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Infectious Virion Capture by HIV-1 gp120-Specific IgG from RV144 Vaccinees

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The detailed examination of the antibody repertoire from RV144 provides a unique template for understanding potentially protective antibody functions. Some potential immune correlates of protection were untested in the correlates analyses due to inherent assay limitations, as well as the need to keep the correlates analysis focused on a limited number of endpoints to achieve statistical power. In an RV144 pilot study, we determined that RV144 vaccination elicited antibodies that could bind infectious virions (including the vaccine strains HIV-1 CM244 and HIV-1 MN and an HIV-1 strain expressing transmitted/founder Env, B.WITO.c). Among vaccinees with the highest IgG binding antibody profile, the majority (78%) captured the infectious vaccine strain virus (CM244), while a smaller proportion of vaccinees (26%) captured HIV-1 transmitted/founder Env virus. We demonstrated that vaccine-elicited HIV-1 gp120 antibodies of multiple specificities (V3, V2, conformational C1, and gp120 conformational) mediated capture of infectious virions. Although capture of infectious HIV-1 correlated with other humoral immune responses, the extent of variation between these humoral responses and virion capture indicates that virion capture antibodies occupy unique immunological space.

The RV144 vaccine in Thailand, a combination of two vaccines, ALVAC HIV canarypox vector expressing HIV-1 protein vaccine (a four-dose prime) and the AIDSVAX B/E protein vaccine (a two-dose boost), provided 31% protection against heterosexual HIV-1 infection (1). Since the standard for humoral responses by an HIV-1 vaccine of tier II neutralization (2) was not met, despite vaccine efficacy (3), effort has focused on understanding the functional attributes of specific but non-neutralizing antibodies. Analysis of the RV144 correlates studies indicate that binding antibody responses contributed to the protective efficacy in RV144: (i) HIV-1 Env V1/V2 IgG correlated with decreased infection risk, and (ii) high levels of anti-HIV-1 Env plasma IgA correlated with decreased vaccine efficacy (4). A possibility is that functional antibody responses not specifically measured in the correlates analysis may have contributed to protection. Thus, further examination of the functional attributes of RV144 vaccine elicited antibodies will provide a comprehensive understanding of the breadth of antibody responses elicited by HIV-1 vaccination. Identification and characterization of the non-neutralizing (but virus inhibitory) antibody responses elicited by RV144 provides potential mechanisms for protection by the vaccine-elicited humoral repertoire.

Vaccine elicited antibodies that can block HIV-1 acquisition at mucosal surfaces might be among the most efficacious types of antibodies (5). In addition to traditional HIV-1 neutralization (6–9) and Fc receptor-mediated antibody inhibition (10), including antibody-dependent cellular cytotoxicity (ADCC) (11, 12), antibody-dependent cellular viral inhibition (ADCVI) (13), and phagocytosis, antibodies may also aggregate virions (14), potentially inhibit movement through cervical mucus (15, 16), inhibit transcytosis (17–19), mediate intraepithelial neutralization, block HIV-1 Env gp120 interaction with $\alpha_4\beta_7$ integrin on $CD4^+$ target cells (20), and inhibit macrophage infection (7, 21). The ability of HIV-1 specific IgG to bind HIV-1 viral particles, especially infectious functional virions, is likely a prerequisite of many biological activities of antiviral antibodies. *In vivo*, the ability of non-neutralizing antibody to capture infectious SHIV may have been related to the protection against mucosal challenge in the macaque model (22).

In the present study, we characterized the magnitude and breadth of IgG antibodies induced by RV144 and determined the ability and breadth of RV144-induced IgG to bind the infectious virus particles. RV144 induced HIV-1-specific antibodies capable of binding Env gp140 of multiple strains with a wide range in binding magnitude. In addition to the capacity to bind heterologous HIV-1 envelope proteins, we tested the ability of purified IgG from vaccinee plasma for binding to whole infectious functional HIV-1 virions. We found that plasma IgG from RV144 vaccinees was capable of capturing multiple strains of infectious HIV-1, including the vaccine strains HIV-1 CM244 and MN, and HIV-1 expressing Env from transmitted/founder virus, B.WITO.c. Only

