

Gag-Specific Cellular Immunity Determines *In Vitro* Viral Inhibition and *In Vivo* Virologic Control following Simian Immunodeficiency Virus Challenges of Vaccinated Rhesus Monkeys

Kathryn E. Stephenson,^a Hualin Li,^a Bruce D. Walker,^{b,c} Nelson L. Michael,^d and Dan H. Barouch^{a,b}

Division of Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA^a; Ragon Institute of MGH, MIT, and Harvard, Boston, Massachusetts, USA^b; Howard Hughes Medical Institute, Chevy Chase, Maryland, USA^c; and U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA^d

A comprehensive vaccine for human immunodeficiency virus type 1 (HIV-1) would block HIV-1 acquisition as well as durably control viral replication in breakthrough infections. Recent studies have demonstrated that Env is required for a vaccine to protect against acquisition of simian immunodeficiency virus (SIV) in vaccinated rhesus monkeys, but the antigen requirements for virologic control remain unclear. Here, we investigate whether CD8⁺ T lymphocytes from vaccinated rhesus monkeys mediate viral inhibition *in vitro* and whether these responses predict virologic control following SIV challenge. We observed that CD8⁺ lymphocytes from 23 vaccinated rhesus monkeys inhibited replication of SIV *in vitro*. Moreover, the magnitude of inhibition prior to challenge was inversely correlated with set point SIV plasma viral loads after challenge. In addition, CD8 cell-mediated viral inhibition in vaccinated rhesus monkeys correlated significantly with Gag-specific, but not Pol- or Env-specific, CD4⁺ and CD8⁺ T lymphocyte responses. These findings demonstrate that *in vitro* viral inhibition following vaccination largely reflects Gag-specific cellular immune responses and correlates with *in vivo* virologic control following infection. These data suggest the importance of including Gag in an HIV-1 vaccine in which virologic control is desired.

A vaccine for human immunodeficiency virus type 1 (HIV-1) would ideally block HIV-1 acquisition as well as control viral replication. Recent studies showed that vaccine regimens expressing simian immunodeficiency virus (SIV) Gag, Pol, and Env reduced SIV infection risk in rhesus monkeys and that the inclusion of Env was required for protection against SIV acquisition (1, 16, 17). These vaccine trials in monkeys also demonstrated that optimal vaccines reduced mean set point SIV viral loads following challenge (1, 17). It remains unclear, however, which antigens or immune responses are most important for virologic control.

A wealth of literature has shown that CD8⁺ T lymphocytes can mediate control of viremia in HIV-1-infected humans (2, 7, 10, 11, 22, 25) and SIV-infected rhesus monkeys (8, 9, 23) and that T lymphocytes specific for HIV-1 Gag epitopes are associated with virologic control *in vivo* (4, 5, 13, 15, 26, 28). Vaccine trials in nonhuman primates have also indicated an association between Gag-specific cellular immune responses and virologic control in vaccinated rhesus monkeys following SIV challenge (1, 12, 14, 18, 27). *In vitro* CD8⁺ T cell-mediated inhibition of HIV-1 has been shown to correlate with *in vivo* virologic control in HIV-1-infected subjects (6, 13, 22, 24). However, the *in vitro* viral inhibition assay has not been extensively studied in vaccinated or SIV-infected rhesus monkeys, and no previous study has shown a correlation between *in vitro* SIV_{MAC251} inhibition and *in vivo* virologic control.

In the present study, we demonstrate that CD8⁺ lymphocytes from vaccinated rhesus monkeys inhibit SIV_{MAC251} *in vitro* in a viral inhibition assay that was originally developed for humans (6, 24) and adapted for use in rhesus monkeys (12, 19). We also show that CD8⁺ cell-mediated viral inhibition *in vitro* correlates with Gag-specific, but not Pol- or Env-specific, CD4⁺ and CD8⁺ T lymphocyte responses and that *in vitro* inhibition prior to chal-

lenge inversely correlates with *in vivo* virologic control following SIV challenge.

