## Title

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# Vaccine-Induced Linear Epitope-Specific Antibodies to Simian Immunodeficiency Virus SIVmac239 Envelope Are Distinct from Those Induced to the Human Immunodeficiency Virus Type 1 Envelope in Nonhuman Primates

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**To evaluate antibody specificities induced by simian immunodeficiency virus (SIV) versus human immunodeficiency virus type 1 (HIV-1) envelope antigens in nonhuman primate (NHP), we profiled binding antibody responses to linear epitopes in NHP studies with HIV-1 or SIV immunogens. We found that, overall, HIV-1 Env IgG responses were dominated by V3, with the notable exception of the responses to the vaccine strain A244 Env that were dominated by V2, whereas the anti-SIVmac239 Env responses were dominated by V2 regardless of the vaccine regimen.**

Analyses of RV144 immune correlates identified V1-V2 IgG as positively correlated with a decreased risk of infection (1–3), and secondary correlate analysis with linear peptide microarrays demonstrated that binding to linear V2 correlated with a decreased risk of infection (4). Follow-up studies (2, 3, 5, 6, 37, 38) demonstrated that the magnitude, specificity, and subclass of the antibody responses are all critical measurements for immune correlate analyses.

The nonhuman primate (NHP) is a valuable model for AIDS vaccine evaluation (7). There are currently two immunization and challenge systems used in NHP. One is simian immunodeficiency virus (SIV), and the other is chimeric simian-human immunodeficiency virus (SHIV), in which the envelope glycoproteins of SIV are replaced with those of human immunodeficiency virus type 1 (HIV-1) (8). The SHIV system has the advantage of being capable of testing immunogens that can be directly related to humans. However, the SHIV strains that were developed early on were X4-tropic, were of the tier 1 neutralization phenotype, and were highly pathogenic compared to HIV-1 strains in human (9). Encouragingly, new SHIV strains (10–15, 39) have been developed in recent years that are R5-tropic, that are of the tier 2 neutralization phenotype that is common for most circulating strains of HIV-1, and that can exhibit pathogenesis after mucosal exposure. The SIV system has the advantage of having relatively well characterized, with consistent challenge models available, and thus has been used widely in vaccine studies (16–21). However, significant differences exist between the SIV and HIV-1 genomes and pathogenesis characteristics (22–24). One key issue for the field is how well NHP vaccine-induced antibody responses translate to human vaccine trials: are antibody responses to SIV vaccines indicative of the responses to HIV-1 vaccines?

To investigate the comparability of antibody responses in the

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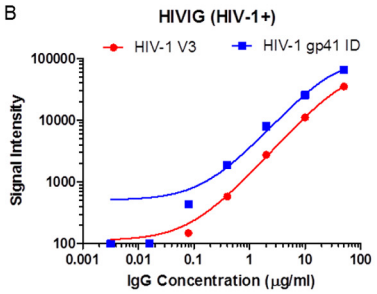
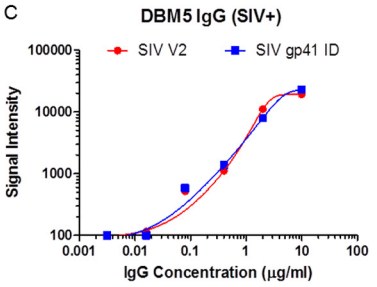
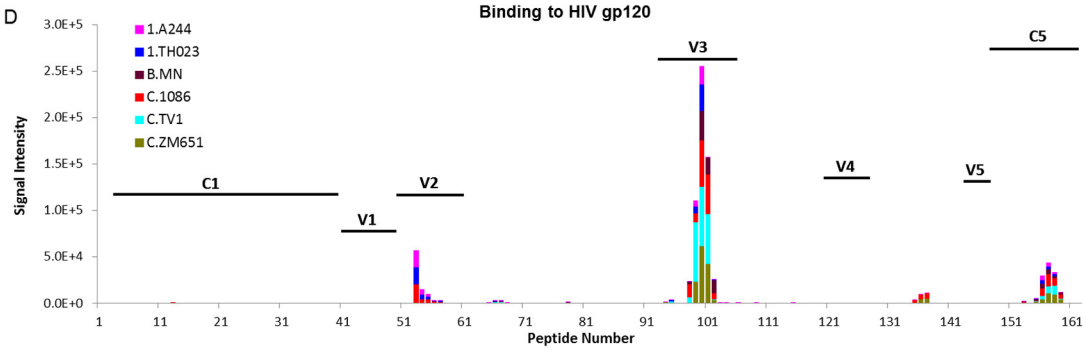
**A.** HIV and SIV Immunization Studies.

