SIV-challenged monkeys. *Science* **312**, 1530–1533 (2006).
13. Wilson, N. A. *et al.* Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. *J. Virol.* **80**, 5875–5885 (2006).
14. Friedrich, T. C. *et al.* Subdominant $CD8^+$ T-cell responses are involved in durable control of AIDS virus replication. *J. Virol.* **81**, 3465–3476 (2007).
15. Reynolds, M. R. *et al.* Macaques vaccinated with live-attenuated SIV control replication of heterologous virus. *J. Exp. Med.* **205**, 2537–2550 (2008).
16. Shen, A. *et al.* 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 (2003).
17. Dinoso, J. B. *et al.* A simian immunodeficiency virus-infected macaque model to study viral reservoirs that persist during highly active antiretroviral therapy. *J. Virol.* **83**, 9247–9257 (2009).
18. North, T. W. *et al.* Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS. *J. Virol.* **84**, 2913–2922 (2010).
19. Ruiz, L. *et al.* Protease inhibitor-containing regimens compared with nucleoside analogues alone in the suppression of persistent HIV-1 replication in lymphoid tissue. *AIDS* **13**, F1–F8 (1999).
20. Blankson, J. N. *et al.* Isolation and characterization of replication-competent human immuno-deficiency virus type 1 from a subset of elite suppressors. *J. Virol.* **81**, 2508–2518 (2007).
21. Anton, P. A. *et al.* Multiple measures of HIV burden in blood and tissue are correlated with each other but not with clinical parameters in aviremic subjects. *AIDS* **17**, 53–63 (2003).
22. Montefiori, D. C. Evaluating neutralizing antibodies against HIV, SIV, and SHIV in luciferase reporter gene assays. *Curr. Protoc. Immunol.* **12**, Unit 12.11 (2005).
23. Cline, A. N., Bess, J. W., Piatak, M. Jr & Lifson, J. D. 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 (2005).
24. Venneti, S. *et al.* Longitudinal *in vivo* positron emission tomography imaging of infected and activated brain macrophages in a macaque model of human immunodeficiency virus encephalitis correlates with central and peripheral markers of encephalitis and areas of synaptic degeneration. *Am. J. Pathol.* **172**, 1603–1616 (2008).

Author Contributions S.G.H. planned and performed experiments and analysed data, assisted by J.C.F., M.S.L., A.B.V., C.M.H., L.C.-J. and N.W. T.S., A.W.L. and M.K.A. managed the animal protocols. M.A.J. designed, constructed and characterized the RhCMV vectors. M.P. Jr and J.D.L. planned and performed SIV quantification studies, assisted by K.O. and R.S. C.L.P. and M.J.C. designed and constructed the DNA and Ad5 vectors used in this study. J.A.N. was involved in conception of the RhCMV vector strategy. L.J.P. conceived the RhCMV vector strategy, supervised experiments, analysed data and wrote the paper, assisted by S.G.H., M.A.J. and J.D.L.