MATERIALS AND METHODS

Animals, immunizations, and challenges. Cryopreserved peripheral blood mononuclear cells (PBMC) from rhesus monkeys of a previously published SIV vaccine study were utilized (1). All monkeys were immunized by the intramuscular route with the following vaccine regimens expressing SIV_{SME543} Gag-Pol and Env immunogens ($n = 7$ per group for this analysis): (i) DNA prime, modified vaccinia Ankara (MVA) boost; (ii) MVA prime, MVA boost; (iii) adenovirus serotype 26 (Ad26) prime, MVA boost; (iv) MVA prime, Ad26 boost; (v) sham controls. Monkeys were primed at week 0, boosted at week 24, and challenged at week 52. All monkeys were challenged intrarectally with the heterologous virus, SIV_{MAC251}. All animal studies were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

SIV infection of PBMC *in vitro*. PBMC obtained at week 20 prechallenge from 7 vaccinated animals were used to determine the minimum multiplicity of infection (MOI) of SIV_{MAC251} required to establish consistent productive infection *in vitro*. Cryopreserved PBMC were thawed and rested overnight in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% heat-inactivated fetal calf serum (FCS), 1% L-glutamine, 1% HEPES, and 1% penicillin-streptomycin (R10). On day -1 of the assay, CD8⁺ lymphocytes were depleted from PBMC by using a magnetically activated cell sorting nonhuman primate CD8 MicroBead kit

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Address correspondence to Dan H. Barouch, dbarouch@bidmc.harvard.edu.

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(Miltenyi Biotec), according to the manufacturer's instructions, and the CD8-depleted fraction was incubated with concanavalin A (ConA; 5 μ g/ml) in R10 containing 50 U of interleukin-2/ml (R10-50) at 37°C for 18 to 24 h. On day 0, aliquots of 10^6 CD8-depleted PBMC were resuspended in 150 μ l of R10-50 and incubated with SIV_{MAC251} at MOIs of 0.01, 0.1, 1, 5, 10, and 100 in 1.5-ml Eppendorf tubes at 37°C for 2 h. After incubation with virus, CD8-depleted PBMC were washed twice and resuspended at 10^6 cells/ml of R10-50. Then, 0.5×10^6 CD8-depleted PBMC were cultured in duplicate in 1 ml of R10-50 in 48-well plates and incubated at 37°C for 7 days. Half of the supernatant was replaced with R10-50 on days 4 and 7. Supernatant SIV p27 content was measured qualitatively on day 7 by enzyme-linked immunosorbent assay (ELISA; Zepetometrix). Wells with p27 levels above 20,000 pg/ml (the upper limit of detection at a 1:10 dilution) were considered positive for productive infection. Based on this titration, an MOI of 10 and 7 days of culture were chosen to establish consistent infections of CD8-depleted PBMC from vaccinated rhesus monkeys prechallenge.

Viral inhibition assay. Cryopreserved PBMC from weeks 28 to 34 prechallenge (reflecting peak vaccine immunity) were available from 23 of 29 vaccinated monkeys and 3 of 8 control monkeys that became SIV-infected following challenge. Cryopreserved PBMC from week 20 and freshly obtained PBMC from weeks 32 to 44 postchallenge (reflecting the viral set point/chronic infection) were available from 28 of 29 vaccinated, SIV-infected monkeys and from 7 of 7 surviving control, SIV-infected monkeys.

The *in vitro* HIV-1 inhibition assay has been described by Fauci et al. (6) and was modified for use with rhesus monkey PBMC. On day -1 of the assay, CD8⁺ lymphocytes were separated from thawed/rested or fresh PBMC, and the CD8-depleted fraction was incubated with ConA in R10-50 at 37°C for 18 to 24 h. The remaining fraction of CD8⁺ lymphocytes was incubated overnight in R10 alone. On day 0, 10^6 CD8-depleted PBMC were resuspended in 150 μ l of R10-50 and incubated with SIV_{MAC251} at MOIs of 0, 5, 10, and 100 in 1.5-ml Eppendorf tubes at 37°C for 2 h. Experimental duplicates were typically performed. After incubation with virus, CD8-depleted PBMC were washed twice and resuspended at 10^6 cells/ml of R10-50. Then, 0.5×10^6 CD8-depleted PBMC were cultured with or without 0.5×10^6 autologous CD8⁺ lymphocytes in 1 ml of R10-50 in 48-well plates and incubated at 37°C for 14 days. Half of the well supernatant was replaced with R10-50 on days 4, 7, 11, and 14. Supernatant SIV p27 content was measured quantitatively on days 7 and 14 by ELISA. An additional CD8⁺ lymphocyte titration experiment was performed on three SIV-infected animals with a low *in vivo* SIV viral load (<3.0 log plasma SIV RNA; "SIV controllers") based on CD8⁺/CD8⁻ ratios of 0:1, 0.5:1, 1:1, and 2:1.

Statistical analyses. CD8⁺ cell-mediated inhibition was expressed as the log reduction in p27 content of cocultures of CD8-depleted/CD8⁺ PBMC, compared with CD8-depleted PBMC alone. Negative values were treated as zero inhibition. Mean viral inhibition in each vaccine group was compared to sham controls by using Wilcoxon signed rank tests. All correlations with immunologic variables were performed using Spearman rank correlation tests. Gag-, Pol-, and Env-specific enzyme-linked immunosorbent spot assay (ELISPOT) and intracellular cytokine staining (ICS) data have been described previously (1). Two-tailed *P* values were calculated for all tests. *P* values of <0.05 were considered significant, except in the case of multiple comparisons when *P* values were adjusted using Bonferroni corrections.

