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Vaccine-Induced T Cells Control Reversion of AIDS Virus Immune Escape Mutants▽

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Many current-generation human immunodeficiency virus (HIV) vaccines induce specific T cells to control acute viremia, but their utility following infection with escape mutant virus is unclear. We studied reversion to wild type of an escape mutant simian-HIV in major histocompatibility complex-matched vaccinated pigtail macaques. High levels of vaccine-induced CD8$^+$ T cells strongly correlated with maintenance of escape mutant virus during acute infection. Interestingly, in animals with lower CD8$^+$ T-cell levels, transient reversion to wild-type virus resulted in better postacute control of viremia. Killing of wild-type virus facilitated by transient reversion outweighs the benefit of a larger CD8$^+$ T-cell response that only maintains the less fit escape mutant virus. These findings have important implications for the further development of T-cell-based HIV vaccines where exposure to escape mutant viruses is common.

CD8$^+$ cytotoxic T lymphocytes (CTL) exert immune selection pressures on human, simian, or simian-human immunodeficiency virus (HIV, SIV, or SHIV), leading to the outgrowth of "escape mutant" (EM) viruses bearing mutations in CTL epitopes and abrogating CTL recognition (2, 11, 20–22). When EM viruses are transmitted to new hosts, two outcomes are observed. If the new host shares the relevant restricting major histocompatibility complex (MHC) allele with the donor and can therefore recognize the CTL epitope, then the escape mutation is maintained during chronic infection. Alternatively, if the epitope cannot be recognized, the T-cell EMs frequently "revert" to wild-type (WT) sequence, as the WT virus has a higher replicative capacity (is "fitter") and outgrows the EM virus (11, 13, 16).

Acute HIV and SIV infection is characterized by rapid viral growth and massive CD4$^+$ T-cell depletion in sites such as the gut (17, 19). Limiting viral fitness and dissemination during this acute phase is therefore a vital strategy for maintaining control of viremia (8, 23). It has been shown during chronic SIV infection of MHC-matched, unvaccinated rhesus macaques that transient late reversions of CTL EM to WT result in the reselection of the EM variant, though with limited impact on the viral load (3). Vaccination could potentially influence this interplay between WT and EM virus during the critical acute phase of infection, blunting the impact of disease.

The relative benefits of T-cell-mediated killing of WT virus-infected cells versus the fitness cost of the escape mutation have not been studied in acute and chronic infection following vaccination. We recently developed a pigtail macaque model that permits the study of these competing immunologic pressures. In pigtail macaques, the MHC class I molecule Mane-A*10 presents an immunodominant SIV Gag CD8$^+$ T-cell epitope (KP9; SIV $Gag_{164\text{-}172}$) (24), and we have developed an MHC class I tetramer (Mane-A*10/KP9) to detect KP9-specific T cells. The primary escape motif at the KP9 epitope is a lysine-to-arginine substitution at amino acid 165 (K165R) (11, 24, 25). In addition, we have characterized a SHIV challenge stock "preescaped" at the KP9 epitope ($SHIV_{mn229}$, with 91% of viral clones bearing the K165R EM and 9% WT). We have also studied the impact of T-cell immunity induced by DNA and recombinant poxvirus vaccine regimens on the rates of escape and reversion of this virus (6, 11, 15). In *Mane-A*10*-negative hosts, the K165R EM $SHIV_{mn229}$ strain rapidly reverts to WT lysine at position 165 during the first 2 weeks of infection, indicative of a strong in vivo selective advantage of WT over EM virus in the absence of immune pressure. This $SHIV_{mn229}$ challenge virus system now allows the in vivo dissection of competition between the EM and WT viruses under the influence of vaccine-induced KP9-specific T cells without the stochastic events required to generate the initial EM clone.

MATERIALS AND METHODS

Mane-A*10 pigtail macaques. Experiments on *Macaca nemestrina* were approved by the University of Melbourne and CSIRO livestock industries Animal Ethics Committees. Both reference strand-mediated conformational analysis and sequence-specific primer PCR were used to identify *Mane-A*10*$^+$ animals capable of responding to the SIV Gag KP9 epitope, as previously described (25).

Vaccination and $SHIV_{mn229}$ challenge. Twenty-four *Mane-A*10*$^+$ animals across two macaque SHIV vaccine studies were analyzed (Fig. 1 and Table 1). Study 1 was a prospective vaccine trial that is now published (9), while study 2 was retrospective on a completed study (6). The vaccination regimens of both studies (except for two animals in study 2) were three doses of DNA vaccines alone or prime-boost vaccination consisting of priming with two doses of DNA vaccinations followed by boosting with recombinant fowlpoxvirus (rFPV) vaccines or priming with a single dose of recombinant vaccinia virus (VV) followed by boosting with rFPV vaccines (Table 1). The vaccine doses for study 1 were pHIS-SHIV-AE DNA (1 mg in saline), recombinant VV (2×10^8 PFU), or rFPV