| | Study | Vaccine Regimen | Adjuvant | Route of Immunization | Animal |
|---|---|---|---|---|---|
| HIV-1 | CAVIMC369 | Clade C 1086 Env gp140 | R848 | IM | Indian Rhesus (n=5) |
| | VAC1003 | Bivalent SF162/TV1 Env gp140 | R848 | IN/IM | Cynomolgus (n=16) |
| | HIVRAD6 | Clade B SF162ΔV2 gp140, no prime | MF59 | IM | Chinese Rhesus (n=10) |
| | AUP513 | [a]NYVAC or ALVAC prime; NYVAC or ALVAC + gp120 boost, clade C or B/E | MF59 | IM | Indian Rhesus (n=10) |
| | P167 | ALVAC prime; gp120 boost, Clade AE+B | Alum | IM | Indian Rhesus (n=7) |
| | PAVEG1112 | DNA prime/Ad5 boost, group M mosaics | -- | IM | Indian Rhesus (n=3) |
| | BM415 | MVA gp140 prime, protein gp120 boost (1086C) | R848/MF59 | IN/IM | Indian Rhesus (n=4) |
| SIVmac239 | CAVIMC031 _No Prime Arm | mac239 gp145 protein | poly I:C | IM | Indian Rhesus (n=11) |
| | VRC145 _No Prime Arm | mac239 gp140 Ad5 | -- | IM or Aerosol | Indian Rhesus (n=2) |
| | CAVIMC031 _MVA/protein Arm | mac239 MVA prime, gp145 protein boost | poly I:C | IM | Indian Rhesus (n=14) |
| | M14 | mac239 gp160 DNA prime/MVA gp150 boost | -- | IM | Indian Rhesus (n=20) |
| | VRC145 _DNA/Ad5 Arm | mac239 DNA prime; Ad5 gp140 boost | -- | IM or Aerosol | Indian Rhesus (n=6) |
| | AUP417 | mac239 DNA prime; gp160 protein (viral particle) boost | -- | IM | Indian Rhesus (n=8) |
| **mac251&smE660:** Pal 4.22 | | mac251 & smE660 DNA prime; gp145 boost | carbohydrate-based | IM | Chinese Rhesus (n=4) |

[a]For AUP513, the NYVAC contained ZM96 Env gp140 and ZM96 Gag+CN54 Pol&Nef; the ALVAC contained either ZM96 Env gp140 and ZM96 Gag+CN54 Pol&Nef (ALVAC-C), or LAI (Clade B) GagPro and 92TH023 Env gp120 (Subtype E)(ALVAC-vCP1521); gp120 proteins were either AIDSVAX (Clade E A244& Clade B MN), or TV1 and 1086C (Clade C).