RESULTS

Development of the *in vitro* SIV inhibition assay. The *in vitro* viral inhibition assay was originally developed for use with HIV-1-infected human PBMC (6, 24). Here we adapted this assay to assess *in vitro* CD8⁺ cell-mediated inhibition of SIV_{MAC251} using PBMC from rhesus monkeys. We first added CD8⁺ lymphocytes to cultures of CD8-depleted PBMC from three SIV-infected rhesus monkeys that spontaneously controlled viral replication (SIV

controllers) and one naïve uninfected animal. We measured endogenous SIV replication in cell cultures with increasing numbers of CD8⁺ lymphocytes, and we observed that p27 levels in culture supernatant decreased with increasing numbers of autologous CD8⁺ lymphocytes (Fig. 1A, left). We also assessed the capacity of CD8⁺ lymphocytes to suppress exogenous SIV_{MAC251} infection of CD8-depleted PBMC at a multiplicity of infection of 100. As a negative control, we observed that CD8⁺ lymphocytes from a naïve uninfected monkey did not suppress viral replication in PBMC, as expected (Fig. 1A, right). In contrast, CD8⁺ lymphocytes from SIV controllers effectively suppressed exogenous viral replication in CD8-depleted PBMC in a dose-dependent manner (Fig. 1A, right).

Viral inhibition in vaccinated animals prior to SIV challenge.

We next evaluated whether CD8⁺ lymphocytes from vaccinated but uninfected rhesus monkeys in our recently published vaccine study (1) could also suppress SIV_{MAC251} replication *in vitro*. In that study we evaluated the immunogenicity and protective efficacy of DNA/MVA, MVA/MVA, Ad26/MVA, and MVA/Ad26 vectors expressing SIV_{SME543} Gag-Pol and Env immunogens against SIV_{MAC251} challenge. Of the 32 vaccinated animals in the study, 29 became SIV infected following challenge; of these 29 animals, 23 had cryopreserved PBMC available for use in the viral inhibition assay. We also assessed *in vitro* viral inhibition in 3 of 8 control animals. CD8⁺ cell-mediated viral inhibition in vaccinated animals ranged from a 0.00 to 1.12 log reduction in p27 levels and was 0.00 for all three control animals. The magnitude of *in vitro* CD8⁺ cell-mediated viral inhibition was less in vaccinated animals than in SIV-infected controllers (Fig. 1B), although *in vitro* viral inhibition in vaccinated animals was still clearly detectable. In particular, there was maximal viral inhibition in the Ad26/MVA group (*P* = 0.016, Wilcoxon signed rank test compared with sham controls), which also showed the highest adaptive immune responses in the parent vaccine study (1). These results demonstrate that CD8⁺ lymphocytes from vaccinated rhesus monkeys suppress heterologous SIV *in vitro*.

***In vitro* viral inhibition correlates with Gag-specific cellular immune responses.** We next investigated the relationship between *in vitro* viral inhibition in vaccinated monkeys and cellular immune responses to various SIV antigens. We observed that *in vitro* CD8⁺ cell-mediated viral inhibition in vaccinated monkeys correlated significantly with Gag-specific, but not Pol- or Env-specific, cellular immune responses. In particular, viral inhibition correlated with Gag-specific ELISPOT responses (*P* = 0.002 and *R* = 0.62) (Fig. 2). In contrast, only a trend was observed between viral inhibition and Pol- and Env-specific responses (*P* = 0.067 and 0.043, respectively; a *P* value of <0.017 was required for significance due to multiple comparison adjustments). Moreover, viral inhibition correlated significantly with Gag-specific total CD8⁺ T lymphocyte responses (*P* < 0.001 and *R* = 0.66) (Fig. 3) and central/transitional memory CD8⁺ T lymphocyte responses (*P* < 0.001 and *R* = 0.68), as measured in multiparameter ICS assays. A trend was observed with Gag-specific effector memory CD8⁺ T lymphocyte responses (*P* = 0.014 and *R* = 0.51; *P* < 0.006 required for significance due to multiple comparison adjustments). However, there were no detectable associations between viral inhibition and Pol- or Env-specific CD8⁺ T lymphocyte responses. Viral inhibition in vaccinated animals also correlated significantly with Gag-specific total CD4⁺ T lympho-