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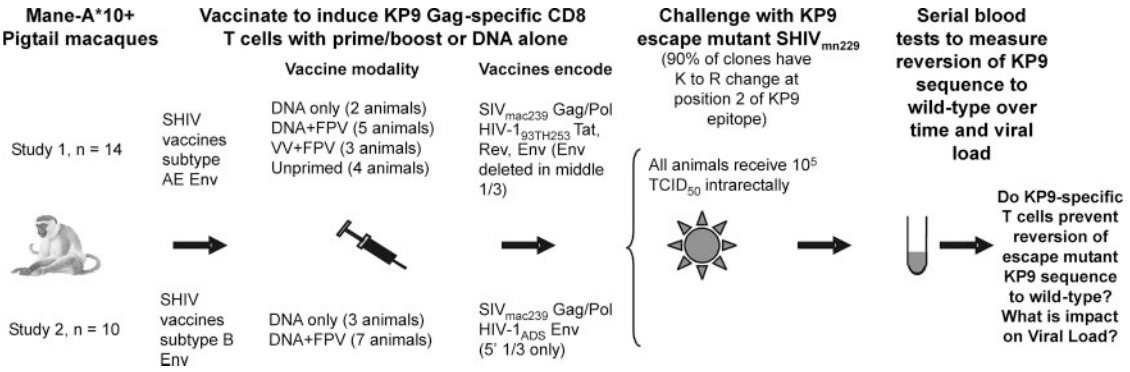

FIG. 1. Experimental strategy to measure the impact of vaccine-induced KP9-specific CD8 T cells on reversion to WT KP9 of EM SHIV$_{mn229}$. Macaques from two separate studies were vaccinated to induce KP9-specific T cells and then challenged 14 weeks (study 1) or 10 weeks (study 2) later. KP9 tetramer$^+$ cells, plasma virus reversion to WT KP9, and plasma viral loads were then correlated.

(a low dose of 5×10^7 or a high dose of 3×10^8 PFU); for study 2, they were pHIS-SHIV-B DNA (1 mg in saline) and rFPV (5×10^7 PFU) (6). All study 1 vectors expressed full-length WT SIV$_{mac239}$ Gag/Pol and subtype AE HIV-1$_{93TH253}$ Tat, Rev, and Env with the middle third deleted (5). These SHIV AE DNA and FPV vaccines were constructed identically to HIV type 1 (HIV-1) AE vaccines described previously (10). Both study 2 vectors expressed full-length WT SIV$_{mac239}$ Gag/Pol and the 5′ third of HIV-1 Env, as described previously (6). All vaccines were given 4 weeks apart intramuscularly. SHIV$_{mn229}$ challenge (10^5 50% tissue culture infectious doses; 500 monkey infectious doses) was administered intrarectally 14 weeks after the last vaccine in study 1 and 10 weeks after the last vaccine in study 2 (6). In study 1, 14 *Mane-A*10*-negative animals were randomly assigned to receive the same vaccines as the *Mane-A*10*$^+$ animals and were also analyzed to compare SIV Gag-specific immune responses and post-acute viral loads. SHIV viremia was quantified by real-time reverse transcriptase PCR as described previously (7).

Cloning and sequencing of plasma SHIV across the KP9 epitope. SHIV RNA was extracted from plasma at each time point, and cDNAs were generated as previously described (11). The Gag region containing the KP9$_{164-172}$ epitope was then amplified using SIV Gag-specific primers under conditions previously outlined (11).

Measurement of T-cell immunity. KP9-specific T-cell responses were monitored by flow cytometry using a KP9/Mane-A*10 tetramer conjugated to phycoerythrin as previously described (1, 25). Fresh whole blood (200 μl; study 1) or frozen peripheral blood mononuclear cells (PBMC) (10^6; study 2) were stained

