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VACCINES AND ANTIVIRAL AGENTS

# Coadministration of CH31 Broadly Neutralizing Antibody Does Not Affect Development of Vaccine-Induced Anti-HIV-1 Envelope Antibody Responses in Infant Rhesus Macaques

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**ABSTRACT** Prevention of mother-to-child transmission (MTCT) is an indispensable component in combatting the global AIDS epidemic. A combination of passive broadly neutralizing antibody (bnAb) infusion and active vaccination promises to provide protection of infants against MTCT from birth through the breastfeeding period and could prime the immune system for lifelong immunity. In this study, we investigate the impact of a single infusion of CD4 binding site (CD4bs) bnAb administered at birth on *de novo* antibody responses elicited by concurrent active HIV envelope vaccination. Four groups of infant macaques received active immunizations with subunit Env protein or modified vaccinia Ankara (MVA)-vectored Env and subunit Env protein, with or without a single intravenous coadministration of CH31 bnAb at birth. Vaccinated animals were monitored to evaluate binding and functional antibody responses elicited by the active vaccinations. Despite achieving plasma concentrations that were able to neutralize tier 2 viruses, coadministration of CH31 did not have a large impact on the kinetics, magnitude, specificity, or avidity of vaccine-elicited binding or functional antibody responses, including epitope specificity, the development of CD4bs antibodies, neutralization, binding to infected cells, or antibody-dependent cell-mediated cytotoxicity (ADCC). We conclude that infusion of CD4bs bnAb CH31 at birth does not interfere with *de novo* antibody responses to active vaccination and that a combination of passive bnAb infusion and active HIV-1 Env vaccination is a viable strategy for immediate and prolonged protection against MTCT.

**IMPORTANCE** Our study is the first to evaluate the impact of passive infusion of a broadly neutralizing antibody in newborns on the *de novo* development of antibody responses following active vaccinations in infancy. We demonstrated the safety and the feasibility of bnAb administration to achieve biologically relevant levels of the antibody and showed that the passive infusion did not impair the *de novo* antibody production following HIV-1 Env vaccination. Our study paves the way for further investigations of the combination strategy using passive plus active immunization to provide protection of infants born to HIV-1-positive mothers over the entire period of risk for mother-to-child transmission.

**KEYWORDS** AIDS vaccine, broadly neutralizing antibody, HIV, nonhuman primate, antibody, infant, passive immunization, pediatric vaccine

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In 2017, there were 1.8 million new human immune deficiency virus type 1 (HIV-1) infections worldwide, 160,000 of which were in children less than 15 years of age (1). Previous studies have found that antiretroviral (ARV) drug treatment of HIV-infected pregnant women decreased the rate of pediatric HIV-1 infections significantly (2). However, early detection and treatment of HIV-1 in pregnant women are difficult in areas where health care is less accessible, leading to women who are treated late in pregnancy, are lost to follow-up visits, or are not adherent to continuous ARVs, particularly in the postpartum period (3). Moreover, despite widespread ARV use in pregnancy, HIV-1 mother-to-child transmission (MTCT) rates remain over 5% (4).

HIV-1 broadly neutralizing antibodies (bnAbs) have unique characteristics that both enable them to neutralize multiple clades of HIV-1 with high potency and render them difficult to develop by the host, including long heavy-chain complementarity-determining region 3 (CDR3), glycan interactions, and high levels of somatic hypermutation (5). As a result, bnAbs develop only after years of infection and only in a small fraction of patients in the setting of natural infection (6, 7). Efforts aimed at eliciting bnAbs through active immunization have proven largely futile (8). Meanwhile, non-neutralizing antibodies (including both binding and functional antibodies) have been associated with decreased risk of infection or protection from challenge virus acquisition in immune correlate analyses of the RV144 Thai trial, the only human vaccine trial that showed evidence of efficacy (9, 10), and of multiple nonhuman primate (NHP) vaccine studies (11–15). Therefore, both broadly neutralizing antibody and non-broadly neutralizing antibody responses are of interest for AIDS vaccine development. While challenges remain for elicitation of bnAbs through active vaccination, passive administration with bnAbs has been proven to provide protection against infection in NHP models (16–21) and is a strategy now being tested in clinical trials (22, 23). The bnAbs included in these passive NHP vaccination studies target the CD4 binding site (CD4bs), membrane-proximal external region (MPER), glycans on the variable loops 1 and 2 (V1V2), and V2 apex.

In the pediatric field, immune-based approaches to prevent perinatal virus infections have included passively administered antiviral antibodies, providing rapid protection of infants after birth against infection with viruses such as respiratory syncytial virus and hepatitis A and B viruses (HBA and HBV, respectively) (24). As HIV-1 exposure for infants born to HIV-1-infected mothers starts *in utero*, includes peripartum transmission, and continues with frequent daily mucosal exposure via breastfeeding, it is critical that infants are provided protection immediately after birth and are covered through early years of high risk of infection. However, protective immunity against HIV-1 via active immunization is likely to require months and multiple doses. Meanwhile, passive immunization with potent bnAbs is a viable strategy for rapidly protecting infants while active vaccine responses are developing. In particular, a combination of passive and active immunization strategies could provide infants with immediate protection by the administration of bnAbs while *de novo* antibody responses develop from the active immunization. In fact, this passive-active vaccine strategy is used successfully to prevent perinatal HBV infections while eliciting lifelong immunity in the neonatal window, a strategy that has the advantage of high vaccine coverage due to integration with standard childhood vaccines (25).

In the current study, we aimed to assess the impact of a single administration of a CD4bs bnAb at birth on the immune responses elicited by concurrent active Env immunizations. The active vaccine regimen was built upon our previous infant macaque study testing accelerated- versus extended-interval immune schedules using Env and modified vaccinia Ankara (MVA)/Env immunogens (26). We showed in the former study that recombinant gp120 protein or MVA/gp120 coadministered immunizations given 6 weeks apart and an extended follow-up period of 32 weeks resulted in antibody persistence up to 20 weeks after the third immunization and induction of HIV-1-specific activated memory B cells (26). The current study utilizes a similar active vaccine regimen and tests the effect of a single bnAb CH31 administration on the development of *de novo* vaccine-elicited antibody responses as an infant vaccine strategy. We hypothe-

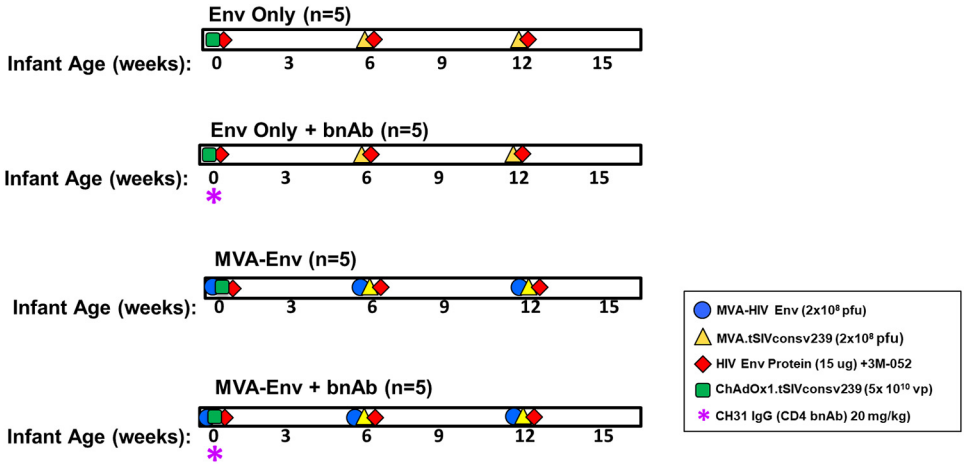


**FIG 1** Animal study design schema. Vaccine components are represented by different symbols. Group identifiers and numbers of animals per groups are indicated at the top of the time scale for each vaccine group.

sized that the single administration of the CH31 bnAb, while providing a biologically relevant *in vivo* level for potential protection against oral virus acquisition in the days immediately following birth, will not impact the *de novo* development in infants of binding or functional Env-specific antibody responses elicited by active vaccination.