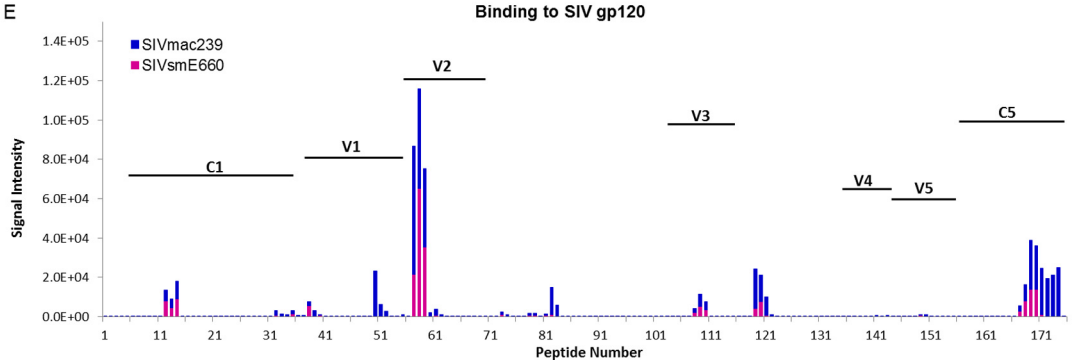




**FIG 1** (A) List of NHP studies characterized in the study and information on vaccine regimens. IM, intramuscular; IN, intranasal; cynomolgus, cynomolgus monkey. (B and C) Binding of serially diluted human immunodeficiency virus immune globulin (HIVIG) from a pool of chronically infected subjects to HIV-1 V3 and gp41 immunodominant (ID) epitopes (B) and binding of serially diluted DBM5 IgG (IgG purified from a SIVmac251-infected macaque) to SIV V2 and gp41 ID epitopes by serially diluted DBM5 IgG (C). Concentrations of the antibodies are indicated on the *x* axis. (D and E) Representative gp120 binding plots for serum samples from macaques immunized with either HIV-1 (VAC1003) (D) or SIVmac239 (CAVIMC031) (E) antigens. Numbers on the *x* axis are peptide numbers in the array library. Different colors of bars represent different strains/clades as indicated in the keys in the panels (A244, TH023, MN, 1086C, TV-1, and ZM651 for panel D and SIVsmE660 and SIVmac239 for panel E).