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a subset of vaccinee plasma IgG was capable of breadth of virion capture, a finding indicative of the heterogeneous response to the RV144 vaccine. Using protein adsorption and monoclonal antibodies (MAbs) of defined specificities, we identified that the capture of infectious virions was specifically directed to multiple epitopes in gp120 Env and that envelope binding and virion capture were distinct measurements. The finding that virion capture was not tightly correlated with envelope binding assays, neutralization, and ADCC suggests that this functional attribute of vaccine-elicited antibodies may represent distinct immunological space. Further studies of virion capture as an immunological mechanism of protection in nonhuman primate and human clinical studies is warranted to help decipher potentially protective antibody mechanisms.

MATERIALS AND METHODS

Clinical trials. Participants in RV144 (1) received vCP1521 (expressing Gag and protease (MN subtype B) and gp120 (92TH023 (CRF01_AE) linked to the transmembrane portion of subtype B gp41 without the gp41 ectodomain (Sanofi Pasteur)) at 0, 1, 3, and 6 months with the coadministration of AIDSVAX B/E gp120 (MN gp120 and CRF01_AE CM244 [A244]) (Global Solutions for Infectious Diseases) (23) at 3 and 6 months. RV144 was conducted in accordance with the Declaration of Helsinki and local Institutional Review Board requirements. Written informed consent was obtained from all subjects. In the present study, plasma from RV144 vaccinees (1), as part of pilot set A, were examined at weeks 0 and 26 (2 weeks after the last vaccination).

Cells and replication-competent reporter viruses. The 5.25.EGFP.Luc.M7 (M7-Luc) (provided by N. R. Landau, NYU Langone Medical Center, New York, NY) is a CEMx174 cell clone that was produced by retroviral vector transduction to express CCR5 (CD4 and CXCR4 are expressed naturally) and transfection to contain Tat-responsive luciferase (Luc) and green fluorescence protein (GFP) reporter genes. The cells were grown in RPMI 1640, supplemented with 12% fetal bovine serum (FBS), 50 μg of Geneticin (G418)/ml, 0.5 μg of puromycin/ml, 1% penicillin-streptomycin, and 200 μg of hygromycin/ml (24, 25). A3R5 cells, a human T-cell leukemia cell line (a subline of A3.01 expressing both CCR5 and CXCR4 in addition to CD4) generated by Jerome Kim, Walter Reed) was grown in RPMI 1640 plus 10% FBS and 1 mg of G418/ml. Wild-type HIV-1 $_{MN}$ was amplified by the human H9 cell line. Replication-competent virus stocks from infectious molecular clones (IMCs) of *Renilla* luciferase (LucR) reporter viruses (designated NL-LucR.T2A-Env.ecto) (26–28) expressing envelope regions from the lab-adapted NL4-3, CRF01_AE 92TH023 (subtype A/E) or transmitted/founder viruses (B.WITO.c [29]) were generated as described previously (26–28, 30). IMCs of *Renilla* luciferase (LucR) reporter viruses subtype A/E transmitted/founder 427299 were made using a backbone derived from CRF01_AE strain CM235 (3). Briefly, proviral DNA was transfected into 293T cells by Fugene HD (Roche). Working stocks of IMCs and CM244 (31, 32) were amplified by passaging virus in peripheral blood mononuclear cells (PBMCs). Virus supernatants were collected every 2 to 3 days and filtered through a 0.45-μm-pore-size syringe filter, and titers were determined on TZM-bl cells.