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METHODS

Rhesus macaques. A total of 68 purpose-bred juvenile and adult male rhesus macaques (*Macaca mulatta*) of Indian genetic descent were used in the experiments reported in this study, including 61 RhCMV⁺ macaques in the vaccination/challenge experiment shown in Fig. 1a (Group A and B: $n = 12$ each; Group C: $n = 9$; and Group D: $n = 28$; 11 concurrent and 17 historical controls), 4 long-term RhCMV/Gag-vector-vaccinated macaques, 2 live attenuated SIV-vector-vaccinated/wild-type SIV$_{MAC239}$-challenged macaques (one controller and one non-controller), and one unvaccinated, uninfected macaque. All macaques were free of cercopithicine herpesvirus 1, D-type simian retrovirus, simian T-lymphotrophic virus type 1 and SIV infection at the start of the study. Group A–C and concurrent Group D included 4 macaques each with the Mamu A*01 allele, but no macaques with the B*08 and B*17 alleles associated with post-peak control of SIV replication[4]. Of the 18 historical Group D rhesus macaques, 11 lacked A*01, B*08 and B*17 alleles, and 6 expressed either B*08 or B*17. The latter macaques were excluded from analysis of viral load in progressive infection, as they expressed protective alleles that were not represented in the vaccine groups. Rhesus macaques were used with approval of the Oregon National Primate Research Center Animal Care and Use Committee under the standards of the US National Institutes of Health Guide for the Care and Use of Laboratory Animals. RhCMV/SIV vectors (RhCMV Gag, Rev/Nef/Tat, Env, Pol1 and Pol2, see later) were given subcutaneously at a dose of 5×10^6 plaque-forming units. Ad5-SIV vectors (Ad5 Gag, Env, Pol, Nef and VVVTR, see later) were given intramuscularly (i.m.) at a dose of 2×10^{10} particle units per vector, and DNA (i.m. at 1.6 mg per vector) was given at weeks 0, 4 and 8 for the DNA prime animals before Ad5-SIV boost at week 14. Plasmids expressing a fusion protein comprised of SIV Vif, Vpr, Vpx, Tat and Rev, or individual Gag, Pol, Env and Nef open reading frames (ORFs) were used for DNA priming. Rhesus macaques were challenged intrarectally with highly pathogenic SIV$_{MAC239}$ using the repeated (weekly), limiting dose protocol described previously[5]. Plasma viral loads were measured weekly, with challenge discontinued the week after detection of >30 copy equivalents (Eq) per ml of SIV RNA (with the challenge preceding the first measured plasma viral load of >30 copy Eq ml⁻¹ considered the day of infection). Macaques were considered controllers if plasma viral load became undetectable within 2 weeks of the initial positive plasma viral load and was then maintained below threshold for at least 4 of the subsequent 5 weeks. Challenged macaques were followed until onset of AIDS or a minimum of 224 days for progressive infection and a minimum of 365 days for controlled infection. For CD8⁺ lymphocyte depletion, rhesus macaques were administered 10, 5, 5 and 5 mg per kg body weight of the humanized monoclonal anti-CD8α antibody cM-T807, on days 0, 3, 7 and 10, respectively[25]. For CD4⁺ lymphocyte depletion, rhesus macaques were administered one dose of the humanized monoclonal anti-CD4 antibody huOKT4A at 50 mg per kg body weight[26]. Mononuclear cell preparations were obtained from blood, bone marrow, broncho-alveolar lavage (BAL), lymph nodes, spleen, liver, tonsil, thymus and intestinal mucosa as previously described[27–29]. For SIV quantification by nested qPCR/RT–PCR, whole tissue pieces obtained at necropsy were then flash frozen in liquid nitrogen and stored at −80 °C before nucleic acid isolation.

RhCMV/SIV vectors. Construction and characterization of RhCMV Gag, RhCMV Retanef and RhCMV Env has been described[5]. Two additional RhCMV viruses expressing either the 5′ (protease/reverse transcriptase; designated RhCMV Pol1), or 3′ (RNase H/integrase; designated RhCMV Pol2) of SIV$_{MAC239}$ Pol were constructed in an identical fashion by using E/T recombination and the RhCMV (68–1) bacterial artificial chromosome (BAC) (pRhCMV/BAC-Cre)[30]. Deletions were introduced into Pol to inactivate protease (Δ25-DTG-27), reverse transcriptase (Δ184-YMDD-187), RNaseH (ΔE478) and integrase (ΔD64, ΔD116, and ΔE152)[31]. Pol protein expression was placed under control of the EF1α promoter to achieve maximal expression. In all RhCMV/SIV vectors, the SIV antigen-expressing cassettes are inserted into the pRhCMV/BAC-Cre at nucleotide 207,630 within a non-coding region between rh213 and Rh214. RhCMV/SIV viruses were reconstituted by transfection of recombinant BAC DNA into RhCMV-permissive macaque fibroblasts. Following virus reconstitution and BAC cassette 'self-excision', RhCMV/SIV vectors contain the entire wild-type (68–1) RhCMV genome[30]. Vector SIV antigen expression was confirmed by western blot analysis using antibodies to Flag (Sigma-Aldrich; RhCMV Gag), V5 (Invitrogen; RhCMV Retanef), c-Myc/KK45 (Sigma-Aldrich; RhCMV Env), and HA (Sigma-Aldrich; RhCMV Pol1 and Pol2).