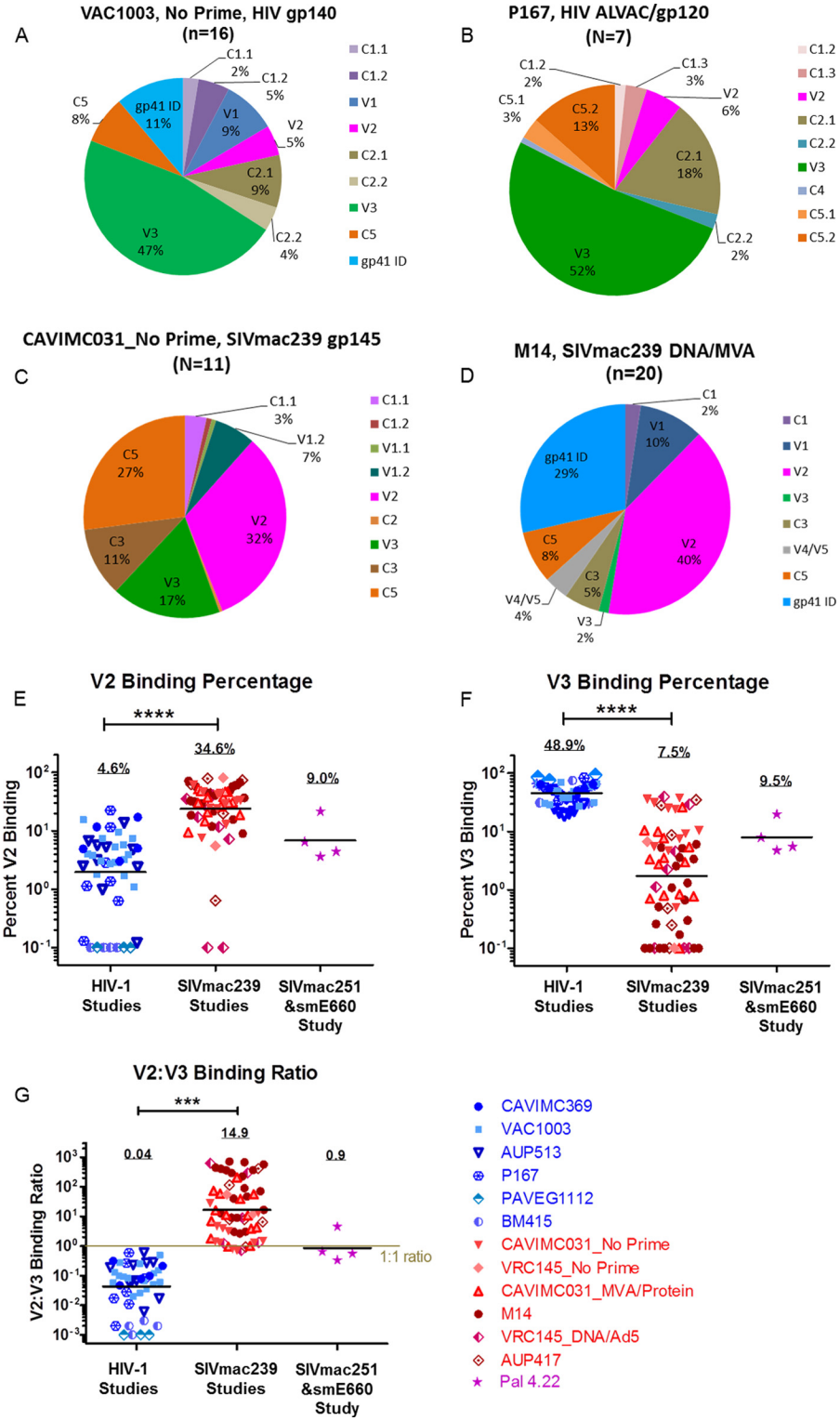



**FIG 2** (A to D) Proportions of linear binding responses to each epitope region in 2 representative HIV-1 Env (VAC1003, 1086C gp140 [A]; P167, ALVAC/MN and A244 gp120 [B]) and 2 representative SIVmac239 Env (MVA gp145 protein [C] and DNA gp160/MVA gp150 [D]) immunization studies. Each slice represents the mean percent binding relative to the total gp160 binding to the specific region in one NHP study. (E to G) Proportions of V2 (V2%) (E) and V3 (V3%) (F) in total Env binding and V2/V3 binding ratio (V2:V3) (G) for all studies/animals. Any V2% or V3% value lower than 0.1% was converted to 0.1%. Animals from individual studies are represented by different symbols as indicated in the key, with data from HIV-1 studies in blue, data from SIVmac239 studies in red, and data from the SIVmac251 and smE660 study in purple. Horizontal bars represent mean values for each category. Numbers on plots above each group of symbols are mean values for the group. Statistical test, 2-tailed *t* test. Statistical analysis was not performed on the data from the mac251 and smE660 study (Pal 4.22) due to the limited number of animals.