## RESULTS

**Vaccine regimens and animal groups.** The current study was designed to test the safety of passive infusion of a bnAb to infants and the effect of the passive administration on the development of *de novo* antibody responses. Our previous study showed that HIV Env protein alone or coadministered with an MVA-vectored immunogen could elicit robust antibody responses in infant macaques (26). In a later study we further compared different vaccine adjuvants in infant macaques and found 3M-052 to significantly improve and better sustain HIV Env-specific antibody responses than an alum adjuvant (27). Based on these findings, our active vaccine regimen for the current study included HIV 1086.C gp120 protein (referred to as Env) with 3M-052-SE adjuvant and MVA-vectored Env (referred to as MVA). The bnAb is CH31, a CD4bs antibody that is similar to VRC01 that is being tested in human clinical trials. Ten infant macaques 4 to 10 days of age were randomly assigned to each of the four study groups as follows: (i) Env only, (ii) Env only plus bnAb (Env+bnAb), (iii) MVA/Env, and (iv) MVA/Env+bnAb (Fig. 1). Active vaccine components Env (15 $\mu$g) and MVA/Env (15 $\mu$g Env plus $2 \times 10^8$ PFU of MVA) were administered intramuscularly (i.m.) at weeks 0, 6, and 12. Env+bnAb and MVA/Env+bnAb groups also received a single infusion of 20 mg/ml body weight CH31 at the time of the first active immunization (week 0). In addition, all animals also received chimpanzee adenovirus expressing simian immunodeficiency virus (SIV) Gag/Pol (ChAdOx1.tSIVconsv239) at week 0 and MVA expressing SIV Gag/Pol (MVA.tSIVconsv239) at week 6 and week 12.

**Kinetics of *in vivo* levels of infused CH31 bnAb.** We first evaluated levels of *in vivo* CH31 concentration in the Env+bnAb and MVA/Env+bnAb groups at days 1, 3, 7, 14, and 42 following the single intravenous CH31 injection by an enzyme-linked immunosorbent assay (ELISA) using an anti-human IgG detection (Fig. 2A). CH31 IgG levels, measured as the CH31 equivalent human IgG concentration, were highest 1 day after infusion for both the Env+bnAb and the MVA/Env+bnAb groups (median, 69 and 55 $\mu$g/ml CH31 equivalent; ranges, 55 to 73 $\mu$g/ml and 50 to 101 $\mu$g/ml CH31 equivalent, respectively) and then declined below detectable levels by 14 days postinfusion. Between day 1 and day 3 postinfusion, the levels of Env-specific human IgG (CH31 equivalent) declined by approximately 47%, with median concentrations of 44 and 30 $\mu$g/ml CH31 equivalent for the Env+bnAb and MVA/Env+bnAb groups, respec-

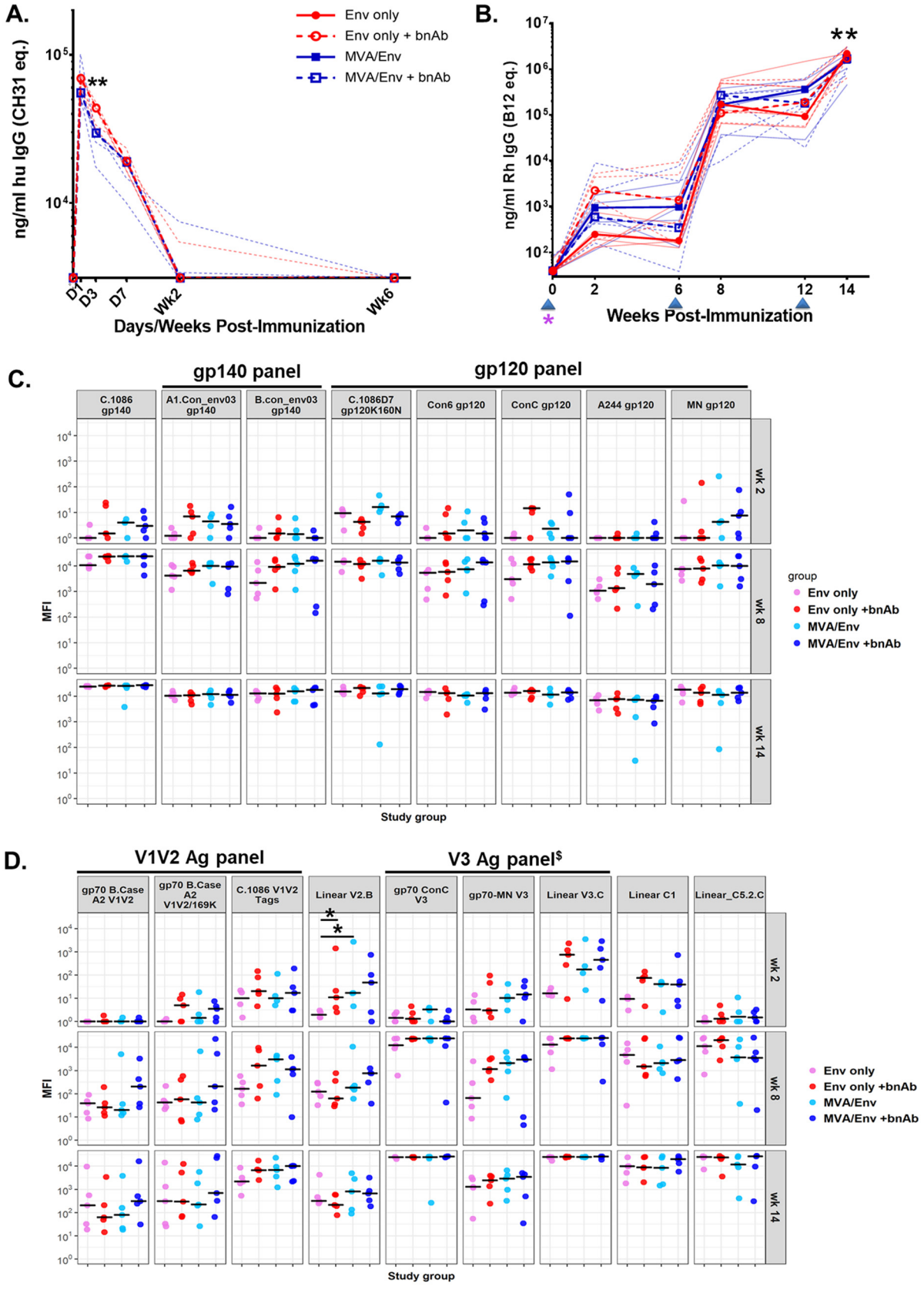


**FIG 2** Level of *in vivo* CH31 and *de novo* binding responses in vaccinated macaques. (A) Levels of CH31 MAb in groups that received CH31 coadministration, measured as Env-specific human (hu) IgG. D, day; wk, week. (B) Levels of IgG binding for vaccine strain 1086.C gp120 over time as measured in an anti-monkey IgG ELISA. (C) Magnitude of binding to a panel of HIV-1 Env antigens as measured in BAMA. (D) Magnitude

(Continued on next page)

**TABLE 1** Statistical test results for between-group comparisons with a *P* value of <0.05

| Immunogen type(s) and groups compared[a] | Assay | Analyte, virus, or epitope | Parameter | Time point | Unadjusted *P* | FDR *P* | Figure reference |
|---|---|---|---|---|---|---|---|
| With and without bnAb | | | | | | | |
| MVA/Env vs MVA/Env+bnAb | sCD4 blocking | sCD4 | % CD4 blocking | wk 8 | 0.032 | 1 | Fig. 6 |
| Env-only vs Env only+bnAb | Epitope mapping | C5.2_159-161 | Signal | wk 8 | 0.032 | 1 | Fig. 3 |
| Env-only vs Env only+bnAb | Infected cell binding | CM235 IMC | Secondary MFI | wk 14 | 0.032 | 1 | Fig. 4E |
| Env-only vs Env only+bnAb | Infected cell binding | CM235 IMC | % Positive | wk 14 | 0.032 | 1 | Fig. 4F |
| Env-only vs Env only+bnAb | BAMA | Bio_V2_B | MFI | wk 2 | 0.040 | 1 | Fig. 2D |
| MVA/Env vs MVA/Env+bnAb | Epitope mapping | C3.2_116-117 | $Log_2$ fold change | wk 8 | 0.048 | 1 | Fig. 3 |
| Env vs MVA/Env | | | | | | | |
| Env+bnAb vs MVA/Env+bnAb | ELISA | 1086.c.gp120 | CH31 equivalent human IgG | day 3 | 0.008 | 0.84 | Fig. 2A |
| Env-only vs MVA/Env | ELISA | 1086.c.gp120 | B12 equivalent rhesus IgG | wk 14 | 0.008 | 0.84 | Fig. 2B |
| Env-only vs MVA/Env | BAMA | V3_mean | MFI | wk 2 | 0.029 | 0.84 | Fig. 2D |
| Env-only vs MVA/Env | BAMA | bio_v2_b | MFI | wk 2 | 0.029 | 0.84 | Fig. 2D |
| Env only+bnAb vs MVA/Env+bnAb | sCD4 blocking | sCD4 | % CD4 blocking | wk 8 | 0.032 | 0.84 | Fig. 6 |
| Env only+bnAb vs MVA/Env+bnAb | Infected cell binding | CM235 IMC | Secondary MFI | wk 14 | 0.032 | 0.84 | Fig. 4E |
| Env-only vs MVA/Env | Avidity | gp70-ConC_V3tags | $k_{off}$[b] | wk 8 | 0.032 | 0.84 | Fig. 5A |
| Env only+bnAb vs MVA/Env+bnAb | Infected cell binding | CM235 IMC | % Positive | wk 14 | 0.032 | 0.84 | Fig. 4F |
| Env only+bnAb vs MVA/Env+bnAb | Epitope Mapping | C3.2_116-117 | $Log_2$ fold change | wk 8 | 0.048 | 0.88 | Fig. 3B |