TABLE 1. Summary of immune responses, viral load, and percent WT KP9 sequence

Study	Animal	Vaccine[a]	KP9 tetramer response[b] (% of blood CD8$^+$ T lymphocytes) on day:							Viral load ($\log_{10}$ SHIV RNA copies/ml plasma) on day:						% WT KP9 sequence (% WT/total clones sequenced[e]) on day:					
			0	7	10	13/14[c]	16	20/21	35/42	7	10	13/14	16	20/21	35/42	7	10	13/14	16	20/21	35/42
1	6352	Unprimed	0	0	0	0	0.03	0.44	0.04	3.18	5.14	6.88	8.09	6.98	5.94	100	30	10	40	44	10
	5619	Unprimed	0	0.01	0	0.27	0.21	0.4	0.11	4.82	6.99	7.56	6.59	5.48	5.02	24	30	68	ND	80	0
	5712	Unprimed	0	0	0.01	0.01	0.22	0.38	0.03	3.36	5.88	7.6	7.62	6.80	5.78	27	40	58	ND	50	0
	6167	Unprimed	0	0	0	0.22	0.49	0.14	0.36	6.03	7.67	7.51	6.67	6.23	5.61	14	33	36	ND	70	42
	6351	DNA only	0.01	0.01	0.02	1.46	30.71	3.85	2.75	3.62	5.5	7.12	7.31	7.18	6.43	0	30	9	0	5	0
	5614	DNA only	0.04	0.05	0.12	15.05	13.15	3.18	0.67	4.66	6.97	7.25	6.58	4.9	6.09	52	22	4	0	ND	0
	5616	DNA/FPV[f]	0.03	0.07	0.13	20.46	32	24.19	4.87	4.65	7.11	7.04	6.25	5.66	5.18	100	100	92	80	40	34
	5396	DNA/FPV[f]	0.22	0.15	0.25	0.67	19.2	17.19	5.01	3.18	6.11	7.69	8.17	6.85	6.02	ND	10	0	ND	0	0
	6370	DNA/FPV	0.14	0.08	0.13	1.65	14.96	23.04	8.79	3.3	5.73	7.88	7.38	6.27	5.33	0	0	9	0	0	0
	6279	DNA/FPV[f]	0.08	0.23	0.22	3.53	19.14	16.46	2.65	4.02	6.07	7.12	6.87	6.39	5.64	0	5	4	ND	ND	0
	6276	DNA/FPV	0.06	0.09	0.8	42.19	30.98	26.91	18.09	4.86	7.53	8.03	7.45	6.42	5.63	41	10	0	ND	ND	0
	6259	VV/FPV	0.03	0.01	0.03	0.05	0.08	0.18	0.08	3.18	5.1	6.61	6.97	6.98	6.3	0	0	0	ND	ND	0
	6349	VV/FPV	0.29	0.17	1.48	21.86	42.71	43.24	4.72	4.44	6.56	7.23	6.24	6.89	5.89	32	0	0	ND	ND	0
	6377	VV/FPV	0.05	0.06	0.06	0.04	35.59	6.97	8.26	3.18	5.36	6.82	7.66	7.26	5.67	ND	11	0	ND	0	0
2	4241	DNA only	0.02	0.39	ND[d]	0.68	ND	0.29	0.15	3.59	ND	7.1	ND	5.99	5.42	80	ND	71	ND	0	0
	4386	DNA only	0.27	0.19	ND	3.3	ND	1.45	1.01	4.67	ND	4.41	ND	4.12	3.84	69	ND	71	ND	41	7
	4299	DNA only	0.27	0.46	ND	8.92	ND	3.07	1.32	4.83	ND	6.2	ND	5.61	5.06	93	ND	73	ND	0	ND
	4247	DNA/FPV	0.3	0.51	ND	6.52	ND	11.59	2.4	3.2	ND	6.21	ND	4.82	4.37	100	ND	39	ND	17	43
	4277	DNA/FPV	0.6	1.18	ND	4.15	ND	1.32	0.81	4.66	ND	7.03	ND	5.29	4.51	10	ND	0	ND	ND	ND
	4290	DNA/FPV	0.45	0.75	ND	2.86	ND	3.03	0.45	4.47	ND	7.85	ND	6.62	5.34	40	ND	0	ND	ND	ND
	4295	DNA/FPV	0.44	1.08	ND	7.65	ND	7.2	6.32	3.32	ND	6.97	ND	6.26	5.5	20	ND	0	ND	ND	0
	4296	DNA/FPV	0.63	1.17	ND	2.02	ND	2.7	2.09	4.46	ND	4.64	ND	4.99	5.14	33	ND	0	ND	13	ND
	4382	DNA/FPV[g]	0.28	0.33	ND	0.69	ND	0.48	1.15	3.35	ND	5.88	ND	5.15	5.38	17	ND	0	ND	ND	ND
	4664	DNA/FPV[h]	0.58	0.56	ND	17.8	ND	ND	1.33	5.1	ND	6.8	ND	6.79	5.06	47	ND	30	ND	0	ND

[a] Vaccines and animals for study 2 were described previously (6). Vaccines for study 1 were similar SHIV DNA vaccines (1 mg in saline intramuscularly three times), VV recombinants (2×10^8 PFU intramuscularly once), and FPV (5×10^7 or 3×10^8 PFU intramuscularly once), all expressing full-length WT SIV$_{mac239}$ Gag/Pol and subtype AE HIV-1$_{93TH253}$ Tat, Rev, and Env with the middle third deleted (5).

[b] KP9-Mane-A*10 tetramer studies were performed on fresh blood in study 1 and on frozen PBMC in study 2 as described previously (25).

[c] Study 1 had day 13, day 20, and day 42 time points; study 2 had day 14, day 21, and day 35 time points.

[d] ND, not done.

[e] Performed by cloning and sequencing plasma SHIV RNA across KP9 SIV Gag epitope (11). The number of total clones sequenced for both studies was 1,923. The mean number of clones per animal sample was 21 (range, 9 to 42 clones).

[f] Animals given the high dose of rFPV vaccine (3×10^8 PFU).

[g] Animal given a prime-boost vaccination consisting of only one DNA vaccine followed by an rFPV boost.

[h] rFPV vaccine vector expressed the human IFN-γ gene in addition to other genes described in Materials and Methods.

with the KP9/Mane-A*10 tetramer (1:200 to 1:400 dilution) and then counterstained with anti-CD3-FITC (Becton Dickinson, San Diego, CA; clone SP34) and anti-CD8-APC (Becton Dickinson; clone SK1). Induction of Gag-specific intracellular gamma interferon (IFN-γ) expression in $CD3^+$ $CD8^+$ T lymphocytes was assessed by stimulating whole blood with 1 μg/ml of overlapping 15-mer peptides, WT for SIV_{mac239} sequence, for 6 h and analyzing them by flow cytometry as previously described (7, 18).