**TABLE 1** Proportions of antibody responses targeting common linear epitopes[a]

| Env immunogen | Study; no. of animals | % total binding[a] | | | | | Top ranking rate (%)[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | C5 | gp41 ID | V2 | V3 | C5 | gp41 ID |
| HIV-1 | CAVIMC369; $n = 5$ | 0 | 8 | **57** | 13 | 17 | 20 | **100** | 40 | 40 |
| | VAC1003; $n = 16$ | 9 | 5 | **47** | 8 | 11 | 6 | **100** | 13 | 31 |
| | HIVRAD6; $n = 10$ | 0 | NA[c,d] | **51** | 10 | 17 | NA[d] | **100** | 40 | 40 |
| | AUP513; $n = 10$ | 0 | 5 | **32** | 14 | 0 | 0 | **90** | 40 | 0 |
| | P167; $n = 7$ | 0 | 7 | **51** | 13 | 0 | 14 | **100** | 43 | 0 |
| | PAVEG1112; $n = 3$ | 0 | 0 | **85** | 0 | 5 | 0 | **100** | 0 | 33 |
| | BM415; $n = 4$ | 0 | 0 | **54** | 6 | 0 | 0 | **100** | 0 | 0 |
| SIVmac239 | CAVIMC031 (protein-only arm); $n = 11$ | 7 | **32** | 18 | 27 | 0 | **73** | 27 | 73 | 0 |
| | VRC145 (Ad5-only arm); $n = 2$ | 3 | **43** | 3 | 6 | 7 | **50** | 0 | **50** | **50** |
| | CAVIMC031 (MVA/protein); $n = 14$ | 11 | **32** | 5 | 19 | 0 | **79** | 0 | **79** | 0 |
| | M14; $n = 20$ | 10 | **40** | 2 | 8 | 29 | 75 | 0 | 20 | **80** |
| | VRC145 (DNA/Ad5 arm); $n = 6$ | 9 | **46** | 12 | 0 | 11 | **67** | 50 | 0 | 33 |
| | AUP417; $n = 8$ | 10 | 17 | 10 | 16 | **26** | **50** | 25 | 38 | **50** |
| SIVmac251 + smE660 | Pal 4.22; $n = 4$ | 15 | 9 | 9 | 1 | **32** | 0 | 0 | 0 | **100** |

[a] Data represent percentages of total gp160 linear binding against specific epitope regions in each study (mean percentage values for all animals analyzed in each study). Boldface data represent the highest percentage value(s) for each study.
[b] Data represent percentages of animals in each study with binding magnitude for the specific epitopes ranked among the top 2 of their epitope specificities. Boldface data represent the highest percentage value(s) for each study.
[c] NA, not applicable.
[d] The V2 loop was not included in the immunogen of HIVRAD6.

NHP model, we profiled the linear epitope serum IgG responses in seven NHP studies using HIV-1 immunogens, six studies using SIVmac239 immunogens, and one study using SIVmac251 and smE660 immunogens, for a total of 120 macaques that were analyzed in this study. The regimens of the 14 NHP studies are listed in Fig. 1A. The seven HIV-1 NHP studies included a DNA and viral vector (NYVAC/ALVAC/MVA) as a prime or no-prime immunogen and Env gp120, gp140, or viral vector (Ad5/NYVAC [40]) as a boosting immunogen. The seven SIV NHP studies include either DNA or viral vector (MVA) as a prime immunogen and either Env protein (monomer or viral particles [25, 26]) or viral vector (MVA [27] or Ad5 [28]) as a boosting immunogen.

We characterized serum IgG responses to HIV-1 and SIV linear epitopes using peptide microarray linear epitope mapping. This technology has been used previously in various studies to characterize antibody responses following infection and after vaccinations in humans and in NHP (29–31, 41). Notably, linear V2 binding data generated by peptide microarray correlated with a decreased risk of infection in the RV144 efficacy trial (4). The HIV-1 peptide libraries contain overlapping HIV-1 peptides covering full-length gp160 of 7 consensus clades/circulating recombinant forms (CRFs): clades A, B, C, and D, group M, CRF01 AE, and CRF02 AG. Samples from four studies (CAVIMC369, VAC1003, P167, and BM415) were mapped against a library that also contained peptides for 6 vaccine strains: 3 clade C, 1 clade B, and 2 CRF01 AE strains. The SIV peptide library contains peptides covering full-length gp160 of SIVmac239 (GenBank accession no. AAA47637, with a premature stop codon at amino acid [aa] 762 converted to W) and SIVsmE660 (GenBank accession no. AFW03363). We were able to detect as little as 0.08 μg/ml HIV-1-positive IgG (Fig. 1B) or 0.016 μg/ml SIV-positive IgG (Fig. 1C) using this technology. The total binding intensity to all linear epitopes identified in the peptide microarray correlates with gp140 protein binding in the binding antibody multiplex assay (BAMA [1, 3, 32]), which measures binding to linear as well as conformational epitopes (data not shown).