HIV-1 specific binding antibody assay. Plasma HIV-1 specific antibodies were measured by a custom HIV-1 binding antibody multiplex assay as previously described (33). HIV-specific antibody isotypes were detected with mouse anti-human IgG (Southern Biotech, Birmingham, AL), conjugated to phycoerythrin, at 4 μg/ml. Antibody measurements were acquired on a Bio-Plex instrument (Bio-Rad, Hercules, CA), and the readout is expressed in MFI or μg/ml equivalents based on an HIVIG (Polymune Scientific, Vienna, Austria) standard curve for gp120 IgG detection (5-PL curve fitting using 21CFR Part 11 compliant software). All assays were run under Good Clinical Laboratory Practices (GCLP)-compliant conditions, including the tracking of positive controls by Levy-Jennings charts. Positivity criteria for antibody-antigen pairs were predetermined by using a set of plasmas from 30 seronegative subjects (mean MFI + 3 standard deviations). Envelope proteins examined from multiple HIV-1 clades included the following: subtype B JRFLgp140 and US1 gp140, subtype A or AE Env HVI3700AEconenv03140CF, 97CNG2F140CF, and clade A 00MSA4076gp140, subtype G HV14000DRCBLgp140, as well as various subtypes recombinant consensus Env Con6 gp120B, ConS gp140CFI, Bconenv03140CF, Gconenv03 140CF, and A1 conenv03140CF (all provided by H.-X. Liao and B. F. Haynes, Duke University, as previously described [4]).

Magnitude and breadth score. The calculated magnitude and breadth score is a weighted average of binding to 14 recombinant gp120 and gp140 Env proteins measured by a binding antibody multiplex assay, Luminex, that included A244 gD-293T gp120, 92TH023 gD-293T gp120, 00MSA4076 gp140, 97CNGX2F gp140CF, A1.con.env03.gp140CF, B.con.env03 gp140CF, C.con.env03 gp140CF, Con6 gp120, ConSgp140CFI, G.con.env03 gp140CF, AE.con.env03 gp140CF, DRCBL gp140, JRFL gp140, and US1SIVcpz gp140 that were produced as described previously (4). Briefly, for each Env, four fluorescence intensity readouts were measured: (i) week 0 binding to blank bead, denoted by y00; (ii) week 0 binding to Env-coated bead, denoted by y01; (iii) week 26 binding to blank bread, denoted by y260; and (iv) week 26 binding to Env-coated bead, denoted by y261. A sample was considered positive if both y261 − y260 was >100 and (y261 − y260)/(y01 − y00) was >3. The binding activity to each Env was defined as 0 if it failed the positivity call and as the log[max(y261 − y260, 0) + 1] otherwise. To compute the weights for the 14 Envs, a bottom-up hierarchical clustering tree with average linkage for all Env but A244 gD-293T gp120, 92TH023 gD-293T gp120, and US1gp140 was first constructed based on a distance measured as 1 minus the Spearman correlation coefficient between pairs of binding activities. Relative weights were computed based on the tree using the Gerstein-Sonnhammer-Chothia method. Average weighting across 10 bootstrap samples (within vaccine recipients) was taken. Second, US1SIVcpz gp140 was given the same relative weight as the highest relative weight among the 11 nonvaccine strain Envs. Together, the 12 nonvaccine strain Envs were assigned two-thirds of the total weight. Finally, A244 gD-293T gp120 and 92TH023 gD-293T gp120 were each assigned one-sixth of the total weight. The weights were selected to give greatest influence to the two vaccine Envs (MN.B and A244.AE) and to the nonvaccine Envs that were least redundant based on cluster analysis (4).

gp120-specific MAbs. CH21, CH22, CH40, CH81, and CH90 MAbs were isolated from antigen-specific memory-B-cell sorting as previously described (3, 34, 35). Briefly, group M consensus ConS gp140 Env was labeled with Pacific Blue and Alexa Fluor 647 using fluorochrome labeling kits (Invitrogen, Carlsbad, CA). Thawed PBMCs were stained as described previously (35), and memory B cells stained with ConS gp140 in both colors were sorted as single cells. HG107 MAb was isolated from memory B cells cultured at nearly clonal dilution, as previously described (36, 37). Immunoglobulin genes were recovered as described previously (38, 39). Gene analysis was performed as described previously (40), and then isolated immunoglobulin V(D)J gene pairs were assembled by PCR into linear full-length immunoglobulin heavy- and light-chain gene expression cassettes and expressed in 293T cells (American Type Culture Collection, Manassas, VA) and purified for use in the virus capture assay (40).