Statistical analysis. Correlations between T-cell responses, reversion to WT virus, and viral load used Spearman rank correlation. Analyses of vaccination modality and reversion to WT used the Kruskal-Wallis test, and paired comparisons of vaccine modalities used Dunn's multiple comparisons posttest to compare subgroups.

RESULTS

Vaccine-induced T cells limit reversion to WT virus. We hypothesized that vaccine-induced $CD8^+$ T-cell immunity present at the time of transmission would have an impact on the degree of reversion of transmitted EM virus to WT during acute infection. Multiple plasma SHIV Gag cDNA clones were sequenced across the KP9 epitope from 14 *Mane-A*10*$^+$ macaques at closely spaced time points during acute infection with the EM $SHIV_{mn229}$. Ten of the 14 macaques in study 1 were primed for KP9-specific $CD8^+$ T-cell responses with DNA only or DNA-vaccinia virus and rFPV expressing the WT SIV Gag CTL epitope KP9 (Fig. 1 and Table 1).

Striking transient reversions to WT virus were observed in some macaques over the first weeks of acute infection (Fig. 2). The degrees of early transient reversion to WT were, however, remarkably variable between macaques (Fig. 2b). Since maintaining the less fit EM virus presumably requires pressure from effector CTL, we evaluated the relationship between the number of KP9-specific T cells in fresh blood samples from the day of challenge and the degree of transient reversion to WT seen subsequently. Significant inverse correlations existed between KP9-specific T cells on the day of challenge and the proportion of virus that had reverted to WT at day 10, day 13, day 20, and the peak reversion to WT virus (Fig. 3a to d) ($r = -0.77$, -0.73, -0.92, and -0.75; $P = 0.001$, 0.003, 0.0004, and 0.003, respectively; Spearman rank correlation). Hence, the vaccine-induced KP9-specific T-cell response at transmission appears to determine the dominance of WT or EM virus during acute infection.

Maintaining the escape variant could depend not only on KP9-specific T cells on the day of challenge, but also on their efficiency of expansion during acute infection. KP9-specific T cells 7 days after challenge inversely correlated with reversion to WT at both day 10 and day 13 (Fig. 3e and f) ($r = -0.63$ and -0.55; $P = 0.02$ and 0.04, respectively). KP9-specific T cells at day 10 also inversely correlated with reversion to WT at both days 10 and 13 (not shown) ($r = -0.60$ and -0.62; $P = 0.02$ and 0.02, respectively).

To confirm these findings, frozen PBMC and plasma samples from 10 additional *Mane-A*10*$^+$ macaques vaccinated with DNA alone or DNA and FPV and also challenged with the EM $SHIV_{mn229}$ from a previous vaccine study (6) were analyzed (Table 1). Again, a significant inverse correlation existed between KP9-specific T cells on the day of challenge and reversion to WT virus 2 weeks later (Fig. 3g) ($r = -0.65$; $P = 0.02$). This correlation, although still significant, was not as strong as observed in the first study, potentially owing to the longer sampling interval and the use of frozen cells for the tetramer analyses.

Vaccine modality influences reversion to WT virus. DNA vaccination alone induces much lower levels of specific T cells than regimens that involve heterologous prime-boost vaccination (6, 10). We therefore examined the relationship between vaccine modality and reversion to WT virus using data from the two vaccine studies described above in which *Mane-A*10*$^+$ animals were immunized with either DNA only or prime-boost vaccine strategies (Table 1). Across the 24 animals studied, there was a significant relationship between vaccine modality and reversion to WT virus at day 14 (Fig. 3 h) (Kruskal-Wallis test; $P = 0.008$). Specifically, prime-boost-vaccinated animals (DNA or VV vaccine priming with FPV vaccine boosting) had significantly lower reversion to WT virus at day 14 than animals vaccinated with DNA alone (mean ± standard error of the mean [SEM] reversion = 12% ± 7% versus 46% ± 16%; $P = 0.03$; Dunn's multiple-comparison posttest to compare subgroups). Thus, the modality of vaccination and efficiency of T-cell priming directly influence the control of virus with WT sequence.

Virologic effect of reversion to WT virus. The transient reversion to WT KP9 followed by reescape detected during acute infection of *Mane-A*10*$^+$ macaques presumably reflects activation of KP9-specific T cells and then killing of cells infected with the "fitter" WT virus. In animals that do not revert to WT, the less fit EM virus is maintained, but these animals do not benefit from the $CD8^+$ T-cell-mediated killing of cells infected with WT KP9. We assessed how the relative benefits of immune recognition of WT virus versus the fitness cost of EM virus affected the overall viral dynamics in the infected animals.

We first studied the virologic effect on acute infection. Since WT virus is "fitter" than the EM virus (11), we might expect a more rapid viral growth and earlier peak viral loads in animals that revert to WT virus due to enhanced viral replication. Indeed, an earlier timing of the peak viral load was significantly associated with the proportion of WT virus at the viral-load peak (Fig. 4a) ($r = -0.45$; $P = 0.03$). This is consistent with more rapid WT virus replication than for EM virus.