We profiled IgG binding responses in these NHP studies and calculated the proportions of binding to each epitope in the total gp160 peptide array. Representative binding plots for serum IgG against HIV-1 sequences (study CAVIMC369) and SIV sequences (study CAVIMC031) are shown in Fig. 1D and E. The proportion of binding to each identified epitope in the peptide arrays was determined as follows: maximum binding intensity to a single epitope/sum of maximum binding intensities to all epitopes identified. The proportions of specificities in two representative HIV-1 and two SIVmac239 studies are shown in Fig. 2A to D. Table 1 summarizes the average percentages of V1, V2, V3, C5, and gp41 immunodominant (ID) responses in these HIV-1 and SIV NHP studies as well as the proportions of animals within each study with V2, V3, C5, or gp41 ID among the top two specificities. We also compared the proportions of V2 (V2%) and V3 (V3%) responses and V2/V3 ratios across all SIV and HIV-1 immunization studies (Fig. 2E to G).

We found that binding antibodies elicited by HIV-1 immunogens targeted epitopes in the C1, V2, V3, C5, and gp41 ID regions, with the V3 response representing the dominant response and demonstrating higher binding intensity than the V2 response in all 6 HIV-1 studies that contained V2 in the immunogens (immunogen in HIVRAD6 is deleted of V2) (Fig. 1D, 2A, B, and E to G, and Table 1). Binding antibodies elicited by SIVmac239 immunogens also targeted C1, V2, V3, and C5 in peptide arrays (Fig. 1E and 2C and D). However, the magnitude of binding to V2 was higher than that to V3 in all 6 SIVmac239 studies (Fig. 1E, 2C, D, and E to G, and Table 1). In addition, binding of antibodies against the SIV V1 region was detected in all SIV studies, while no anti-V1 response was seen in 6 of the 7 HIV-1 vaccine studies (Fig. 2A to D and Table 1).