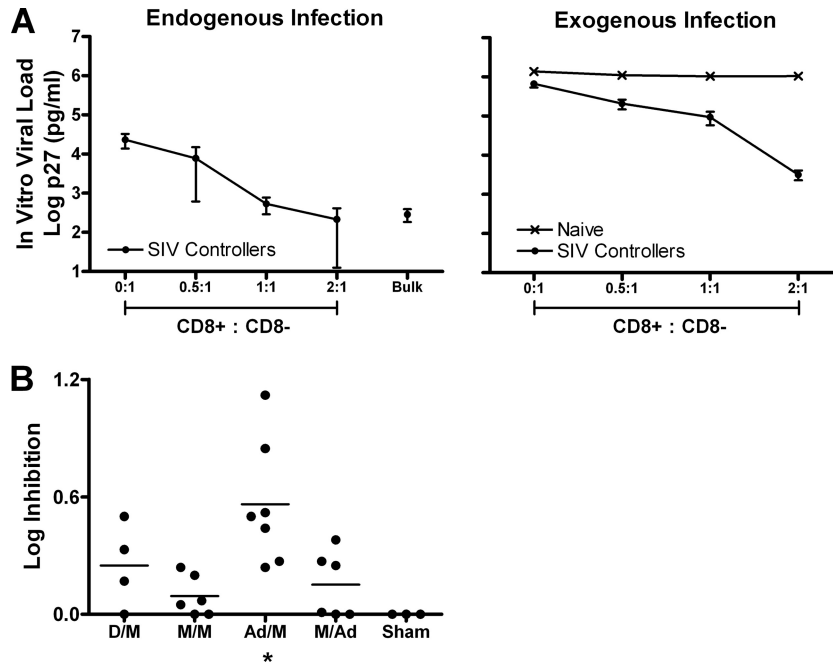


FIG 1 *In vitro* SIV inhibition assay results. (A) Mean SIV_{MAC251} log p27 levels (in pg/ml) at day 7 of culture in PBMC from SIV-infected rhesus monkeys with excellent *in vivo* viral control (SIV controllers; $n = 3$) and one naïve monkey. *In vitro* viral load is plotted for endogenous infection (left) and exogenous infection at a multiplicity of infection of 100 (right) in cocultures of CD8⁺/CD8-depleted PBMC using increasing numbers of CD8⁺ lymphocytes. Bars represent standard errors of the means. Bulk, unfractionated PBMC. (B) CD8⁺ cell-mediated viral inhibition in vaccinated rhesus monkeys is depicted for each vaccine group. *, $P = 0.016$, Wilcoxon signed rank test (Ad/M versus sham). The horizontal lines represent mean log inhibition levels. Ad, adenovirus serotype 26; D, DNA; M, MVA.

cyte responses ($P = 0.002$ and $R = 0.63$) (Fig. 4) and central/transitional memory CD4⁺ T lymphocyte responses ($P = 0.001$ and $R = 0.64$), whereas there were no associations between viral inhibition and Pol- or Env-specific CD4⁺ T lymphocyte responses. These data demonstrate that *in vitro* viral inhibition is largely determined by Gag-specific cellular immune responses in vaccinated rhesus monkeys.

***In vitro* SIV inhibition in vaccinated animals prior to challenge inversely correlates with *in vivo* virologic control following challenge.** We next assessed whether *in vitro* viral inhibition in vaccinated rhesus monkeys predicted *in vivo* set point virologic control. We observed that *in vitro* CD8⁺ cell-mediated viral inhibition at weeks 28 to 34 prechallenge (reflecting peak vaccine immunity) inversely correlated with postchallenge *in vivo* set point

plasma SIV RNA levels at week 12 following challenge ($P = 0.014$ and $R = -0.50$, Spearman rank-correlation test) (Fig. 5). To the best of our knowledge, this is the first study to demonstrate that *in vitro* SIV_{MAC251} inhibition following vaccination is significantly associated with *in vivo* virologic control following challenge.

Viral inhibition in vaccinated animals after SIV challenge also correlates with Gag specificity and *in vivo* virologic control. To elucidate further the relationship between *in vitro* viral inhibition and *in vivo* virologic control, we performed additional viral inhibition assays in these same animals 5 to 11 months after SIV_{MAC251} infection. CD8⁺ cell-mediated viral inhibition of endogenous virus in the 28 surviving vaccinated, SIV-infected monkeys ranged from 0.00 to 3.02 log reduction in p27 levels. In 7 control animals, viral inhibition ranged from 0.27 to