Does transient reversion to WT during acute infection make a difference in the longer term? We analyzed postacute plasma viral levels 6 weeks after challenge across all 24 *Mane-A*10*$^+$ animals and found that animals with higher levels of reversion to WT during acute infection had significantly lower viremia levels (Fig. 4b) ($r = -0.46$; $P = 0.03$). This was a surprising result; lower levels of primed KP9-specific T-cell immunity were unable to limit reversion to WT but resulted in lower levels of chronic viremia. The results suggested that reversion to WT virus and subsequent killing of this revertant WT virus by restimulated T cells is more efficient in controlling viremia than simply maintaining the less fit EM virus from early infection with higher levels of primed T cells. It is notable that previous studies have suggested that *Mane-A*10*$^+$ animals are unable to prime a new response to the K165R EM peptide in animals infected with the EM virus (reference 11 and data not shown).

Effect of "redundant" immunodominant T-cell response following EM virus challenge. We had previously shown that *Mane-A*10*$^+$ macaques had an improved virologic outcome compared to *Mane-A*10*-negative macaques following chal-

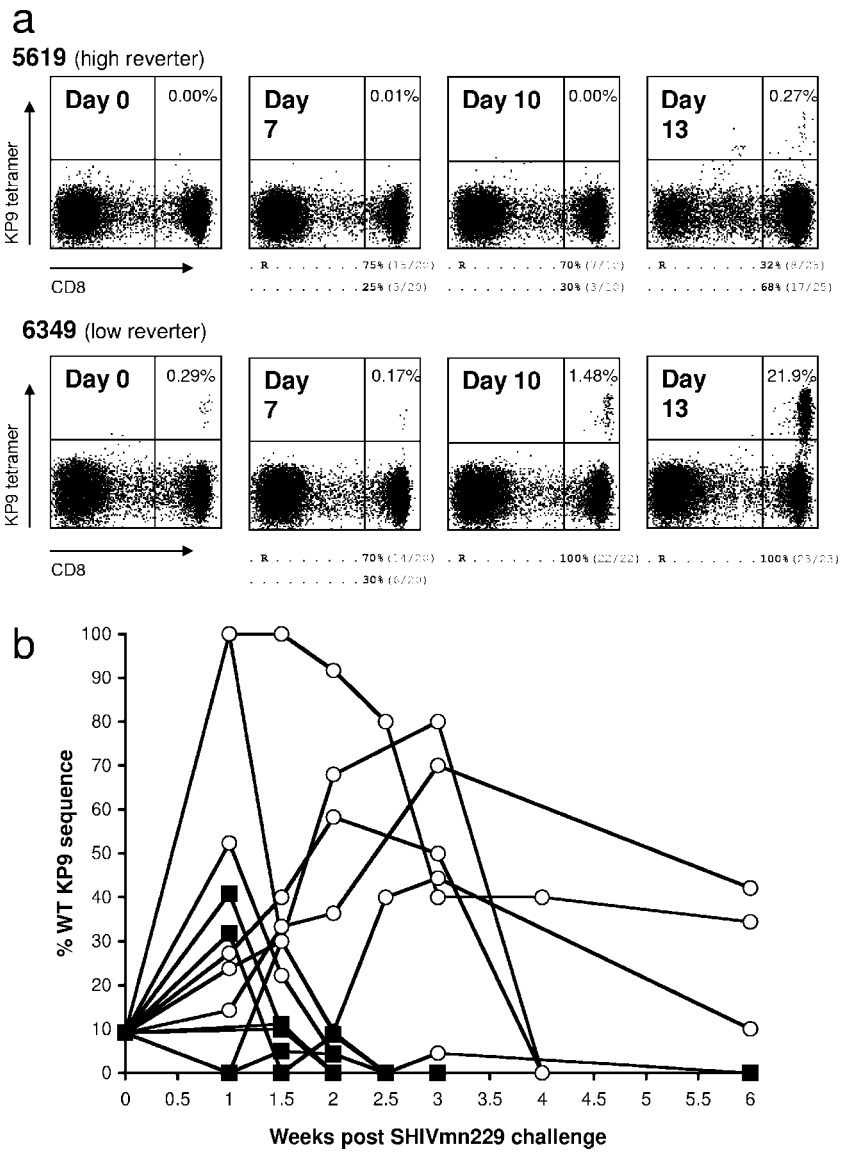

FIG. 2. Relationship of $CD8^+$ T cells and control of WT SHIV viremia. (a) KP9-specific $CD8^+$ T cells quantified by MHC tetramer staining of fresh blood (25) were studied at closely spaced intervals after challenge with the KP9 EM virus $SHIV_{mn229}$ (11). The proportions of KP9-specific $CD8^+$ T cells are indicated in the upper-right quadrants. Serial samples from two representative macaques are shown. Animal 5619 showed low levels and poor expansion of the KP9 T cells, and animal 6349 showed higher levels prechallenge and better expansion of the T cells. Clones derived from plasma SHIV RNA at each time point were sequenced across the KP9 epitope (the WT amino acid epitope of KKFGAEVVP is denoted by dots). Virus from animal 5619 reverted to WT (R to K at position 165 of Gag) in 68% of clones by 13 days postchallenge. Viral clones from animal 6349 (with higher T-cell immunity) displayed minimal reversion to WT (0% at day 13). (b) Reversion to WT was highly variable across the 14 macaques from study 1. The six animals with higher levels of baseline KP9-specific T cells by MHC tetramer studies (>0.04%; black squares) had less reversion to WT virus than animals with lower baseline levels of KP9-specific T cells (open circles).

lenge with SIV_{mac251}, which is WT at KP9 (25). This presumably reflects efficient killing of virus-infected cells by KP9-specific CTL, since the KP9-specific response is highly immunodominant (24, 25) and responses to non-Gag antigens are low and infrequent (6). In the setting of infection with an EM virus, such as $SHIV_{mn229}$, however, induction of an immunodominant KP9-specific $CD8^+$ T-cell response could be counterproductive by limiting T-cell immunity to other epitopes and inflicting only the more modest virologic benefit of maintaining the EM virus. Previous studies have shown no specific $CD8^+$ T-cell response to the K165R EM peptide in SIV-infected *Mane-A*10*$^+$ animals with either the WT or EM virus present (reference 11 and data not shown).