[a]Six out of 208 comparisons between groups with and without bnAb had unadjusted *P* values of <0.05. Nine out of 212 comparisons between groups with Env versus MVA/Env immunogens had unadjusted *P* values of <0.05. Rows are ordered by unadjusted *P* value (ascending order). A full list of statistical test results for all comparisons is given in Tables S1 and S2 in the supplemental material. The underlined group in each pair is the higher one in the comparison.
[b]Off-rate constant.

tively, at day 3. Env-specific human IgG (presumably the CH31 human IgG infused) levels were comparable between the two groups except at day 3, when the Env+bnAb group showed a higher Env-specific human IgG level than the MVA/Env+bnAb group though the difference was not statistically significant once values were corrected for multiple comparisons (unadjusted $P = 0.008$; false discovery rate-adjusted *P* value [FDR *P*] $> 0.05$) (Table 1; see also Table S2 in the supplemental material).

**CH31 passive immunization did not impact the magnitude, specificity, and breadth of the active vaccine-elicited binding antibody response.** Levels of the vaccine-elicited IgG binding response against vaccine strain 1086.C gp120 in plasma were assessed at the times of each immunization and 2 weeks after each immunization by an ELISA using anti-rhesus IgG detection (Fig. 2B). The 1086.C gp120-specific IgG response was detected as early as 2 weeks postpriming and was further boosted for subsequent immunizations and peaked after the third immunization. There was no difference between paired groups with and without CH31 administration (Env-only versus Env+bnAb group and MVA/Env versus MVA/Env+bnAb group) at any time point tested (unadjusted $P > 0.05$) (Table S1). Therefore, human bnAb CH31 IgG administration infusion neither inhibited nor enhanced the magnitude of the vaccine-elicited HIV-1 Env-specific IgG response compared to the response with the respective active vaccination groups that did not receive CH31. Magnitudes of the vaccine-elicited IgG responses were also comparable between vaccine groups using Env versus MVA immunogens (Env-only versus MVA/Env group and Env+bnAb versus MVA/Env+bnAb group) at various time points, except for higher 1086.C gp120 binding for the Env-only group than for the MVA/Env group at week 14 though the difference was not significant once values were corrected for multiple comparisons (unadjusted $P = 0.008$; FDR $P > 0.05$).

The magnitude, breadth, and epitope specificity of the vaccine-elicited HIV-1 Env-binding plasma IgG responses following each immunization were further characterized by binding antibody multiplex assay (BAMA) using a panel of HIV-1 Env proteins, gp70 scaffolded V1V2 and V3 proteins, and linear peptide antigens (Fig. 2C and D; Table 2).

**FIG 2** Legend (Continued)
of binding to a panel of region/epitope-specific antigens as measured in BAMA. *, unadjusted $P < 0.05$; **, unadjusted $P < 0.01$. All *P* values are >0.05 once values were adjusted for multiple comparison (Tables 1 S1, and S2 give both unadjusted *P* and FDR *P* values for all comparisons). Black horizontal bars represent group median values. For panel D, binding to the V3 Ag panel ($) (calculated as mean MFI for gp70 Con V3, gp70-MN V3, and linear V3.C by each animal) was lower for the Env-only group than for the MVA/Env group at week 2 (unadjusted $P < 0.05$).

**TABLE 2** Sequence information for peptide antigens used in BAMA

| Peptide | Sequence |
|---|---|
| Bio-RV144_C5.2_C | Biotin-KKKSELYKYKVVEIKPLGIAPTKAKRRVVEREKRAV |
| Bio-V2.1086C | Biotin-KKKTELKDKKHKVHALFYKLDVVP |
| Bio-V3.C | Biotin-KKKNNTRKSIRIGPGQTFYATGDIIGDIRQAHC |
| C1 biotin | Biotin-KKKMQEDVISLWDQSLKPCVKLTPLCV |
| Bio-V2.B | Biotin-KKKTSIRDKVQKEYALFYKLDVVP |

Levels of plasma IgG binding to the gp140 panel (two antigens), gp120 panel (three antigens), V1V2 panel (three antigens), and V3 panel (three antigens) were all comparable between groups with and without CH31 administration and between groups using Env-only versus Env/MVA immunogens at all three time points tested. For IgG binding to the V3 antigens, a higher level was seen for the MVA/Env group at week 2 than for the Env-only group though the difference was not significant once values were corrected for multiple comparisons (unadjusted $P < 0.029$; FDR $P > 0.05$). No difference was seen between the vaccine groups in the magnitudes of IgG binding to linear C1 and C5.2.C peptides at any time point tested. For IgG binding to V2.B linear peptide at week 2 (after the first immunization), levels were higher for the Env+bnAb group than for the Env-only group (unadjusted $P = 0.04$) and for the MVA/Env group than for the Env-only group though the differences were not significant once values were corrected for multiple comparisons (FDR $P > 0.05$).

We next characterized the specificities of plasma IgG against overlapping peptide covering the entire Env gp160 by a peptide microarray mapping assay at study week 2 (after the first immunization) and week 8 (after the second immunization) to investigate any potential effects of CH31 infusion on the fine specificity and magnitude of vaccine-elicited IgG responses early after immunization when CH31 levels were high and after vaccine boosting. Plasma IgG from the vaccines was found to target the linear epitopes in the C1, V2, C2, V3, C3, V5, and C5 regions of gp120, with the anti-V3 response being the dominant specificity, followed by C5 for both the early and boost time points (Fig. 3A) (regions for each epitope are defined in Table 3). Importantly, magnitudes of IgG binding to the linear epitopes were mostly comparable between treatment groups with and without CH31 infusion for both the early and boost time points, with the exception of week 8 binding for epitope C3.2, for which the MVA/Env+bnAb group showed a higher level than the MVA/Env group, and for C5.2, for which the Env+bnAb group showed a higher level than the Env-only group (Fig. 3B). Differences for both C3.2 and C5.2 in the two comparisons were not significant once values were corrected for multiple comparison (unadjusted *P* values of 0.032 and 0.048, respectively; FDR $P > 0.05$) (Tables 1, S1, and S2).

**CH31 passive immunization did not impact antibody-mediated functions of the active vaccine-elicited antibodies.** Antibody-mediated functions of the plasma antibodies, including neutralization, binding to infected cells, and antibody-dependent cell-mediated cytotoxicity (ADCC), were characterized following each HIV Env immunization. Consistent with the kinetics of binding antibody response, neutralization (Fig. 4A) and HIV-infected cell IgG binding (Fig. 4B) activities peaked at week 14 (i.e., 2 weeks following the third immunization), whereas ADCC activity peak at week 8 (2 weeks after the second immunization) and maintained relatively the same level at week 14 (Fig. 4C).