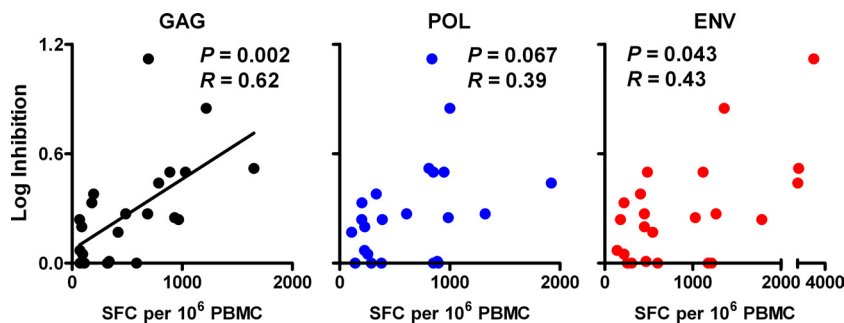


FIG 2 Correlation of prechallenge CD8⁺ cell-mediated viral inhibition and peak ELISPOT responses in vaccinated animals. CD8⁺ cell-mediated viral inhibition in vaccinated rhesus monkeys ($n = 23$) is plotted versus cellular immune responses to SIV_{MAC239} Gag, Pol, and Env as determined in gamma interferon ELISPOT assays at week 26 prechallenge. P values reflect Spearman rank correlation tests. P values of <0.017 were required for significance after multiple comparisons adjustments. SFC, spot-forming cells.

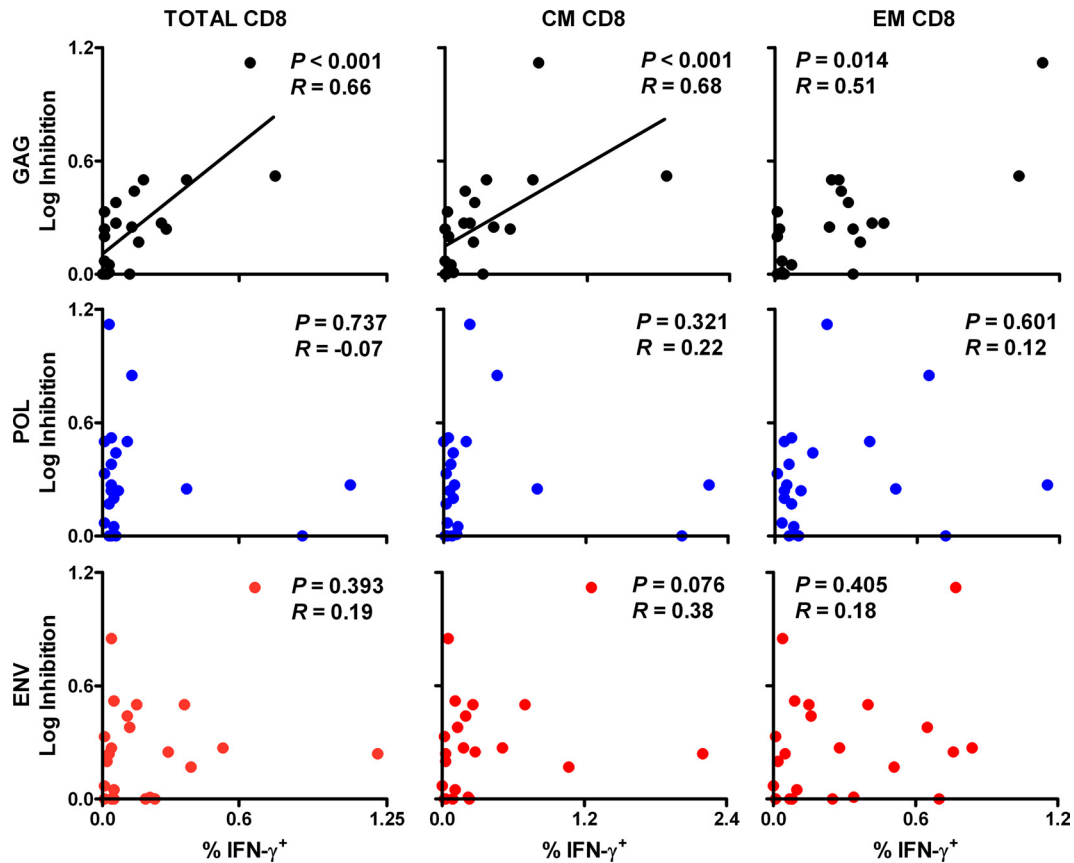


FIG 3 Correlation of prechallenge CD8⁺ cell-mediated viral inhibition and ICS responses among CD8⁺ T lymphocytes in vaccinated animals. CD8⁺ cell-mediated viral inhibition in vaccinated rhesus monkeys ($n = 22$) is plotted versus CD8⁺ total, central/transitional memory (CM; CD28⁺ CD95⁺), and effector memory (EM; CD28⁻ CD95⁻) responses to Gag, Pol, and Env as determined in multiparameter gamma interferon ICS assays at week 26 prechallenge. ICS data are missing for one vaccinated animal. P values reflect Spearman rank correlation tests. P values of <0.006 were required for significance after multiple comparison adjustments.