To evaluate this further, we studied the virologic outcome of infection with the EM $SHIV_{mn229}$ in vaccinated *Mane-A*10*$^+$ animals able to mount a KP9-specific $CD8^+$ T-cell response compared to *Mane-A*10*-negative animals in the same vaccine study. The EM KP9 sequence reverts uniformly and rapidly to WT following $SHIV_{mn229}$ infection of *Mane-A*10*-negative animals (11). Each of the four immunization cohorts of six animals (DNA/FPV, DNA/FPV with a high dose of FPV, DNA only, or VV/FPV) was randomly stratified before vaccination

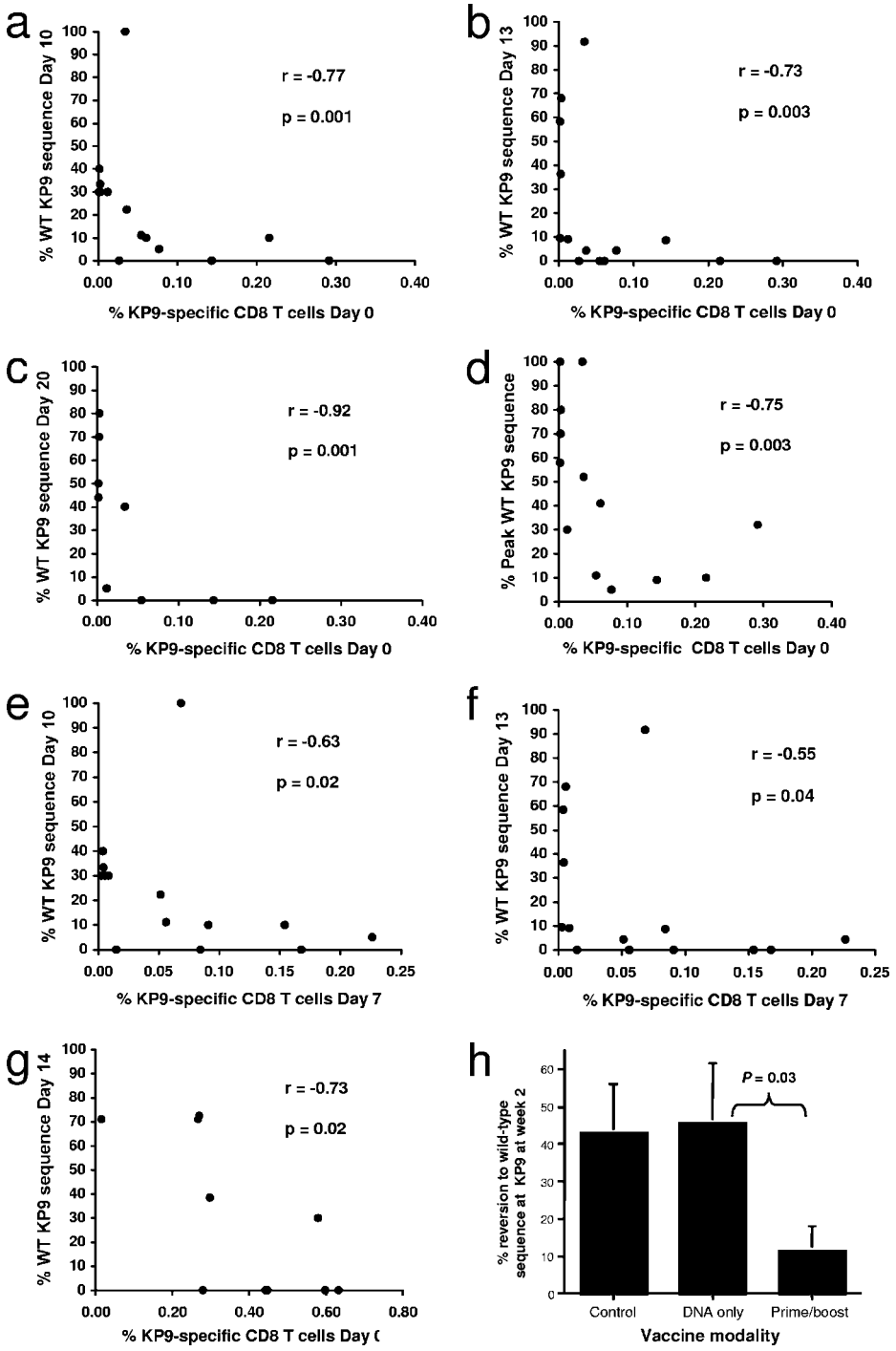

FIG. 3. Reversion to WT virus is correlated with vaccine-induced $CD8^+$ T cells. (a to d) An inverse correlation between KP9-specific T cells on the day of EM SHIV challenge and reversion to WT at the KP9 epitope 10 days, 13 days, and 20 days later and to the peak of reversion in study 1. (e to f) Expansion of KP9-specific $CD8^+$ T cells by day 7 inversely correlated with reversion to WT at days 10 and 13 after challenge. (g) A study of 10 additional vaccinated *Mane-A*10*$^+$ macaques (study 2) confirmed an inverse correlation between KP9-specific T cells on the day of EM SHIV challenge and reversion to WT at the KP9 epitope 14 days later. (h) Effect of vaccine modality on reversion to WT virus at day 14. Twenty-four animals across both studies were divided into unprimed, DNA-only vaccinated, or prime-boost vaccinated (either DNA/FPV or VV/FPV) animals as shown in Table 1. Spearman correlation coefficients and significance values are shown for panels a to g, and in panel h, the significance value using the Kruskal-Wallis test and Dunn's multiple comparisons posttest to compare subgroups is shown. The error bars indicate SEM.

to contain two or three *Mane-A*10*$^+$ animals and three or four *Mane-A*10*-negative animals.

The immunodominant effect of the KP9 response in *Mane-A*10*$^+$ animals in comparison to *Mane-A*10*-negative animals was assessed by intracellular IFN-γ staining of $CD8^+$ T cells following stimulation with a pool of overlapping 15-mer peptides spanning the Gag protein. The 10 *Mane-A*10*$^+$-vaccinated animals studied had a mean 3.5-fold-higher peak pro-

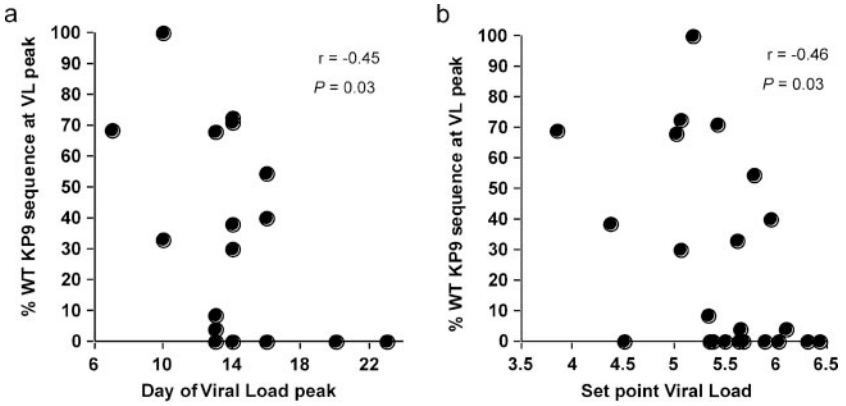