DNA vaccines. The plasmid vaccine immunogens used in this study covered the full SIV$_{MAC239}$ (NCBI M33261) genome. ORFs were sequence-optimized for expression in mammalian cells and cloned into a plasmid DNA expression vector. Expression of the intronless coding sequences was controlled by the human CMV (HCMV) promoter/enhancer and the bovine growth hormone polyadenylation signal[32]. Gag (12S), Pol (91S), Env (99S) and Nef (pCMV-Nef) were expressed as single polypeptides, whereas Vif, Vpr, Vpx, Tat and Rev were expressed as a fusion protein (pCMV-VVVTR). The 12S Gag plasmid expresses a myristoylated Gag protein spanning amino acids (aa) 1–508 and lacks two C-terminal aa, but is otherwise similar to a Gag plasmid reported previously[33]. Plasmid 99S expresses a native form of gp160, as previously reported[31,33]. Sequence-optimized Nef without a myristoylation signal was PCR-amplified from 179S plasmid[31], and subsequently transferred into the pCMV vector. The Pol (91S) coding sequence contained deletion mutations to inactivate protease, reverse transcriptase, RNaseH and integrase, as described[31]. Large-scale plasmid production for immunization was prepared by Aldevron, LLC. Expression of plasmids after transient transfection of HEK 293 cells was corroborated by western blot using polyclonal antibodies against SIV$_{MAC239}$ Gag, Env, Nef and Rev proteins made in-house, and anti-SIV$_{MAC251}$ serum from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

Adenovirus vectors. Plasmid SIV$_{MAC239}$ sequences, again covering the full SIV$_{MAC239}$ genome, were cloned into a human adenovirus serotype 5 (Ad5), which lacks E1A and most of E1B and ΔE3 using the Adeasy Adenoviral vector system (Stratagene) to make the Ad5-SIV Gag, Ad5-SIV Env, Ad5-SIV Pol, AD5-SIV Nef and Ad5-SIV VVVTR vectors. SIV$_{MAC239}$ genes were inserted into the E1 region of the Ad5 under the control of the HCMV immediate early promoter/enhancer and the SV40 polyadenylation signal. All vectors were rescued and propagated in HEK 293 cells and purified by double cesium chloride centrifugation[34]. Dosing was based on the physical number of particles (PU) of Ad5 as measured by spectrophotometry[35]. Expression of SIV proteins from Ad5 vectors after A549 infection was confirmed by western blot as described earlier.

SIV$_{MAC239}$ challenge virus. The pathogenic SIV$_{MAC239}$ challenge virus stock (provided by C. Miller) was generated by expanding the SIV$_{MAC239}$ clone in rhesus macaque peripheral blood mononuclear cells (PBMCs), and was quantified using the sMAGI cell assay and by quantitative RT–PCR for SIV genomic RNA[5].

Immunological assays. SIV- and RhCMV-specific CD4⁺ and CD8⁺ T-cell responses were measured in mononuclear cell preparations from blood and tissues by flow cytometric intracellular cytokine analysis, as previously described in detail[5]. Briefly, sequential 15-mer peptides (overlapping by 11 amino acids) comprising the SIV$_{MAC239}$ Gag, Rev/Nef/Tat, Env, Pol, Vif and Vpr/Vpx proteins were used in the presence of co-stimulatory CD28 and CD49d monoclonal antibodies (BD Biosciences). Cells were incubated with antigen and co-stimulatory molecules alone for 1 h, followed by addition of Brefeldin A (Sigma-Aldrich) for an additional 8 h. Co-stimulation without antigen served as a background control. Cells were then stained with fluorochrome-conjugated monoclonal antibodies, flow cytometric data collected on an LSR II (BD Biosciences) and data analysed using the FlowJo software program (version 8.8.6; Tree Star). Response frequencies (CD69⁺/TNF⁺ and/or CD69⁺/IFN-γ⁺) were first determined in the overall CD4⁺ and CD8⁺ population and then memory corrected (with memory T-cell subset populations delineated on the basis of CD28 and CD95 expression), as previously described[5,27]. The data presented as 'end of vaccine phase' response frequencies represent an average of values obtained from samples collected on days 379, 392, 401 and 413 after initial vaccination. Titres of SIV Env-specific antibodies were determined by neutralization of tissue culture-adapted SIV$_{MAC251}$ using a luciferase reporter gene assay[22].