Plasma IgG purification. IgG was isolated from plasma using MultiTrap 96-well plates (GE Healthcare, Inc.) as described previously (25). Briefly, plasma was 2-fold diluted with Tris-buffered saline (TBS; pH 7.5), and 200 μl of diluted plasma was incubated in the plates for 1 h. Unbound fractions were removed by centrifugation at 1,500 × *g* for 3 min. Wells were then washed three times with 200 μl of TBS. IgG was eluted with 2.5% glacial acetic acid and immediately neutralized with 120 μl of 1 M Tris-HCl (pH 9.0). The eluted IgG fractions were concentrated and desalted using Amicon Ultra centrifugal filters (Millipore) with a 30K cutoff at 14,000 × *g* for 12 min. The IgG was then concentrated to the desired

volume and assayed for protein concentration using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific).

Microplate infectious virion capture assay. Infectious virion capture assay was performed as previously described (25, 33). Briefly, microplates (Nunc) were coated overnight at 4°C with mouse monoclonal anti-human IgG (Southern Biotech) at a concentration of 1 μg/ml diluted in phosphate-buffered saline (PBS). After coating and washing, coated plates were blocked for 2 h with PBS supplemented with 5% goat serum, 5% milk, and 0.05% Tween. The indicated concentrations of antibodies were mixed with the viral stock containing 1.5×10^7 viral RNA (measured by reverse transcription-PCR) and then centrifuged for 90 min at 2,000 rpm. The mixture was then centrifuged at 21,000 × *g* for 1 h at 4°C to remove the virus-free antibody (41), and the pellet was resuspended in the same volume of PBS. Next, 50 μl of the immune complexes (IC) mixture was added to each coated well in triple wells for a 90-min incubation. The wells were washed four times, and the susceptible target cells (M7-Luc indicator cells or A3R5 cells [for virus T/F B.WITO.c]) in 200 μl were added. HIV-1 infection was assessed on day 7 after infection for M7-Luc and on day 6 for A3R5 cells by luciferase assays. For the M7-Luc firefly luciferase assay, 100 μl of supernatant was removed, and 100 μl of Britelite (Perkin-Elmer) was added to each well. After a 2-min incubation, the 150-μl lysate was used to measure HIV-1 infection as expressed in relative luciferase units (RLU) (42). For the A3R5 *Renilla* luciferase assay, the luciferase expression of infected cells was measured with a *Renilla* luciferase assay kit (Promega) and read on a Berthold E&G luminometer using MicroWin software. Polyclonal HIV immunoglobulin (HIVIG) obtained from the NIH AIDS Research and Reference Reagent Program, the HIV-1 broadly neutralizing antibody 2G12 MAb (Polymune Scientific), and the gp41 immunodominant non-neutralizing 7B2 MAb (from James Robinson, Tulane University) were utilized as positive controls. The negative controls, G8 MAb (an IgG MAb derived from an Epstein-Barr virus-transformed B cell, provided by Kwan-Ki Hwang, Duke University), normal human serum (Sigma), and a virus-only control, were utilized to establish the positivity cutoff.

Protein G infectious virion capture assay. Monoclonal IgG was mixed with a stock containing 2×10^7 RNA copies of HIV-1/ml at a final concentration of 10 μg/ml (300 μl). The IgG and virion IC mixture were prepared *in vitro* and absorbed by a protein G MultiTrap 96-well plate as previously described (25). The infectious virus in the flowthrough fraction was measured by infecting TZM-bl with 25 μl of flowthrough. Each sample was run in triplicate. TZM-bl infection (RLU readout) was measured by a firefly luciferase assay at 48 h postinfection as described previously (25). V1/V2-directed quaternary broadly neutralizing MAbs PG9 and PG16 were kindly provided by D. Burton (Scripps Institute, La Jolla, CA, and IAVI, New York, NY) (43). The percentage of captured infectious virion was calculated as follows: [(100 − flowthrough infectivity)/virus-only infectivity] × 100. The cutoff is 14% based on the mean of negative controls ± the standard error of the mean (SEM).

Neutralizing antibody assays. Neutralizing antibody assays were performed as previously described (3).