Overall, the anti-V3 response was the dominant linear binding

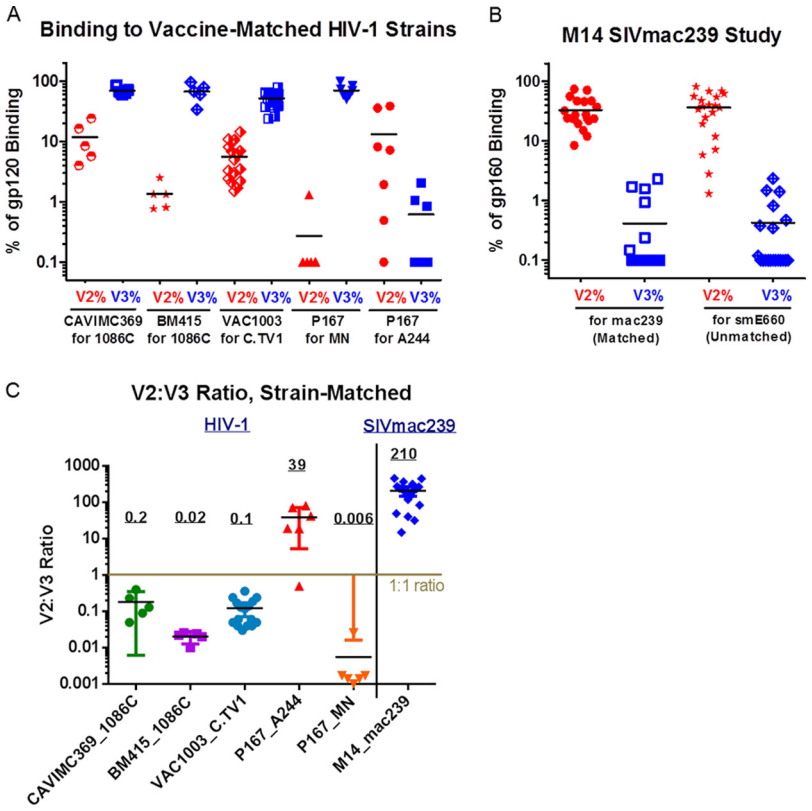



**FIG 3** (A) Proportions of vaccine strain-specific V2 and V3 binding in the total gp120 binding in four HIV-1 studies. CAVIMC369, BM415, and VAC1003 utilized 1086C, 1086C, and C.TV1 Env immunogens, respectively, and data were mapped against these strains. The P167 study utilized gp120 from both MN and A244 strains, and data were mapped against both strains. (B) Proportions of mac239 (vaccine-matched strain)- and smE660 (unmatched with vaccine strain)-specific V2 and V3 binding in total Env binding in study M14. (C) V2:V3 binding values for the 4 HIV-1 studies and 1 SIVmac239 study against the respective vaccine-matched strains. Bars represent mean and 95% confidence interval (CI) values. Numbers above each group of symbols are group mean values.

response in the HIV-1 studies and accounted for an average of 48.9% (range, 32% to 85%) of the total linear Env-binding response (Fig. 2F and Table 1). The anti-V2 response was lower, representing an average of 4.6% (range, 0% to 8%) of the total responses in these HIV-1 studies (Fig. 2E and Table 1). In the SIVmac239 studies, the anti-V2 responses accounted for an average of 34.6% (range, 17% to 46%) of the total Env-binding responses (Fig. 2E and Table 1) and dominated the responses in 5 of the 6 SIVmac239 studies. The sixth SIVmac239 study (AUP417) had a dominant response to the gp41 ID region. The anti-V3 response, in contrast, was much lower in all SIVmac239 studies and accounted for only 2% to 18% of the total Env-binding responses (average, 7.5%) (Fig. 2F and Table 1). The anti-V1 response, albeit absent in 6 of 7 HIV-1 studies, accounted for 3% to 11% of total response in the 6 SIVmac239 studies (Table 1).

To address the possibility that the mean proportion values obtained from a study could be biased by nonrepresentative responses from a small number of animals, we ranked the epitope specificities for each animal and counted how many animals in each study ranked each epitope as being among the top two specificities. As shown in Table 1, the anti-V3 response ranked among the top 2 specificities for 90% to 100% of the 55 animals in the HIV-1 immunization studies, compared to 0% to 20% for V2, whereas the anti-V2 response ranked among the top 2 specifies for 50% to 79% of the 61 animals in the SIVmac239 immunization studies, compared to 0% to 50% for V3 (Table 1).

Differences in either V2% or V3% between the HIV-1 and SIVmac239 studies overall were statistically significant, with $P < 0.0001$ (two-tailed $t$ test; Fig. 2E and F). The difference in the V2/V3 ratio between HIV-1 and SIVmac239 studies overall was also statistically significant ($P < 0.001$) (two-tailed $t$ test; Fig. 2G).

In one available study that utilized non-SIVmac239 immunogens (mac251 plus smE660 DNA/protein), we found that neither the V2 response nor the V3 response was the dominant response (Fig. 2E and F and Table 1) (mean V2/V3 ratio = 0.9). Instead, the anti-Env response was dominated by gp41 ID, another common dominant/codominant response in both HIV and SIV studies, when the epitope region was included in the vaccine (Table 1) (the limited sample size precludes statistical analysis of this study).

We further examined vaccine strain-specific responses in four HIV-1 studies where sequences from vaccine-matched strains were included in the peptide library. CAVIMC369, BM415, and VAC1003 studies elicited a V3-dominant IgG response to strains 1086C and C.TV1 (Fig. 3A). In contrast, P167 elicited a V3-dominant response to vaccine strain MN gp120 but a V2-dominant response to vaccine strain A244. Binding to 1086C, C.TV1, and MN V3 in these four studies accounted for 51% to 70% of the total gp120 binding, whereas for A244 in P167, the anti-V3 response was minimal, accounting for only 1% of the total gp120 binding (Table 2). This resulted in a "reversed" V2/V3 ratio for A244 in P167 (a ratio of 39, compared to 0.006 to 0.2 for other strain-matched HIV-1 V2/V3 values; Fig. 3C). For comparison, we also