Viral detection assays. Quantitative real-time RT–PCR and PCR assays targeting a highly conserved sequence in Gag were used for standard measurements of plasma SIV RNA and cell-associated SIV RNA and DNA within peripheral blood and lymph node mononuclear cells, as previously described[23,24]. For plasma testing of a sample to score as positive, duplicate amplification reactions yielding ≥30 copy Eq ml⁻¹ were required. Isolated viral blips were also repeated in a duplicate sample in almost all instances, and in macaques where infection was manifest only by isolated viral blips (Group A and B controllers), infection was confirmed by the development (Group A) or boosting (Group B) of T-cell responses specific for SIV Vif, an SIV antigen not included in the RhCMV/SIV vectors (Supplementary Fig. 12), as described[5]. To further address the possibility of false positive amplification reactions, we analysed 136 known SIV-negative (pre-challenge) samples from the present study, and from other studies from the same facility and investigators and others. Samples were run in the same laboratory, over approximately the same period of time, using the same procedures, with negative specimens interspersed with positive samples in assay runs. Zero of 136 known SIV-negative samples scored positive by the above criteria, which was significantly different from 84 positive samples (viral 'blips') of 678 total samples (12.4%) obtained from RhCMV/SIV-vector-vaccinated controllers in vaccine Groups A and B ($P < 0.0001$, Fisher's exact test). To more precisely characterize levels of SIV DNA and RNA from tissues of RhCMV/SIV-vector-vaccinated controllers (which were below the sensitivity threshold of the standard assays), we used a new ultra-sensitive, nested, quantitative real-time PCR/RT–PCR method. Tissue pieces were collected directly into extraction tubes and immediately frozen using liquid nitrogen. Samples were stored at −80 °C and handled on dry ice until stabilized in extraction solution. Specimens of approximately 100 mg or less were homogenized in 1 ml of TriReagent (Molecular Research

Center) in 2 ml extraction tubes of Lysing Matrix D using FastPrep instrumentation (MP Biomedicals) according to the manufacturer's recommendations. Total RNA and DNA were prepared from the homogenates following manufacturer's recommendations, but specifically following the alternative, back-extraction method for DNA extraction. Recovered RNA and DNA were dissolved in minimal volumes of 10 mM TrisCl, pH 8.0 and 10 mM TrisCl, pH 9.0, respectively, as appropriate for replicate testing in qRT–PCR and qPCR protocols. Tissue specimens greater than 100 mg in mass were initially pulverized to powder under cryogenic conditions before extraction of RNA and DNA. Pulverization was accomplished in 15 ml polycarbonate extraction tubes with stainless steel grinding balls using rapid vertical shaking, all being maintained at appropriate temperatures in aluminium blocks pre-chilled in liquid N2 (GenoGrinder, SPEX SamplePrep). Pulverized tissue powder was then suspended in 3–10 ml of TriReagent, depending on the starting amount of tissue. Total RNA and DNA were then prepared from 1 ml of TriReagent suspension as described earlier; residual suspension was archived at $-80\,^{\circ}$C for additional analysis, as necessary. To maximize sensitivity, nested quantitative real-time PCR/RT–PCR protocols were designed to accommodate higher amounts of input nucleic acid, and potential inhibitors, than are typically tolerated in standard assays. Reaction conditions and thermal profiles followed those referenced above for the plasma and isolated cell assays[23,24] with two exceptions: (1) in the quantitative RT–PCR assay, the 'nested' reverse primer, as opposed to random hexamers, was used to prime cDNA synthesis specifically for SIV sequence, thereby avoiding generation of non-specific targets and further enhancing the direct sensitivity of detection of SIV RNA; and (2) 2.5 units of PlatinumTaq (Invitrogen), rather than 1.25 units of TaqGold (Applied Biosystems), were used in the amplification steps. A 'nested' or 'pre-amplification' of cDNA or DNA was performed for 12 cycles with the application of primers, SIVnestF01 (GATTTGGATTAGCAG AAAGCCTGTTG) and SIVnestR01 (GTTG GTCTACTTGTTTTTTGGCATAGTTTC), flanking the SIV Gag target region. Five microlitres of this first amplification were then transferred to 50 microlitres of cocktail for amplification of the SIV *gag* DNA target sequence in duplex with amplification of a single copy rhesus CCR5 target sequence for normalization, as referenced earlier[23,24]. Real-time PCR was then performed. For both RNA and DNA determinations, 12 replicate reactions were tested per sample including a spike of 10 copies of RNA or DNA internal control sequence standard in two of the 12 reactions to assess overall amplification efficiency and assess potential inhibition of the PCR or RT–PCR. Samples showing greater than a 5 cycle shift in amplification of the spiked standard, compared to amplification in the absence of specimen nucleic acid, corresponding to less than 74% overall amplification efficiency, were diluted and re-assayed. Quantitative determinations for samples showing amplification in all replicates were derived directly with reference to a standard curve. Quantitative determinations for samples showing less than 10 positive amplifications in replicates were derived from the frequency of positive amplifications, corresponding to the presence of at least one target copy in a reaction, according to a Poisson distribution of a given median copy number per reaction. It should be noted that this assay yielded no positive reactions out of a total of 1,100 total reactions (RNA and DNA) from tissues derived from an SIV-uninfected rhesus macaque, which was significantly different from 178 positive reactions of 4,310 total reactions (4.1%) from tissues derived from the 4 RhCMV/SIV-vector-vaccinated controllers studied at