Antibody adsorption assay. Antibody adsorptions to determine specificity of virion capture were performed as previously described (25). Briefly, purified plasma IgG (2.4 μg total) was incubated with 0.36×10^6 carboxylated fluorescent beads (Luminex Corp, Austin, TX) conjugated with 1.5 μg of HIV-1_{MN} gp41 (Immunodiagnostics, Woburn, MA), HIV-1_{MN} gp120 protein, herpes simplex virus gD peptide kgD27.3 (4), or a blank bead for 1.5 h. After incubation for 1.5 h, the beads were centrifuged for 2 min at 8,000 × *g*, and the supernatant was harvested. The absorption was repeated twice more. The supernatant was used to make an IgG-virion IC mixture *in vitro*, and the captured infectious IgG-virion IC by coated anti-human IgG were measured by an M7-luc assay, as described above. The HIV-1 specific IgG depletion efficiency of absorbed supernatant was confirmed to be significantly depleted of HIV-1-specific antibodies by a quantitative HIV-1-specific binding antibody assay as described above.

ADCC. ADCC activities in purified IgG were measured by using an adapted GranToxiLux (OncoImmunin, Inc.) protocol as described by Pollara et al. (44).

Statistical analysis. The statistical nonparametric correlation between the mean values of the HIV-1 IgG-virion IC of purified plasma IgG and the other HIV-1 antibody function activities of purified plasma IgG were determined by an unpaired two-tailed Spearman test. Differences were considered significant if the *P* value was ≤0.05. All statistical analyses were exploratory in nature and were performed using SAS v9.2 (SAS Institute, Cary, NC) or GraphPad Prism (GraphPad Software, Inc.).

RESULTS

Broad dynamic range of HIV-1 Env IgG binding magnitude and breadth in RV144. In order to quantify the quality of HIV-1 specific IgG induced by RV144 prime-boost regimen, we calculated the breadth score of HIV-1 specific IgG based on the binding magnitude of 14 recombinant HIV-1 Env (2 vaccine strains Env 92TH023 gD−293T gp120 and A244 gD−293T gp120; 12 non-vaccine strain Envs representing clades A, AE, B, C and G) as described previously (4). RV144 vaccine-elicited IgG antibodies bound both multiple subtype primary isolate Envs and recombinant consensus Envs; however, vaccinees had divergent magnitudes and specificities of binding when assayed against a panel of Env proteins (Fig. 1). The magnitude of the binding response (MFI log_e) was broadly distributed among the vaccinees, with only a fraction of vaccinees binding broadly at high levels to the majority of envelope proteins. This different magnitude and breadth profile for each vaccinee demonstrates the heterogeneity in the vaccine-elicited antibody response by RV144.

RV144 elicited HIV-1 virion capture antibodies. To measure the ability of HIV-1 specific IgG elicited by RV144 vaccination to bind to infectious virions, IgG was purified from 42 vaccinees that were among those with HIV-1 binding antibody responses to MN gp120, A244 gD− gp120, and ConSgp140. Total IgG antibody was purified from each vaccinee at visit 1 (prevaccination, week 0) and visit 8 (week 26, 2 weeks after the last gp120 boost). To address the relative heterologous nature of virion binding breadth, four different HIV-1 viruses were tested: the two vaccine strains B.MN and AE.CM244, the laboratory-adapted clade B NL4-3, and HIV-1 expressing transmitted/founder Env from B.WITO.c. Three positive controls (2G12 MAb [broadly neutralizing], 7B2 MAb [non-neutralizing], and HIVIG [purified IgG from HIV-1^+ subjects]) that efficiently captured all four infectious HIV-1 strains were run in each assay (Fig. 2A). Among these RV144 vaccinees selected for high Env binding, notably the majority were capable of capturing vaccine strain HIV-1 virions, with 64% (27/42) and 78% (18/23) capable of capturing the vaccine strains MN and CM244, respectively (Fig. 2B). Approximately 48% of the RV144 vaccinees tested captured the laboratory-adapted strain NL4-3. A smaller number of vaccinees (26%) had vaccine-elicited IgG that captured virions with the transmitted/founder Env from B.WITO.c. For each sample tested, the corresponding purified IgG from prevaccination (visit 1) was below the preset cutoff and did not bind infectious virions. Similar to the binding antibody breadth results, the responses among vaccinees for virion capture were heterogeneous both in terms of the magnitude of capture among the vaccinees for a given virus (greater than one log) and in terms of the viruses captured. These results indicate that RV144 elicited anti-Env IgG capable of binding infectious virions; however, the range of responses among vaccinees was heterogeneous. Notably, the majority of those vaccinees tested (78%) had vaccine-