Neutralizing activity was measured against the clade C tier 1 viruses MW965 at weeks 2, 8, and 14 (Fig. 4A and D) and SO032 at week 14 (Fig. 4D). Neutralizing activity was first detected after the second immunization (week 8). At this time point, a trend of higher neutralization titers was seen for the Env groups (Env-only and Env+bnAb) than for the MVA/Env groups (MVA/Env and MVA/Env+bnAb) although the differences were not significant (unadjusted $P > 0.05$). Neutralizing activities peaked at week 14 after the third immunization for all four groups, with 50% inhibitory dilution ($ID_{50}$) titers ranging from 384 to 41,131. No significant difference was seen between any groups at this time point for neutralization of either MW965 or SO032 (Fig. 4D; Tables S1 and S2).

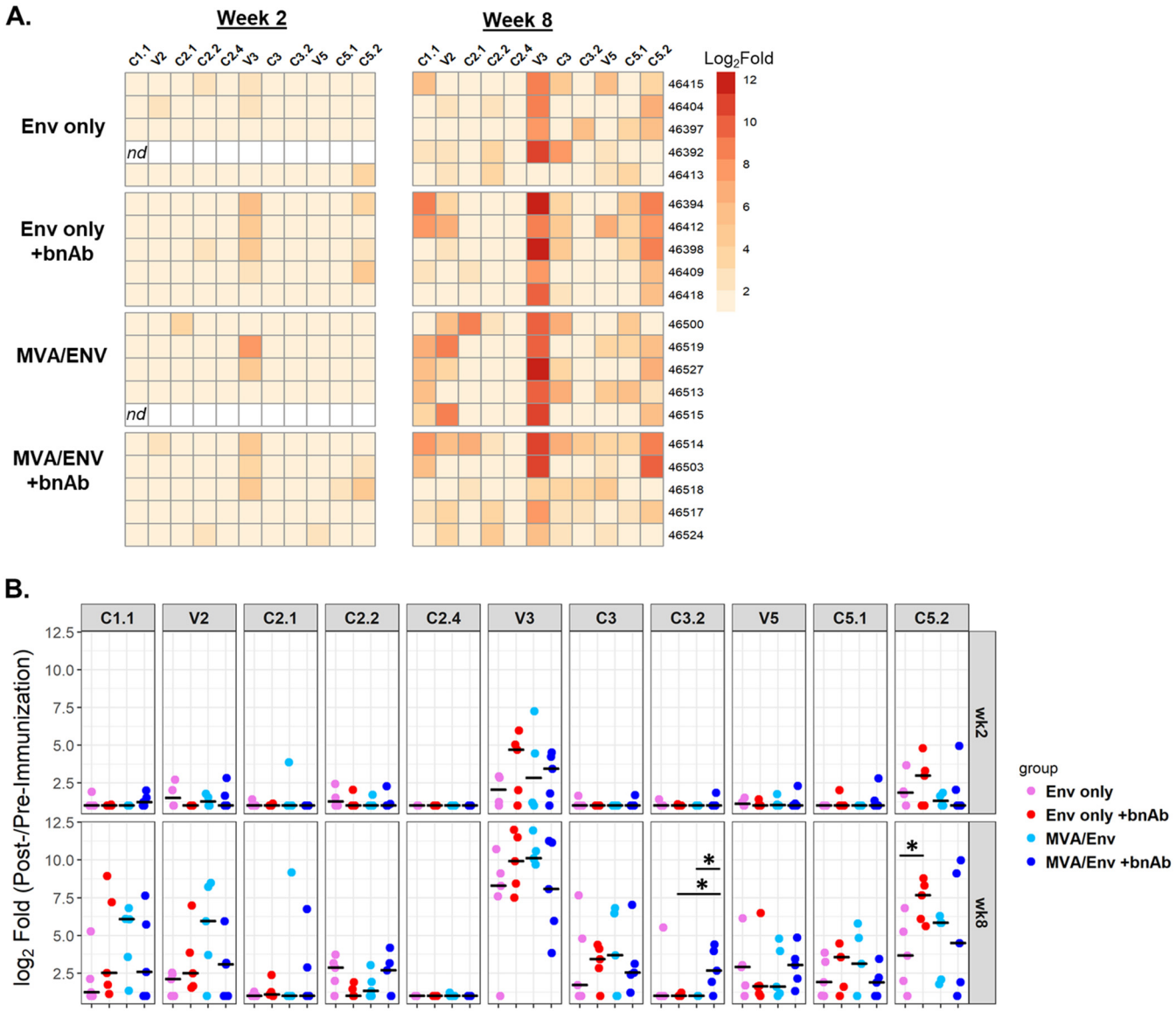


**FIG 3** Magnitude of binding to linear epitopes measured in peptide microarray. (A) Heat map showing binding magnitude to 1086.C linear epitopes at week 2 and week 8 by all animals. nd, not done (2 samples were not available for week 2 and, thus, not analyzed in linear epitope mapping). (B) Scatter plot for 1086.C linear epitope binding magnitude for the vaccine groups at week (wk) 2 and week 8. Black cross bars represent group median values. *, unadjusted $P < 0.05$. All $P$ values were >0.05 once values were adjusted for multiple comparison (Tables 1, S1, and S2 give both unadjusted $P$ and FDR $P$ values for all comparisons).

The ability of the vaccine-elicited IgG to bind to HIV-1 Env antigens expressed on cells infected with three different strains of HIV-1 (1086.C, AE.CM235, and B.WITO) was observed as early as study week 8, 2 weeks after the second immunization for all groups against all three strains (Fig. 4E). No difference among groups in either the levels of secondary mean fluorescence intensity (MFI) (Fig. 4E) or the proportions of positive lymphocytes (Fig. 4F) was observed for week 8. At week 14, both levels of secondary MFI and the proportions of positive lymphocytes (Fig. 4F) were lower for the Env+bnAb group than for the Env-only and MVA/Env+bnAb group (Fig. 4E and F) though the differences were not significant once values were corrected for multiple comparison (Tables 1, S1, and S2).

ADCC activity against 1086.C-coated cells was first detected and peaked after the second immunization (week 8) (Fig. 4C and G). Serum titers ranged from $4.5 \times 10^4$ to $1.5 \times 10^6$. ADCC levels after the third immunization remained similar to those after the second immunization. No difference was detected between groups for either ADCC

**TABLE 3** Key for linear epitopes targeted by binding antibodies in the study

| Epitope | Peptide region[a] | Amino acid position[b] |
|---|---|---|
| C1.1 | p32–p37 | 95–124 |
| V2 | p53–p55 | 163–183 |
| C2.1 | p65–p67 | 199–219 |
| C2.2 | p70–p73 | 214–237 |
| C2.4 | p88–p89 | 268–285 |
| V3 | p98–p103 | 298–329 |
| C3 | p112–p114 | 341–361 |
| C3.2 | p116–p117 | 354–372 |
| V5 | p146–p149 | 454–477 |
| C5.1 | p152–p153 | 472–489 |
| C5.2 | p156–p162 | 484–516 |

[a]Peptide regions as defined in micrography library.
[b]HXB2 numbering.

serum titers (Fig. 4C) or maximum percent granzyme B (GzB) activity levels (Fig. 4G) for either week 8 or week 14.

**CH31 administration did not impact the avidity of vaccine-elicited HIV-1 Env-specific IgG.** The avidity values of vaccine-elicited HIV-1 Env-specific IgG to a panel of gp140, gp120, V1V2, and V3 analytes were measured by surface plasmon resonance (SPR) after the second (week 8) and third (week 14) immunizations (Fig. 5A and B). Avidity data could be determined only when a sufficient magnitude of IgG binding could be determined. Therefore, avidity values were not available for some samples against V1V2, V2, and V3 analytes, especially at week 8. We compared avidity values between groups when at least four data points were obtained. No significant difference was observed in either dissociation rates (Fig. 5A) or avidity scores (Fig. 5B) for any analyte among the groups for all time points tested.

**CH31 administration did not impact *de novo* development of CD4bs-specific antibodies.** Development of CD4bs-specific antibody responses in plasma was evaluated at multiple time points using a soluble CD4 (sCD4) blocking ELISA (Fig. 6). Relative levels of vaccine-elicited HIV-1 CD4bs Abs, as indicated by levels of inhibition of sCD4 binding, were first detected after the second immunization and increased further with the third immunization. As CH31 levels had dropped to below the detection limit of 3 $\mu$g/ml by week 2 and continued to decline and as no detectable sCD4 blocking activity was detected at week 2 and earlier, we were certain that the sCD4 blocking activity at week 8 and week 14 in the assay was mediated by *de novo*-developed antibody (CD4bs antibody) responses. The MVA/Env+bnAb group demonstrated the greatest magnitude of sCD4-specific antibody blocking at week 8 (median, 93.3%; range, 56.7 to 96.1%), and this value was higher than those of both the MVA/Env group and the Env+bnAb group although the differences were not significant once values were corrected for multiple comparison. By week 14, all four groups reached a median blocking level of $>$90%. There was no significant difference in the sCD4 blocking activities between any of the groups at all time points tested.