necropsy ($P < 0.0001$; Fisher's exact test). The presence of inducible, replication competent SIV in mononuclear cell preparations derived from different tissue sites at necropsy was detected by co-cultivation with CEMx174 cells, as previously described[16,36]. To detect shedding of RhCMV/SIV vectors in the urine of vaccinated rhesus macaques, virus was concentrated from cleared pan-collected urine and co-cultured with macaque fibroblasts. Cell lysates were collected after development of cytopathic effect or after 28 days, and assessed for vector replication based on expression of SIV antigen-specific epitope tags by western blot analysis[5].

Statistical analysis. We performed statistical analysis with SAS version 9.1 (Statistical Analysis System). Individual tests are described in the figure legends for all analyses. Briefly, Fisher's exact test was used to determine significance of categorical data such as the fraction of controllers versus non-controllers in the different vaccine groups. The Wilcoxon rank sum and Kruskal–Wallis tests were used to compare populations of continuous data for groups of 2 and $\geq$3, respectively. Analysis of variance and a test for linear trend were used to determine the significance of the reduction in viral blip frequency over time in RhCMV-vector-vaccinated controllers. In all analyses, we used a two-sided significance level (alpha) of 0.05, with correction made for multiple comparisons using the Bonferroni method.

25. Okoye, A. *et al.* Profound CD4$^+$/CCR5$^+$ T cell expansion is induced by CD8$^+$ lymphocyte depletion but does not account for accelerated SIV pathogenesis. *J. Exp. Med.* **206,** 1575–1588 (2009).
26. Vaccari, M. *et al.* Reduced protection from simian immunodeficiency virus SIVmac251 infection afforded by memory CD8$^+$ T cells induced by vaccination during CD4$^+$ T-cell deficiency. *J. Virol.* **82,** 9629–9638 (2008).
27. Pitcher, C. J. *et al.* Development and homeostasis of T cell memory in rhesus macaque. *J. Immunol.* **168,** 29–43 (2002).
28. Veazey, R. S. *et al.* Gastrointestinal tract as a major site of CD4$^+$ T cell depletion and viral replication in SIV infection. *Science* **280,** 427–431 (1998).
29. Schmitz, J. E. *et al.* Simian immunodeficiency virus (SIV)-specific CTL are present in large numbers in livers of SIV-infected rhesus monkeys. *J. Immunol.* **164,** 6015–6019 (2000).
30. Chang, W. L. & Barry, P. A. Cloning of the full-length rhesus cytomegalovirus genome as an infectious and self-excisable bacterial artificial chromosome for analysis of viral pathogenesis. *J. Virol.* **77,** 5073–5083 (2003).
31. Kulkarni, V. R. *et al.* Comparison of immune responses generated by optimized DNA vaccination against SIV antigens in mice and macaques. *Vaccine* doi:10.1016/j.vaccine.2010.12.056.
32. Rosati, M. *et al.* DNA vaccines expressing different forms of simian immunodeficiency virus antigens decrease viremia upon SIVmac251 challenge. *J. Virol.* **79,** 8480–8492 (2005).
33. Rosati, M. *et al.* DNA vaccination in rhesus macaques induces potent immune responses and decreases acute and chronic viremia after SIVmac251 challenge. *Proc. Natl Acad. Sci. USA* **106,** 15831–15836 (2009).
34. Rosenfeld, M. A. *et al.* In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell* **68,** 143–155 (1992).
35. Mittereder, N., March, K. L. & Trapnell, B. C. Evaluation of the concentration and bioactivity of adenovirus vectors for gene therapy. *J. Virol.* **70,** 7498–7509 (1996).
36. Shen, A. *et al.* Novel pathway for induction of latent virus from resting CD4$^+$ T cells in the simian immunodeficiency virus/macaque model of human immunodeficiency virus type 1 latency. *J. Virol.* **81,** 1660–1670 (2007).