**TABLE 2** Epitope-specific binding in HIV-1 studies that were mapped against vaccine-matched strains

| Study; no. of animals (strain) | % total binding[a] | | | Top ranking rate (%)[b] | | |
|---|---|---|---|---|---|---|
| | V2 | V3 | C5 | V2 | V3 | C5 |
| CAVIMC369; $n = 5$ (1086C) | 12 | **69** | 16 | 40 | **100** | 60 |
| BM415; $n = 4$ (1086C) | 1 | **67** | 6 | 25 | **100** | 0 |
| P167; $n = 7$ (MN) | 0 | **70** | 16 | 0 | **100** | 71 |
| P167; $n = 7$ (A244) | 13 | 1 | **30** | 28 | 0 | **57** |

[a] Data represent percentages of total gp160 linear binding against specific epitope regions in each study (mean percentage values for all animals analyzed in each study). Boldface data represent the highest percentage value for each study.
[b] Data represent percentages of animals in each study with binding magnitude for the specific epitopes ranked among the top 2 of their epitope specificities. Boldface data represent the highest percentage value for each study.

examined strain-specific binding to vaccine-matched mac239 and unmatched smE660 for the SIVmac239 study M14 and observed no difference in V2 versus V3 binding patterns for these two strains (Fig. 3B).

In summary, our comparison of epitope-specific binding antibody responses as measured with overlapping peptide arrays spanning the entire gp160 of HIV-1 and SIV revealed that the antibody specificities generated by SIVmac239 Env immunizations were not representative of those generated from most HIV-1 Env immunizations in NHP. The dominant response to HIV-1 Env in the seven studies (55 animals) was to the V3 loop, with the exception of A244-specific binding in one study that included A244 gp120 as an immunogen. In contrast, the dominant response to SIVmac239 Env (6 studies, 61 animals) was to the V2 loop of gp120. Whether the V2-specific antibody responses in these studies were dominated by lambda light chains as has been recently shown for V2-specific antibodies (33) is unknown and is worth further study. In addition, the anti-V1 antibody responses were more frequent in SIV Env immunization studies than in HIV-1 Env immunization studies. These overall differences in binding antibody specificities between SIVmac239 and HIV-1 Env immunogens in NHP are likely due to differences in V2 and V3 loop structures, including differences in number of disulfide bonds, which can result in differences in epitope exposure. Moreover, differences in Env glycosylation, as well as the capacity of Env glycoproteins to modulate expression of genes relevant to innate and adaptive immune responses (34, 35) in the context of different vaccine vectors and adjuvants, may have contributed to the observed differences. One caveat of this study is that the HIV-1 and SIV studies we examined involved different immunogen designs, adjuvants, and immunization schedules. There was no direct comparison between matched SIV and HIV-1 vaccine regimens. Moreover, we focused on linear epitopes using technology that could profile the entire envelope region to directly compare HIV-1 and SIV epitopes in this study. The linear epitope mapping did not include conformational or glycan-dependent epitopes. However, the differences seen here suggest substantial differences in epitope focusing of the IgG responses elicited by SIVmac239 and HIV-1 Env immunogens. Notably, NHPs immunized with A/E A244, the RV144 vaccine strain immunogen, developed a strong V2-specific response, consistent with the antigenic features of this immunogen (36), whereas binding to the MN gp120 vaccine strain in the same study was strongly dominated by the anti-V3 response. Our study results suggest that understanding how vaccine inserts and regimens induce differential dominant antibody specificities is important for vaccine immunogen design. Last, further studies are needed to improve the NHP immunization model to infer the linear, conformational, and subclass/isotype-specific antibody responses that would be generated in human clinical trials with the same immunogen.

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