## DISCUSSION

With the challenge of rapidly eliciting a protective broad neutralization response via active HIV vaccination, interest in the passive administration of bnAbs as prophylaxis has risen. Passive transfer of bnAbs is particularly relevant for pediatric HIV vaccine development due to the well-established timing of exposure and low body weight of the infant, making dosing to achieve high serum levels easier. Furthermore, it has been reported that infants can develop neutralizing antibody responses as frequently as, or more frequently than, adults do, and a cross-clade neutralization response can be developed as early as 1 year following infection in some infants (28, 29), supporting active infant HIV immunization as a strategy to achieve lifelong protective immunity. We aim for a combination vaccine strategy with which infants are protected by passively infused bnAbs until *de novo* neutralization breadth develops. Certain

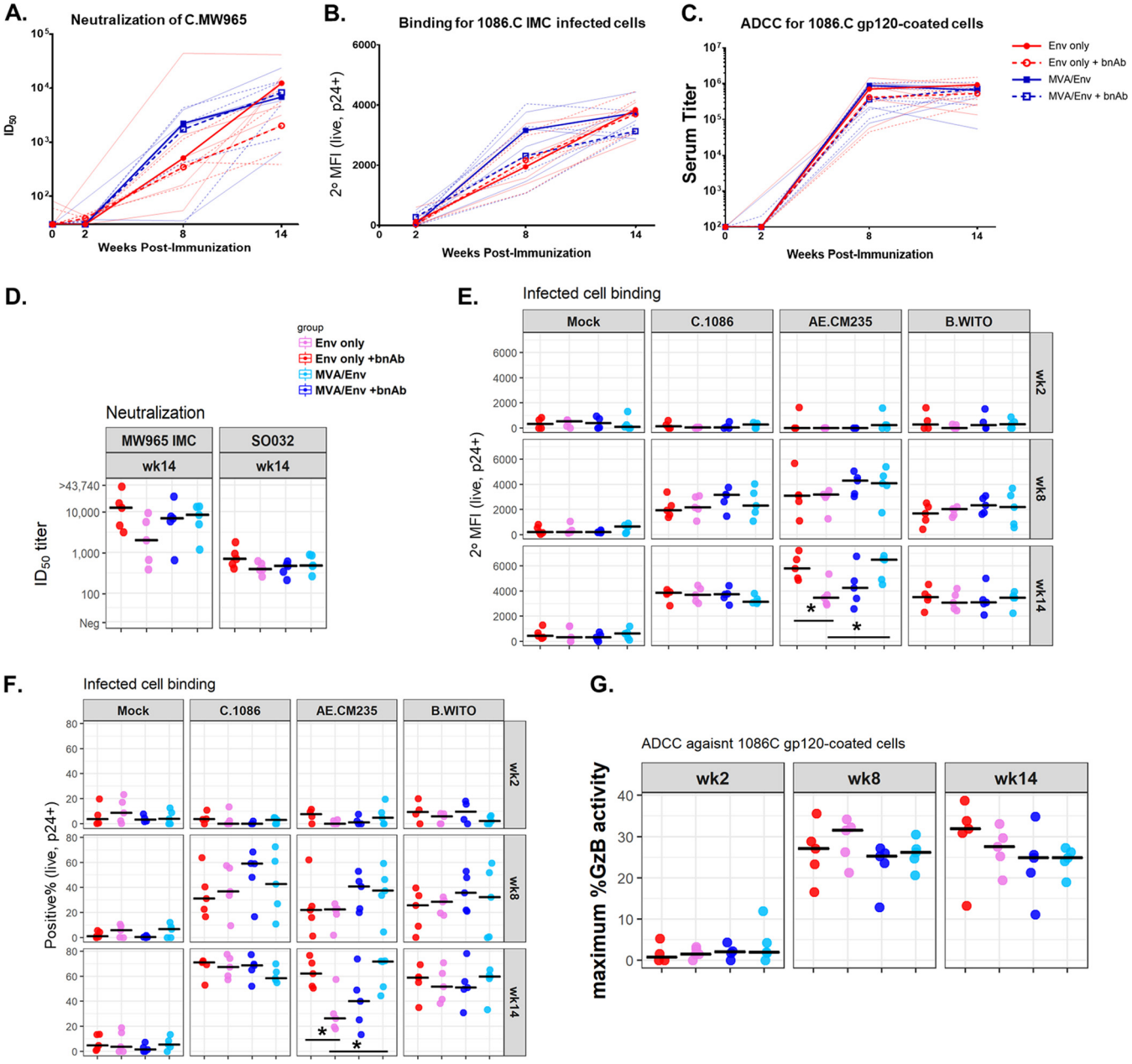


**FIG 4** Functional antibody responses in vaccinated macaques. (A to C) Levels of neutralization (of C.MV965 on TZM-bl cells), infected cell binding (for 1086.C IMC-infected cells), and ADCC (against 1086.C gp120-coated cells) responses over time. (D) Neutralizing activity against MW965 and SO032 at week 14. (E) Levels of infected cell binding (secondary MFI) to a panel of HIV-1 IMC-infected cells at key time points. (F) Levels of infected cell binding (positive percent cells) to a panel of HIV-1 IMC-infected cells at key time points. (G) Levels of ADCC response against 1086.C gp120-coated cells (maximum percent granzyme B activity) over time. *, unadjusted $P < 0.05$. All $P$ values are $>0.05$ once values were adjusted for multiple comparison (Tables 1, S1, and S2 give both unadjusted $P$ and FDR $P$ values for all comparisons). For panels A, B, and C, the thin lines represent individual animals while thick lines indicate group median values. For panels D to G, black horizontal bars represent group median values.

concerns, however, have to be addressed before this method of passive/active vaccination can be used in infants, including *in vivo* levels and persistence of the bnAb following administration, as well as potential interference with *de novo* vaccine-elicited antibody development. Moreover, a combined passive/active HIV-1 vaccine strategy administered in neonates would be an ideal strategy for both early passive protection against peripartum HIV-1 acquisition and priming of the immune system for lifelong immunity, mimicking the successful strategy to prevent perinatal and lifelong HBV infection. With the comprehensive characterization of binding and