FIG. 4. Virologic effects of reversion to WT during acute infection. Twenty-four animals in both vaccine studies were analyzed. The proportion of WT sequences at the KP9 epitope at peak viral load was interpolated from data at surrounding time points in four animals for which it was not measured precisely at the time of peak viral load. (a) The timing of peak viral load (day postchallenge) was significantly inversely associated with the proportion of WT virus at the viral-load peak—animals with higher levels of reversion to WT had an earlier peak viral load. (b) The viral load at day 42 was significantly inversely associated with the proportion of WT sequence at KP9 during peak viremia—animals with higher levels of reversion to WT had lower viral loads.

portion of Gag-specific T cells producing IFN-γ after vaccination than 14 *Mane-A*10*-negative animals receiving the same vaccines in study 1 (Fig. 5a) (0.42% versus 0.12%; $P = 0.015$; Mann-Whitney). The vast majority of Gag-specific IFN-γ^+ CD8 T cells in *Mane-A*10*$^+$ animals are specific for the immunodominant KP9 epitope (reference 11 and data not shown). There was no difference in the levels of responses to the non-Gag antigens expressed by the vaccines (Pol, Env, Tat, and Rev) between *Mane-A*10*$^+$ and -negative animals, and they were low in both groups (reference 6 and data not shown). Despite challenge with the EM virus, there was also a nonsignificant trend toward higher peak anamnestic responses of Gag-specific CD8$^+$ T cells after challenge in *Mane-A*10*$^+$ animals than in *Mane-A*10*-negative animals (11.3% versus 6.2%; $P = 0.12$).

In spite of lower levels of Gag-specific CD8$^+$ T-cell immunity and rapid reversion to WT KP9, the *Mane-A*10*-negative animals had an improved postacute viral load compared to *Mane-A*10*$^+$ animals (Fig. 5b). There was a significant and sustained reduction in plasma viremia of ~0.5 $\log_{10}$ copies/ml in the *Mane-A*10*-negative group from week 4 postchallenge ($P = 0.013$ at day 42; Mann-Whitney).

DISCUSSION

A definitive link between vaccine-induced CD8$^+$ T-cell immunity and control of WT virus at the relevant epitope was demonstrated in this macaque model. This study of 24 MHC-matched pigtail macaques found that vaccine-induced CD8$^+$ T-cell responses quashed reversion to WT of an immune escape variant SHIV. The levels of reversion were influenced by the efficiency of priming of T-cell immunity induced by DNA or prime-boost vaccine strategies.