1.54 log reduction in p27 levels. These results show that CD8⁺ lymphocytes from vaccinated monkeys postchallenge suppress SIV replication *in vitro*. Moreover, viral inhibition correlated with Gag-specific ELISPOT responses ($P < 0.001$ and $R = 0.62$) (Fig. 6), but not Pol- or Env-specific responses ($P = 0.493$ and $P = 0.371$, respectively; $P < 0.013$ required for significance after multiple comparison adjustments). Interestingly, viral inhibition also weakly correlated with Nef-specific ELISPOT responses ($P = 0.010$), a protein not included in the vaccine (data not shown). These data suggest that Gag-specific cellular immune responses during chronic infection contribute substantially to *in vitro* viral inhibition, although responses to other SIV antigens may also contribute.

Finally, we evaluated whether CD8⁺ cell-mediated viral inhibition in vaccinated monkeys following challenge correlated with *in vivo* virologic control. Consistent with studies in HIV-1-infected humans (6, 22, 24), we found that viral inhibition was inversely correlated with concurrent *in vivo* plasma SIV RNA levels in these animals ($P = 0.007$ and $R = -0.51$) (Fig. 7). Taken together, these results demonstrate that Gag-specific cellular immune responses contribute to *in vitro* viral inhibition both before and after SIV challenge as well as *in vivo* virologic control in vaccinated rhesus monkeys.

DISCUSSION

The antigenic determinants of *in vitro* viral inhibition and *in vivo* virologic control in vaccinated rhesus monkeys following SIV challenge have been unclear. In this study, we demonstrated that *in vitro* viral inhibition prior to challenge was inversely correlated with *in vivo* set point plasma SIV viral loads after challenge. Moreover, *in vitro* viral inhibition correlated significantly with total and central/transitional memory CD8⁺ and CD4⁺ T lymphocyte responses to Gag, but not to Pol or Env. These data demonstrated that the *in vitro* viral inhibition assay following vaccination largely reflected Gag-specific cellular immune responses and correlated with virologic control following challenge.

CD8⁺ T lymphocytes have been shown to be critical for the control of HIV-1 infection in persons with very low levels of plasma HIV-1 RNA in the absence of antiviral therapy (7, 10, 15, 22, 25). A similar observation has also been made in SIV-infected rhesus monkeys (8, 9, 12, 14). For example, Hansen et al. demonstrated in nonhuman primates that peak SIV-specific CD8⁺ T lymphocyte responses following immunization were associated with virologic control in rhesus monkeys immunized with rhesus cytomegalovirus vaccines that included Gag antigens (9). In HIV-1-infected elite controllers, multiple mechanisms have been proposed to account for the potency of CD8⁺ T lymphocytes, includ-