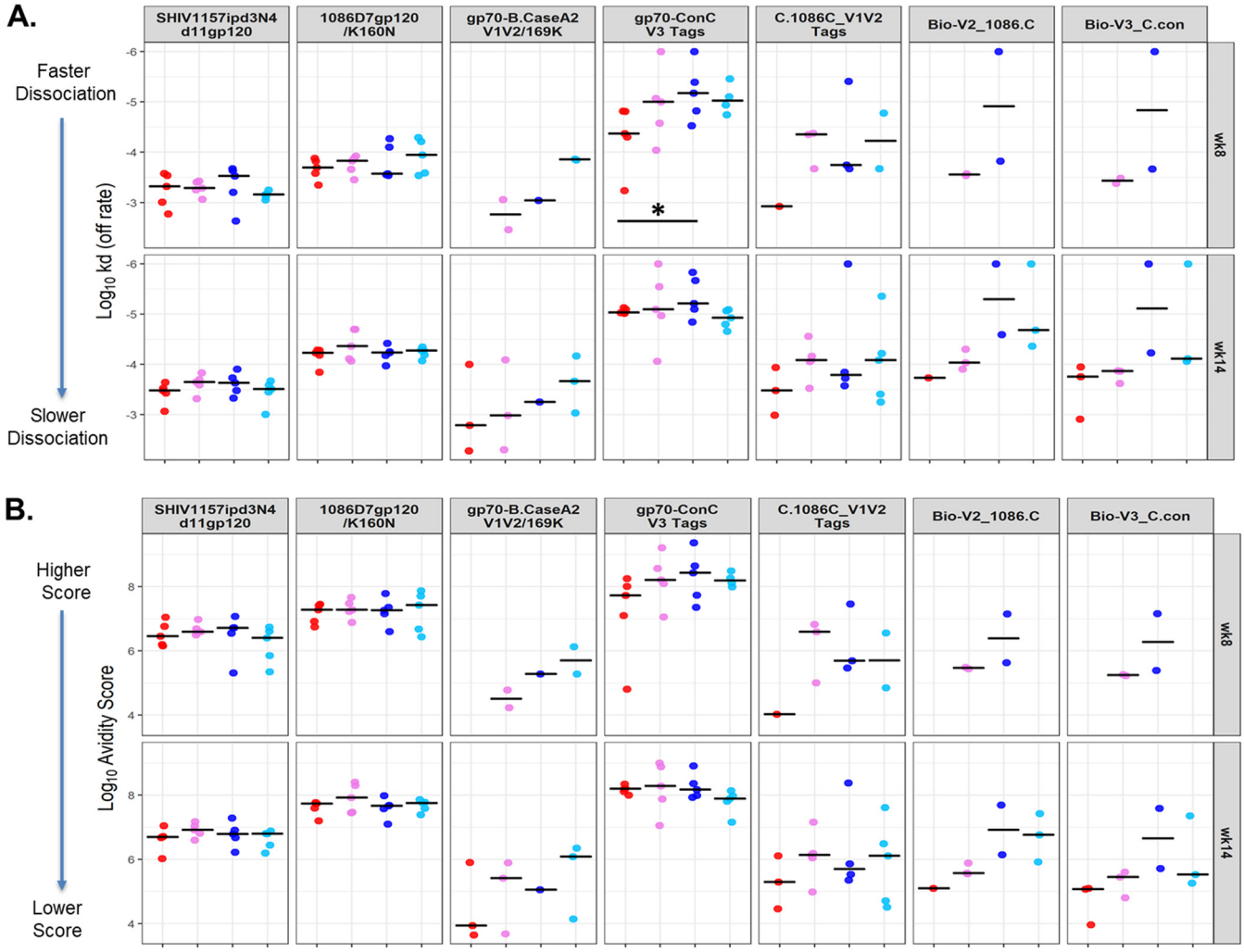


**FIG 5** Avidity of plasma IgG binding to a panel of Env, V1V2, and V3 antigens at week 8 and week 14 shown as disassociation ($k_d$) rate (A) and avidity score (B), as measured by SPR. *, unadjusted $P < 0.05$. All unadjusted $P$ and FDR $P$ values are >0.05 (Tables 1, S1, and S2 give details). Black horizontal bars represent group median values. Avidity data are not available for all samples as avidity could not be measured for samples that did not bind sufficiently to the analytes.

functional antibody responses to active vaccination, our study demonstrated no adverse effects of using bnAb administration in combination with active immunization in infant macaques.

In this current study, we characterized longitudinal binding and functional antibody

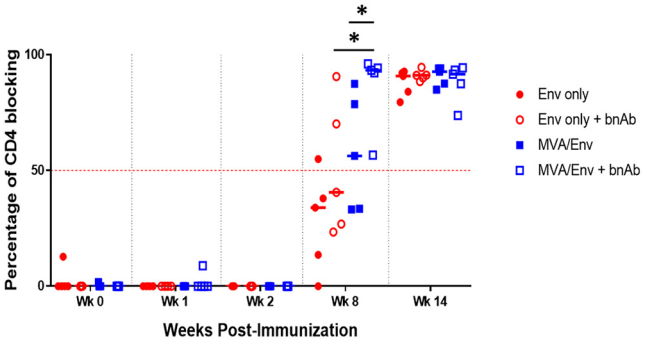


**FIG 6** Level of soluble CD4 blocking activity measured in sCD4 blocking ELISA as an indicator for a potential CD4bs binding antibody response. *, unadjusted $P < 0.05$. All $P$ values are >0.05 once values were adjusted for multiple comparison (Tables 1, S1, and S2 give both unadjusted $P$ and FDR $P$ values for all comparisons). Horizontal black bars represent group median values.

responses following HIV vaccination with and without coadministration of a CD4bs-directed bnAb, CH31, and compared these responses to investigate the effects of the bnAb administration on antibody development. CH31 was selected based on its similarity to VRC01 (30), which is currently being tested in human clinical trials, including an infant passive immunization clinical trial, with satisfying safety profiles demonstrated (31). Note that we monitored and compared antibody responses for 14 weeks (through the active prime/boost vaccination period) in this study. It is possible that effects of the bnAb infusion could manifest at a later stage and in other parameters such as neutralization breadth and durability of responses, which will be monitored with follow-up studies. With the 14 weeks of follow-up, sporadic differences were found in comparisons of responses for paired groups with and without bnAb administration. Among 208 comparisons of vaccine-elicited immunity that we performed between matched groups with and without CH31 administration (Env-only versus Env+bnAb group; MVA/Env versus MVA/Env+bnAb group), 6 comparisons resulted in unadjusted *P* values of $<0.05$. Differences for all six comparisons were not significant once we corrected for multiple comparisons (FDR $P > 0.05$). Furthermore, what seemed different in one pair of the with- and without-bnAb groups often did not show the same trend in the other pair. Therefore, we consider the potential differences seen in antibody responses between HIV Env vaccine groups with and without passive bnAb administration as sporadic and not associated with bnAb administration. In addition, no significant difference between vaccinees with and without bnAb administration was observed for neutralization, ADCC, and avidity of IgG responses (Table 1; see also Table S1 in the supplemental material). Tier 1 neutralizing activity was first developed after the second immunization and peaked after the third immunization, consistent with the observation in a previous infant macaque vaccination study that tested accelerated Env-only or MVA/Env regimens and an extended-interval MVA/Env regimen (26). Importantly, CH31 administration showed no significant impact on the development of a neutralizing antibody response, as shown by comparable levels of neutralizing titers by matched groups with and without bnAbs (Table S1 and Fig. 4A and D). And despite CH31 being a CD4bs-directed bnAb, levels of binding antibodies targeting CD4bs, measured by blocking of sCD4 binding (Fig. 6), were not negatively impacted. We thus conclude that one administration of bnAb CH31 at the time of active HIV Env vaccination priming did not impair the development of antibody responses following Env-only or MVA/Env immunizations.

We assessed the kinetics of CH31 *in vivo* after passive administration and discovered that CH31 levels peaked at 1 day following administration and then declined until they were undetectable for most animals by 2 weeks postadministration. The $IC_{50}$s of CH31 against most of the tier 2 clade C transmission/founder virus isolates are $<1$ $\mu$g/ml (32). In the current study the median concentration of the bnAb in the animals remained higher than 1 $\mu$g/ml for the first 1 week (median, 19 $\mu$g/ml; range, 10 to 24 $\mu$g/ml on day 7), suggesting the feasibility of an administered bnAb to reach serum levels that will neutralize a range of HIV-1 strains and help thwart an infection.

Even though the *in vivo* CH31 concentration was maintained at a higher than typical neutralizing $IC_{50}$ of CH31 against tier 2 clade C viruses, the concentration did drop to below detection in most animals by 2 weeks postadministration. The prolonged time required for an effective antibody response to develop against HIV antigens (6, 7) means that, unlike the success in in pediatric combination vaccine for HBV prevention (25), maintaining a biologically relevant level of the infused bnAbs over an extended period is particularly important for HIV combination vaccines. BnAbs with longer half-lives and/or with higher potencies will be required to cover the length of time before *de novo* neutralizing and other functional antibody responses are developed. Of particular interest, a simianized VRC01 IgG has been shown to maintain *in vivo* therapeutic levels for $>5$ months after the last dose and protected macaques against repeated mucosal challenge for 52 days (17), and VRC01-LS, which was engineered to increase binding to neonatal Fc receptor (FcRn), was shown to have a 3-fold-longer half-life in macaque than VRC01 (33). The CH31 administered in this study is a human

IgG, exogenous to the macaques. The clearance of the human bnAb through a potential anti-human IgG response could have shortened the half-life of CH31 *in vivo*. Future studies could explore methods to enhance the persistence of the administered bnAb through simianization of the IgG (17) or Fc modifications known to extend passive antibody half-life (33, 34) or to lower the biologically relevant *in vivo* concentration required through optimizing potency of the bnAbs by combinations of bnAbs with different specificities and bi- or trispecific bnAbs (20, 35). Nevertheless, the potential effects of the bnAbs on vaccine-elicited antibody responses in studies where longer persistence of the bnAbs is achieved, especially persistence through the boosting period, should also be carefully examined.