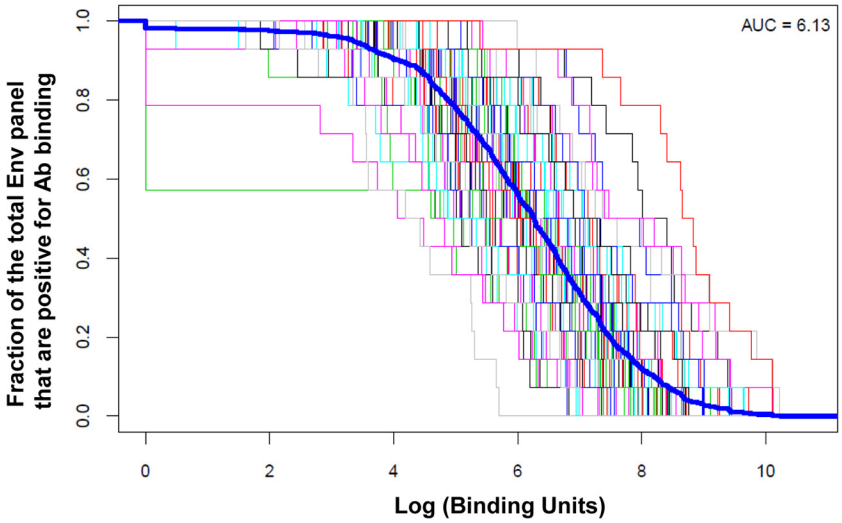

FIG 1 Heterogeneity of vaccine elicited IgG anti-Env breadth. The magnitude and breadth of plasma IgG against a panel of cross-clade envelopes representing the vaccine strains (MN and A244), clades A (clade A consensus, primary clade A, VRC clade A), AE (clade AE consensus), B (clade B consensus, JRFL, VRC clade B), C (clade C consensus), and G (primary clade G, clade G consensus), and group M consensus are shown. Plasma IgG Env binding (log_e MFI) is shown on the *x* axis; the fraction of participants with positive IgG Env binding is shown on the *y* axis. Each line indicates one participant in RV144 pilot set A ($n = 100$).

elicited plasma IgG that captured AE.CM244 virus, which is both the vaccine strain and the clade matched (AE) to circulating strains in Thailand.

Infectious virion capture is mediated by anti-gp120 antibodies of multiple specificities. To determine the specificity of virion capture antibodies from RV144 vaccinees, HIV-1 Env gp120 antibodies were preadsorbed from plasma and then tested for virion capture (Fig. 2C). Anti-gp120 IgG from total purified plasma IgG from three different vaccinees were depleted by HIV-1 Env gp120-conjugated microsphere beads. After depletion of Env gp120 antibodies, infectious virion capture (Fig. 2C and D) was abrogated. In contrast, purified IgG depleted by gp41-conjugated beads (negative control) or blank beads (negative control) still maintained the ability to capture infectious MN virions (Fig. 2C and D).

In the RV144 ALVAC-HIV and AIDSVAX B/E vaccine regimen, AIDSVAX B/E protein HIV-1 Env gp120 A244 and MN contains a gD peptide at N-terminal from herpes simplex virus (4). We found that AIDSVAX induced high anti-gD IgG responses and that anti-gD IgG was present in 100% of RV144 vaccinee plasmas at visit 8 (4). To exclude the possibility of nonspecific virion capture at the key immunogenicity time point, we tested infectious virion capture after depletion of the anti-gD IgG using gD peptide-conjugated beads. After three rounds of depletion, 99% of the anti-gD IgG was removed. The depletion of anti-gD IgG did not affect the ability of plasma IgG to capture HIV-1 virions (Fig. 2D). Taken together, these results demonstrate that anti-gp120 IgG was responsible for the capture of infectious virions in RV144 vaccinee plasma.