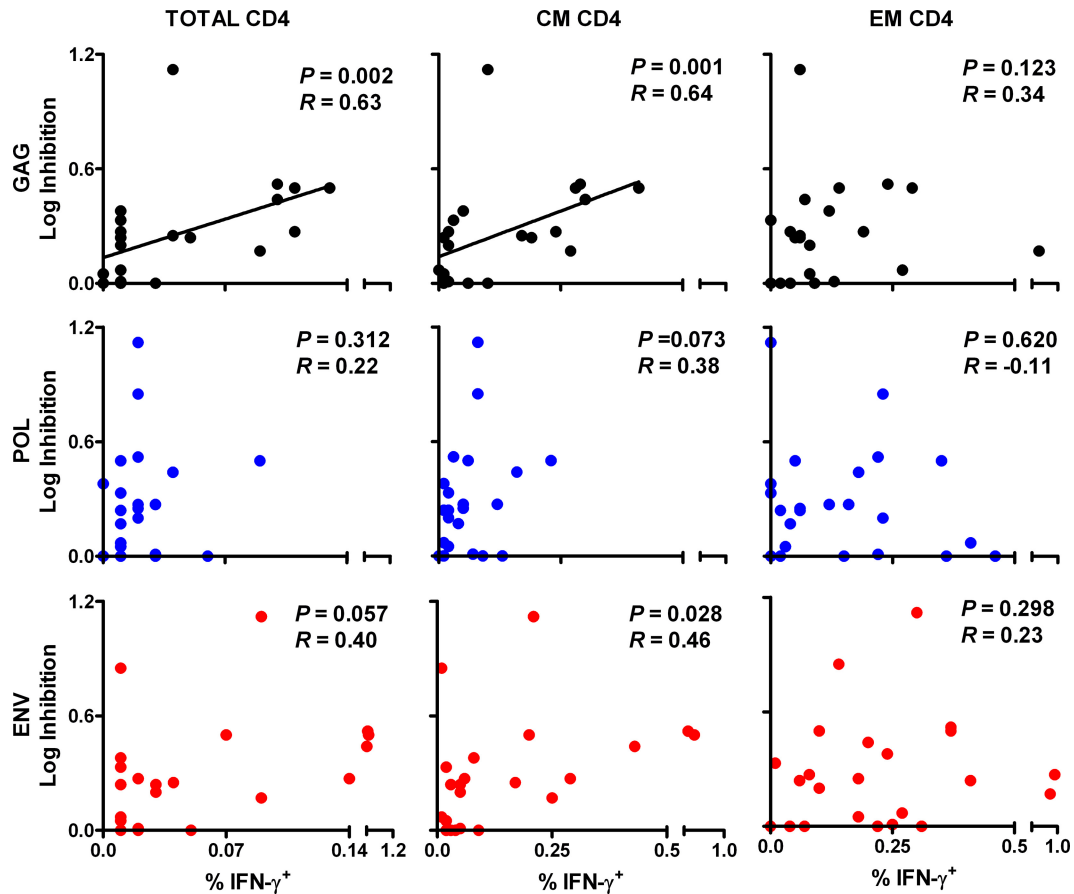


FIG 4 Correlation of prechallenge CD8⁺ cell-mediated viral inhibition and ICS responses among CD4⁺ T lymphocytes in vaccinated animals. CD8⁺ cell-mediated viral inhibition in vaccinated rhesus monkeys ($n = 22$) is plotted versus CD4⁺ total, central/transitional memory (CM; CD28⁺ CD95⁺), and effector memory (EM; CD28⁻ CD95⁻) responses to Gag, Pol, and Env as determined by multiparameter gamma interferon ICS assays at week 26 prechallenge. ICS data are missing for one vaccinated animal. P values reflect Spearman rank correlation tests. P values of <0.006 were required for significance after multiple comparison adjustments.

ing increased perforin expression (11), preserved proliferative capacity (20), and increased granule exocytosis-mediated elimination of HIV-1-infected CD4⁺ T cells (21). Moreover, studies have shown that *in vitro* CD8⁺ T cell-mediated inhibition of viral replication correlates significantly with *in vivo* HIV-1 control and

clinical status in HIV-1-infected subjects (2, 3, 22, 24). As a result, the *in vitro* viral inhibition assay has been proposed for use in the evaluation of HIV-1 vaccine candidates (24). Our findings extend these prior studies by adapting the *in vitro* viral inhibition assay for use in vaccinated rhesus monkeys. Specifically, we demonstrated that *in vitro* viral inhibition following vaccination correlates with virologic control following SIV challenge.

We observed that CD8⁺ cell-mediated viral inhibition was significantly associated with Gag-specific cellular immunity and not Pol- or Env-specific cellular immunity. This result is consistent with studies demonstrating the association of Gag-specific cellular immune responses with virologic control in HIV-1-infected individuals (4, 5, 13, 15, 26, 28) and SIV-infected rhesus monkeys (1, 12, 14, 18), as well as *in vitro* studies showing that Gag-specific cell lines and clones of cytotoxic T lymphocytes are more efficient than cell lines and clones with other specificities at killing exogenously peptide-loaded target cells (3, 27a, 28). In addition, our results are congruent with data from the parent vaccine trial that noted a correlation between Gag-specific ELISPOT magnitude and breadth and virologic control (1).

Our study also builds on previous work by Iwamoto et al. (12), who demonstrated that in a group of vaccinated rhesus monkeys ($n = 8$) with excellent SIV control after challenge, CD8⁺ T lym-

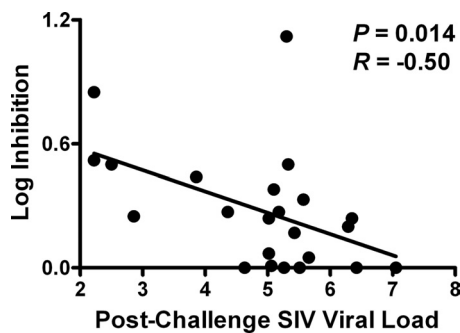


FIG 5 Correlation of prechallenge CD8⁺ cell-mediated viral inhibition with postchallenge *in vivo* set point plasma SIV RNA levels. CD8⁺ cell-mediated viral inhibition in vaccinated rhesus monkeys ($n = 23$) is plotted versus postchallenge set point plasma SIV_{MAC251} RNA levels (in log copies/ml). P values reflect Spearman rank correlation tests. P values of <0.05 were required for significance.