With steady declines in new HIV-1 infections worldwide due to early diagnosis and treatment options, scientists have put more focus on vaccine development to further reduce the infection rates worldwide. The effort will have to include protection of infants born to and breastfed by HIV-infected mothers. A combination of passive transfer of bnAb and active HIV Env vaccination to elicit a protective memory antibody response has the potential to provide protection from birth through the breastfeeding period and into adolescence. The study described in this paper demonstrates that passive CD4bs-directed bnAb administration will not have a large impact on the development of HIV Env vaccine-elicited binding and functional *de novo* antibody responses from active HIV-1 vaccination. With further testing of this combination strategy, developing an infant vaccine that protects against neonatal HIV-1 infection is one step closer.

## MATERIALS AND METHODS

**Animal care and sample collection.** Twenty newborn rhesus monkeys (*Macaca mulatta*) of Indian origin, born to animals from a colony free of HIV-2, SIV, type D retrovirus, and simian T-cell lymphotropic virus type 1 were hand reared in the nursery of the California National Primate Research Center (CNPRC). Animals were maintained in accordance with the American Association for Accreditation of Laboratory Animal Care standards and the *Guide for the Care and Use of Laboratory Animals* (36). All protocols were reviewed and approved by the University of California at Davis Institutional Animal Care and Use Committee prior to the initiation of the study. The infant macaques were 4 to 10 days of age at the time of the first procedure (bnAb inoculation). When necessary, for sample collections and immunizations, animals were immobilized with ketamine HCl (Parke-Davis, Morris Plains, NJ) injected at 10 mg/kg of body weight. EDTA-anticoagulated blood was collected at study weeks 0, 6, 8, 12, and 14, in addition to the early time points of days 1, 3, 7, and 14, via peripheral venipuncture.

**Vaccine regimen preparation.** The 20 infants were randomly assigned to four groups of 5 animals each (Fig. 1). As all groups were immunized with the same simian immunodeficiency virus (SIV) Gag/Pol-expressing constructs, the group nomenclature was based on (i) how the infants received HIV envelope, either only as protein (Env only) or as both protein and an MVA construct that expresses HIV Env (MVA-HIV-Env/Env, and (ii) whether the infants received the bnAb CH31 IgG. At week 0, two experimental groups (Env+bnAb and MVA/Env+bnAb) received a single dose of human CH31 IgG administered intravenously at 20 mg/kg (4.2 ml/kg of a 4.79-mg/ml solution); the other two groups (Env-only and MVA/Env) did not receive the bnAb. At week 0, the Env-only and Env+bnAb groups were primed intramuscularly (i.m.) with HIV-1 Env 1086.C gp120-protein and 3M052-SE adjuvant (15 $\mu$g of protein mixed with 250 $\mu$l of 3M052-SE [27] and diluted with extra saline, for a total dose volume of 0.5 ml, divided over left and right quadriceps), and $5 \times 10^{10}$ viral particles (VP) of chimpanzee adenovirus (ChAdOx1.tSIVconsv239)-SIV Gag/Pol (0.25 ml divided over left and right gluteus). At weeks 6 and 12 of the study, the infants received two successive i.m. boosts with 1086.C gp120 protein (same dose as the prime) and $2 \times 10^8$ particle-forming units (PFU) of MVA.tSIVconsv239 boosts (in 0.25 ml, divided over left and right gluteus). Animals in the MVA/Env and MVAEnv/Env+bnAb groups received the same vaccine regimens as the corresponding Env-only group, but at every time point (weeks 0, 6, and 12) an additional immunization was given with $10^8$ PFU of an MVA-HIV-Env construct (in a volume of 0.25 ml divided over left and right biceps); the MVA-HIV Env construct expressing 1086.C gp120 was generated as previously described (18).

**Enzyme-linked immunosorbent assay (ELISA), recombinant protein, and soluble CD4 blocking.** Env-binding IgG was assessed in plasma in a 384-well plate format. The plates were coated overnight with HIV 1086.C gp120 (30 ng/well) and then blocked with the assay diluent (phosphate-buffered saline containing 4% whey, 15% normal goat serum, and 0.5% Tween 20). Dilutions of plasma were then added to the plates and incubated for 1 h, followed by detection with a horseradish peroxidase (HRP)-conjugated antibody, polyclonal goat anti-monkey IgG (Rockland, Gilbertsville, PA). The plates were developed by using an ABTS-2 [2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid)] peroxidase substrate system (KPL, Gaithersburg, MD). *Macaca mulatta* purified IgG (NIH Nonhuman Primate Reagent Resource, project numbers R24 OD010976 and U24 AI126683) was used to develop standard curves, and the concentration of IgG antibody was calculated relative to the standard using a 5-parameter fit curve

(WorkOut, version 2.5; PerkinElmer, Waltham, MA). For monoclonal antibodies (MAbs), the half-maximal effective concentration ($EC_{50}$) was calculated by the concentration of antibody which resulted in a 50% reduction in optical density (OD) from the maximum value.

For soluble CD4 (sCD4) blocking ELISAs, 384-well plates (Corning Life Sciences, Lowell, MA) were coated with 1086.C gp120 at 30 ng/well. Following the same steps as previously stated, the plates were blocked with assay diluent, and serial dilutions of MAb and plasma were distributed to the plates. Once the MAbs and the plasma were added and incubated for 1 h, sCD4 (NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH; human soluble CD4 recombinant protein [sCD4] from Progenics) was added at 0.64 $\mu$g/ml. The sCD4 binding was detected using a biotinylated human anti-CD4 (Thermo Fisher Scientific, San Diego, CA), followed by HRP-conjugated streptavidin. Percent sCD4 binding inhibition was calculated as follows: 100 – (average of serum duplicate OD/average of negative-control OD) $\times$100. BnAb VRC01 was used as a positive control, consistently blocking soluble CD4 by 80% at 32 $\mu$g/ml.

**Binding antibody multiplex assay (BAMA).** HIV-1 antigens were conjugated to polystyrene beads (Bio-Rad, Hercules, CA) as previously described (18), and then binding of IgG to the bead-conjugated HIV-1 antigens was measured in plasma samples. The positive control was purified IgG from a pool of plasma of HIV-vaccinated rhesus macaques from a previous study (RIVIG), produced internally via IgG depletion. The conjugated beads were incubated on filter plates (Millipore, Stafford, VA) for approximately 30 min before plasma samples were added. The plasma samples were diluted in assay diluent (1% dry milk, 5% goat serum, and 0.05% Tween 20 in 1$\times$ phosphate buffered saline, pH 7.4.) at a 1:500-point dilution. Beads and diluted samples were incubated for 30 min, and then IgG binding was detected using a phycoerythrin (PE)-conjugated mouse anti-monkey IgG (Southern Biotech, Birmingham, Alabama) at 4 $\mu$g/ml. The beads were washed and acquired on a Bio-Plex 200 instrument (Bio-Rad, Hercules, CA), and IgG binding was expressed as mean fluorescence intensity (MFI). To assess assay background, the MFIs of binding to wells that did not contain beads or sample (blank wells) and nonspecific binding of the samples to unconjugated blank beads were evaluated during assay analysis. High background detection, defined as an MFI of $>$100, for plasma samples was noted, and those plasma samples were repeated if necessary. An HIV-1 Env-specific antibody response was considered positive if it was above the lower limit of detection (100 MFI). To check for consistency between assays, the $EC_{50}$ and maximum MFI values of the positive control (RIVIG) were tracked by Levy-Jennings charts. The antigens conjugated to the polystyrene beads were as follows: C.1086 gp140, C.1086 gp120, A1. Con_env03 gp140, A233 gp120, B.Con_env03 gp140, Con6 gp120, Con C gp120, MN gp120, linear V2.B, V3.C, C5.2.C, C1, conformational V1V2, Con C V3, MN V3, and C.1086 V1V2.