To determine the epitope specificity of vaccine-elicited virion capture antibodies, we examined MAbs generated from RV144/RV135 vaccinees representing a range of specificities elicited by vaccination: C1 conformational, V3 and V2 specific (Fig. 2E and Table 1). All three gp120 MAbs captured NL4-3 virus, as well as at least one vaccine strain (MN or CM244 virus). The linear V3-specific MAb from RV144 (CH22) and the C1 conformational MAb from RV144 (CH40) captured HIV-1 NL4-3 and CM244 viruses. CH21, a conformational gp120 MAb, captured HIV-1 NL4-3, MN, and B.WITO.c viruses. We determined that, in addition to CH21, CH22, and CH40, two other C1 conformational MAbs generated from RV144 vaccinees (CH81 and CH90) mediated virion capture. The C1 conformational specific antibody (CH81 MAb) mediated low-level infectious virion capture. We compared the gp120 binding, ADCC, and neutralization activities of all of these MAbs (Table 1). CH22 MAb, targeting gp120 V3 linear epitope, did not mediate ADCC, did not neutralize CM244 virus but it captured infectious virions at low levels. CH40 MAb both captured a low level of CM244 virions and mediated robust ADCC but did not neutralize CM244. In contrast, CH81 and CH90 MAbs mediated ADCC and virus capture (only CH81), but neither bound to matched Env gp120 or neutralized CM244 (Table 1) (34). These data demonstrate that C1 conformational antibodies, V3- and V2-specific antibodies elicited by vaccination, could capture virions, albeit at different levels with V2-specific antibodies capturing weakly (Table 1) (37).

IgG-mediated infectious virion capture represents an overlapping but distinct immunological space other than binding breadth, ADCC, and neutralization. To examine whether virion capture represents an antiviral function distinct from or overlapping with binding, neutralization, and ADCC, we examined correlations among these humoral measurements in the same subset of subjects in the RV144 pilot study (Table 2). The breadth score of HIV-1 plasma IgG weakly correlated with virus capture of the two vaccine strains MN ($r = 0.35$, $P < 0.05$) and CM244 ($r = 0.43$, $P < 0.05$) and did not correlate with NL4-3 virion capture or with the capture of virions bearing the transmitted/founder Env from B.WITO.c (Table 2 and data not shown). The magnitude of IgG binding against the vaccine strain MN correlated with the matched HIV-1 MN viral capture ($r = 0.52$, $P < 0.001$); however, binding to the other vaccine HIV-1 strain, CM244, did not correlate. There was a correlation between the HIV-1 virus capture of vaccine strains MN and the IgG binding against ConS gp140 ($r = 0.41$, $P < 0.01$). In addition, the virion capture of vaccine strains