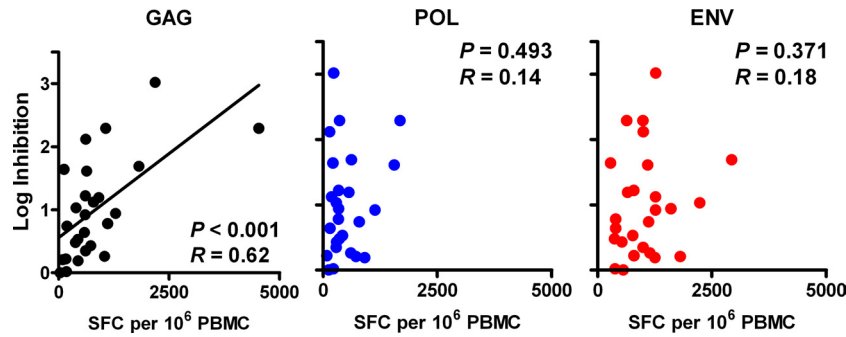


FIG 6 Correlation of CD8⁺ cell-mediated viral inhibition and concurrent ELISpot responses in vaccinated animals postchallenge. CD8⁺ cell-mediated viral inhibition in vaccinated rhesus monkeys at weeks 20 to 44 postchallenge ($n = 27$) is plotted versus concurrent cellular immune responses to SIV_{MAC239} Gag, Pol, and Env as determined in gamma interferon ELISpot assays. P values reflect Spearman rank correlation tests. P values of <0.013 were required for significance after multiple comparison adjustments. SFC, spot-forming cells.

phocytes from the prechallenge phase were able to suppress homologous SIV *in vitro*. Our data extend these findings with larger numbers of animals, as well as by using a heterologous strain of SIV and assessing for correlations with postchallenge viral loads. In contrast, Martins et al. (19) reported that vaccine-induced CD8⁺ T lymphocytes from a small group of rhesus monkeys ($n = 7$) suppressed SIV replication *in vitro* but did not predict virologic control *in vivo*. We suspect that these differences may reflect the larger sample size in our study ($n = 23$) as well as differences in the assays utilized, such as the use of a carefully defined limiting amount of SIV to infect cells in our present study.

Despite its potential utility, the *in vitro* viral inhibition assay also has limitations in terms of potential clinical applications. The magnitude of viral inhibition in vaccinated animals was lower than in SIV-infected animals, suggesting that only relatively large vaccine-elicited viral inhibition responses would have been detectable. Moreover, this assay would be difficult to scale up for high-throughput analysis. In addition, it is not clear whether the *in vitro* viral inhibition assay has superior predictive power compared to Gag-specific ELISpot responses. More studies are therefore warranted to confirm and compare these immune correlates of virologic control. Finally, it is likely that *in vitro* viral inhibition is only one determinant of *in vivo* virologic control and that other factors may also play a role.

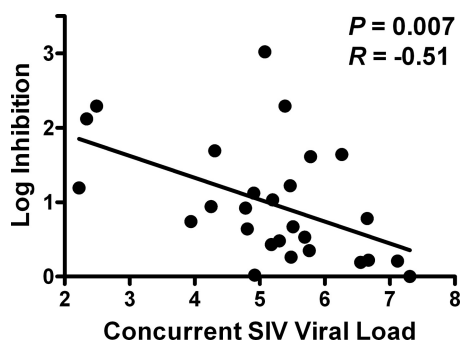


FIG 7 Correlation of postchallenge CD8⁺ cell-mediated viral inhibition with concurrent *in vivo* set point plasma SIV RNA levels. CD8⁺ cell-mediated viral inhibition in vaccinated rhesus monkeys at weeks 20 to 44 postchallenge ($n = 27$) is plotted versus concurrent plasma SIV_{MAC251} RNA levels (in log copies/ml). P values reflect Spearman rank correlation tests. P values of <0.05 were required for significance.

In summary, our data demonstrate that CD8⁺ lymphocytes from vaccinated rhesus monkeys inhibited replication of a heterologous strain of SIV *in vitro*. Moreover, the magnitude of *in vitro* viral inhibition prior to challenge was inversely correlated with *in vivo* plasma viral loads following challenge. In addition, CD8 cell-mediated viral inhibition correlated significantly with Gag-specific, but not Pol- or Env-specific, cellular immune responses. Taken together, the above findings suggest that Gag should be included in HIV-1 vaccine regimens if virologic control following infection is a goal.

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