**Linear peptide microarray mapping and data analysis.** Solid-phase peptide microarray epitope mapping was performed as previously described (37), with minor modifications. Briefly, array slides were prepared by JPT Peptide Technologies GmbH (Germany) by printing a library designed by B. Korber, Los Alamos National Laboratory, onto epoxy glass slides (PolyAn GmbH, Germany). The library contains 15-mer peptides overlapping by 12, covering consensus Env (gp160) clade A, B, C, and D, group M, CRF1, and CRF2 and vaccine strains (gp120) 1.A244, 1.TH023, MN, 1086.C, C.TV1, and C.ZM651. Sera were diluted 1/50 and applied to the peptide array, followed by washing and detection using goat anti-human IgG-Alexa Fluor 647. Array slides were scanned at a wavelength of 635 nm with an InnoScan 710 AL scanner (Innopsys, France) using extended dynamic range (XDR) mode. Scan images were analyzed using Mapix, version 8.0, software to obtain binding intensity values for all peptides. Microarray data were then processed using the R package pepStat (38) to obtain binding signal for each peptide, which is defined as the $\log_2$ value of the intensity of the postimmunization sample/intensity of matched baseline sample. Binding magnitude to each identified epitope is defined as the highest binding signal by a single peptide within the epitope region.

**ADCC.** We used a GranToxiLux assay to detect plasma antibody-dependent cell-mediated cytotoxicity (ADCC) activity directed against $CEM.NKR_{CCR5}$ cells (catalog no. 4376; NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH; from Alexandra Trkola) (20) coated with recombinant gp120 as previously described (25). The $CEM.NKR_{CCR5}$ target cells were coated with gp120 representing the vaccine immunogen, 1086.C D7 gp120 K160N. NHP plasma samples were tested for ADCC activity after 4-fold serial dilutions starting at 1:100. Cryopreserved human peripheral blood mononuclear cells (PBMCs) from an HIV-1-seronegative donor with the heterologous 158 F/V genotype for Fc$\gamma$ receptor IIIa were used as the source of effector cells (26). The percent granzyme B (GzB) activity was defined as the percentage of cells positive for proteolytically active GzB out of the total viable target cell population. Final results were calculated after subtracting the background percent GzB activity observed in wells containing effector and target cells in the absence of plasma samples. The maximum percent GzB activity represents the peak ADCC activity observed for the plasma dilutions tested. ADCC endpoint titers were determined by interpolating the dilutions of plasma that intercept the positivity cutoff using GraphPad Prism, version 7 (GraphPad Software, Inc., La Jolla, CA).

**Plasma binding to the surface of HIV-1-infected cells.** Indirect surface staining was used to measure the ability of plasma samples to bind HIV-1 envelope expressed on the surface of infected cells using methods similar to those previously described (21). $CEM.NKR_{CCR5}$ cells were mock infected or infected with an HIV-1 infectious molecular clones (27) expressing the 1086.C, WITO.B, or CM235.AE envelope proteins. The cells were incubated with a 1:100 dilution of plasma samples for 2 h at 37°C and then stained with Live/Dead Aqua Dead Cell Stain (Thermo, Fisher Scientific, Waltham, MA) to exclude dead cells from analysis. Cells were washed and then permeabilized with Cytofix/Cytoperm solution (BD Biosciences, San Jose, CA) prior to staining with fluorescein isothiocyanate (FITC)-conjugated goat anti-rhesus IgG(H+L) polyclonal antiserum (Southern Biotech, Birmingham, AL) and RD1-conjugated anti-p24 MAb KC57 (Beckman Coulter, Inc., Indianapolis, IN). Cells positive for plasma binding were

defined as viable, p24 positive, and FITC positive. Final results are reported as the FITC MFI of the live infected cell population (p24-positive cells) after subtraction of the background observed for the prevaccination samples.

**SPR measurements of purified plasma IgG avidity.** IgG avidity to a panel of HIV-1 antigens (C.1086 gp120, C.1086 V1V2, linear C.1086 V2 and C.V3, conformational V1V2 and C.V3, as well as SHIV1157ipd3N4 d11 gp120) was measured by surface plasmon resonance (SPR) (BIAcore 4000; GE Healthcare) analysis. Using a multiplex array format (2 by 8), binding response was measured by SPR in duplicate following immobilization by amine coupling of envelope protein (19) or capture of biotinylated antigens to immobilized streptavidin on series S CM5 sensor chips (BIAcore/GE Healthcare, Pittsburgh, PA). Purified plasma IgG samples at 150 $\mu$g/ml were flowed (2.5 min) over duplicate spots of antigen, followed by a dissociation phase (postinjection/buffer wash) of 10 min. Nonspecific binding of a preimmune (zero time point) sample was subtracted from binding data for each postimmunization IgG sample. Data analyses were performed with BIAevaluation 4000 and BIAevaluation, version 4.1, software (BIAcore/GE Healthcare). Binding responses were measured by averaging postinjection response units (RU) over a 10-s window, and the dissociation rate constant, $k_d$ (second$^{-1}$), was measured during the postinjection phase after stabilization of signal. A response was defined as positive when both replicates had an RU value of $\geq$10. The relative avidity binding score was calculated as follows: avidity score (RUs) = (binding response units/$k_d$).

**Neutralization.** Plasma neutralization of C.MW965, a clade C, tier 1, infectious molecular clone (IMC), was measured in TZM-bl cells via a reduction in luciferase reporter gene expression (20). In brief, dilutions of plasma were incubated with an optimized amount of virus for 45 min at 37°C in a 96-well plate, and then freshly trypsinized TZM-bl cells in growth medium were added to each well. Following a subsequent 48-h incubation, the culture medium was removed and replaced with a luciferase reagent, Bright-Glo (Promega, Madison, WI), causing cell lysis and luminescence proportional to the amount of infection. The luminescence was measured using a Victor X3 Multilabel Plate Reader (Perkin-Elmer). The 50% inhibitory dilution ($ID_{50}$) was calculated as the dilution that resulted in a 50% reduction in relative luminescence units (RLU) compared to the levels in virus control wells.

**Statistical methods.** The sample size of $n = 5$ animals per group was designed to ensure sufficient power to detect a 2-fold difference in antibody responses between groups. In addition, note that a sample size of $n = 4$ animals per group is the minimal amount necessary to be able to achieve a statistically significant result (i.e., $P$ value of $<$0.05 without multiplicity adjustment) when an exact Wilcoxon rank sum test is used to compare continuous outcomes between two groups. Adding one additional animal per group (i.e., $n = 5$) guards against the possibility that an animal may not be available for analysis, e.g., due to death or missing data. The sample size of $n = 5$ animals per group was not selected to provide adequate power after adjustment for multiple comparisons.

Immunological measurements of antibody responses were compared between immunization groups using Wilcoxon rank sum tests with exact $P$ values, followed by a false discovery rate (FDR) adjustment for multiple comparisons using the Benjamini-Hochberg (BH) procedure (39) per prespecified analysis plan. Separate adjustments to control the FDR at and $\alpha$ of 0.05 were performed for the following: (i) comparisons between paired groups with and without bnAb (i.e., Env-only versus Env+bnAb and MVA/Env versus MVA/Env+bnAb), for a total of 208 comparisons; (ii) comparisons between groups with Env versus MAV immunogens (i.e., Env-only versus MVA/Env and Env+bnAb versus MVA/Env+bnAb), for a total of 212 comparisons. Both the unadjusted Wilcoxon exact $P$ values (unadjusted $P$) and FDR-adjusted $P$ values are reported in Table S1 in the supplemental material for comparison between groups with bnAb infusion versus those without and in Table S2 for comparisons between groups using Env-only versus MVA/Env immunogens. In addition, comparisons for which the unadjusted $P$ value is $<$0.05 are summarized in Table 1. All statistical tests were performed using SAS, version 9.4 (Cary, NC, USA).

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JVI.01783-18.

**SUPPLEMENTAL FILE 1**, PDF file, 0.1 MB.

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data interpretation, and contributed to manuscript preparation; A.S.M. contributed to data generation and visualization and data interpretation; A.D.C assisted in data interpretation and immunogen selection; K.D.C. contributed to data generation and interpretation; P.T.S. and M.G.H. performed statistical analysis and helped with data interpretation; M.G.H. also provided critical reviews of the manuscript; G.G.F., G.F., M.A., and K.D.P. contributed to vaccine study design and data interpretation, and K.D.P. also provided critical reviews of the manuscript; K.K.A.V.R. oversaw the animal study, contributed to data interpretation, and provided critical reviews of the manuscript; S.P. led in study design, oversaw the entire study, contributed to data analysis and interpretation, coordinated manuscript preparation, and made significant contributions to the writing; X.S. participated in study design, contributed data for the study, helped with data visualization and interpretation, and led in manuscript preparation.

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