Studying the virologic outcome of reversion to WT viremia revealed an intriguing paradox. Reversion to a fitter WT viral phenotype resulted in more rapid viral growth and an earlier peak in the acute viral load. Prevention of reversion to WT by the immunodominant KP9-specific T-cell response correlated with a significantly lower postacute viral load following EM virus challenge. Further, *Mane-A*10*-negative animals incapa-

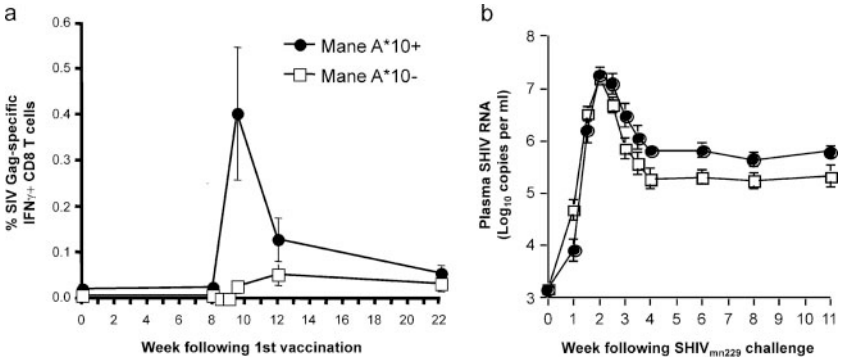

FIG. 5. Effect of vaccine-induced immunodominant Gag-specific T cells in *Mane-A*10*$^+$ animals in comparison to animals negative for *Mane-A*10*. Ten *Mane-A*10*$^+$ animals were compared to 14 *Mane-A*10*-negative animals. The animals were matched for receiving either DNA, DNA/FPV, or VV/FPV vaccines expressing WT Gag, and all were challenged with the EM $SHIV_{mn229}$. (a) Gag-specific CD8$^+$ T cells (mean ± SEM) measured by intracellular cytokine staining after vaccination. (b) Plasma $SHIV_{mn229}$ viral load (mean ± SEM) after challenge.

ble of recognizing this immunodominant epitope also had an improved postacute viral load following infection with virus escaped at this epitope compared to *Mane-A*10*$^+$ animals, which respond strongly to KP9. This was despite both lower levels of vaccine-induced CD8$^+$ T-cell immunity in the *Mane-A*10*-negative animals and uniform reversion to the fitter WT virus by 2 to 3 weeks postinfection (11). Thus, assessing the efficacy of vaccination regimes by focusing on the magnitude of responses to a few immunodominant epitopes may be counterproductive, as these highly focused responses may select for escape mutations and result in a worsening of the long-term virologic outcome (2).

This is the largest reported study of analysis of reversion to WT virus in MHC-matched subjects to date. However, there are some important limitations to our data, and several future studies are suggested by these findings. First, many of the control and poorly protected animals had to be euthanized by week 11 after challenge with this virulent X4-tropic $SHIV_{mn229}$ stock, which precluded longer-term analyses of the virologic outcome. Since R5-tropic viruses are more commonly transmitted between humans and permit longer-term macaque studies, an analysis of longer-term virologic and immunologic outcomes following an R5-tropic infection, such as SIV_{mac}, needs to be undertaken. Some studies analyzing the long-term control of SIV_{mac239} by vaccination have shown diminished protection over time (14). Second, our data study in depth one immunodominant epitope, and additional studies are needed to assess if these findings can be generalized to other epitopes. The contributions of subdominant epitopes in the setting of viral escape at a dominant epitope requires further studies, although this would require analyzing multiple macaques with at least two MHC class I alleles restricting known CTL epitopes. Third, we did not study the multitude of potential functions of the KP9-specific CTL detected by our MHC tetramer (4). Despite the very strong relationship between numbers of KP9-specific CTL and rates of reversion that we observed, it is possible that functional aspects of these CTL could also have an important additional bearing on rates of reversion and reescape at this epitope. In particular, since our studies infer that killing of WT virus infected by CTL provides an important benefit, correlating the cytolytic potential of CTL with the outcome of infection will be important.

HIV strains containing multiple CD8$^+$ T-cell escape mutations are the rule rather than the exception in HIV-1-infected subjects (20). What are the potential implications of these data for vaccination of humans against circulating EM HIV-1 strains? First, the benefit of CTL killing virus-infected cells appears significantly greater than the fitness cost extracted by immune escape. Even partial, transient reversion to WT resulted in more efficient virologic control after postacute viremia was established than did complete maintenance of escape mutations. Second, the generation of higher levels of immunodominant T cells may be detrimental in the setting of infection with immune escape variants in comparison to broader recognition of subdominant epitopes (12). Immunodominant CD8$^+$ T-cell responses result in a more narrowly directed response that, when immune escape occurs (or is already present in the infecting inoculum), will most likely be of more limited utility in the control of viremia than CTL-mediated killing of infected cells. Vaccine strategies should avoid inducing narrowly focused immunodominant CD8$^+$ T-cell responses restricted by commonly expressed MHC class I alleles where exposure to circulating EM viruses is common.

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