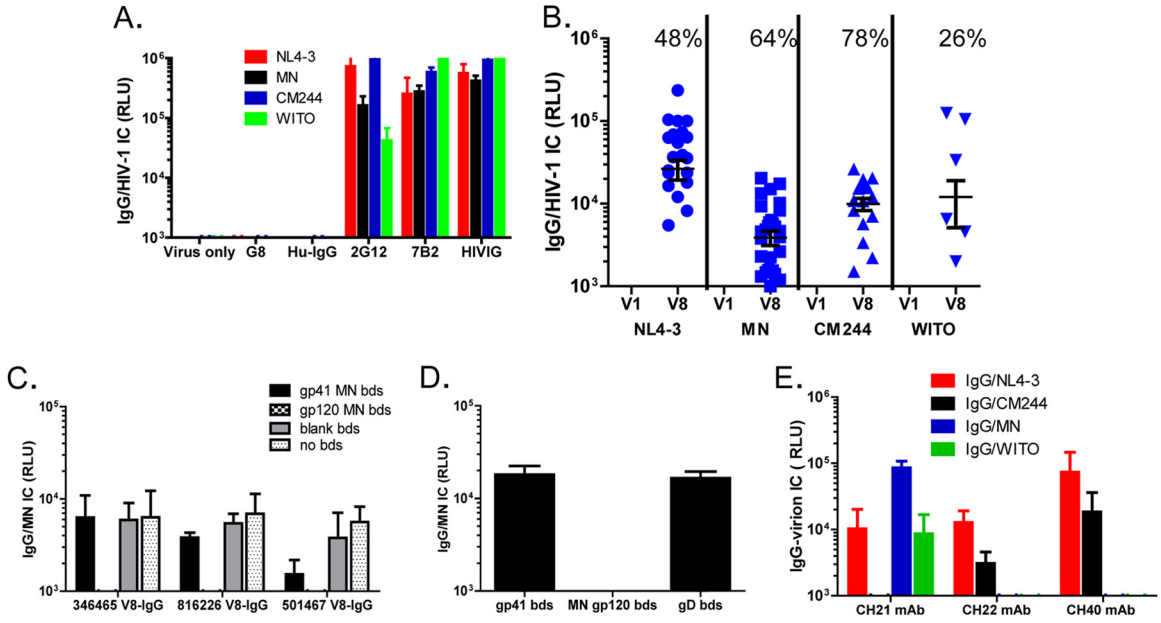

FIG 2 RV144 vaccination elicited antibodies of multiple gp120 specificities capable of infectious HIV-1 virion capture (including clade AE virus). Purified plasma IgG at visit 1 (prevaccination) and visit 8 (2 weeks after the last vaccination) from 23 (CM244 or WITO) and 42 (NL4-3 and MN) vaccinees with detection of HIV-1 Env binding antibody responses were selected and measured in infectious HIV-1 capture assays. (A) Assay controls. Positive controls 2G12 MAb (broadly neutralizing), 7B2 MAb (non-neutralizing), and HIVIG (purified IgG from HIV-1$^+$ subjects) captured all four viruses tested. Negative controls G8 MAb, purified HIV-1 negative human IgG (Hu-IgG), and the virus alone were run in each assay (positivity criteria, RLU ≥ 1,000). All data are mean RLU of triple wells + the SEM. (B) Purified IgG from vaccinees at the baseline (V1) and key immunogenicity visit (V8) were tested for infectious virion capture. The percentages of tested vaccinees that had vaccine-elicited HIV-specific antibodies that could capture infectious virions were 48% (20/42) NL4-3, 64% (27/42), and 78% (18/23) for vaccine strains MN and CM244, respectively. The transmitted/founder WITO was captured by 26% (6/23) vaccinees. (C) The capture of infectious HIV-1 was mediated by HIV-1 gp120 IgG. Purified plasma IgG at visit 8 from three RV144 vaccinees was adsorbed by Env gp41 or Env gp120 conjugated or blank microsphere beads and tested for virion capture. (D) Purified plasma IgG at visit 8 from RV144 vaccinee 501467 was adsorbed by Env gp41, Env gp120, or gD peptide-conjugated or blank microsphere beads prior to the virion capture assay. (E) Env gp120 MAbs from RV144 vaccinees capture multiple strains of HIV-1 virions. Anti-gp120 MAbs (10 μg/ml), generated from RV144 vaccinees, with different specificities (CH21 is a conformational gp120 MAb, CH22 is a linear V3 MAb, and CH40 is conformational C1 MAb [A32 blockable]), captured infectious virions. The data represent three independent experiments and are reported as mean RLU of triplicate + the SEM.

MN and CM244 correlated with the binding antibody avidity to Env ConS gp140 but not to Env gp120 MN or the A244 avidity score (Table 2). Virion capture of the vaccine strain MN significantly correlated with ADCC activity to HIV-1 A/E CM235 Env ($r = 0.52$, $P < 0.001$) and the plasma neutralization titer to MN ($r = 0.54$, $P < 0.001$). However, there were no significant correlations of neutralization or ADCC with virion capture of NL4-3 or CM244. Furthermore, in vaccinees measured for the capture of T/F B.WITO.c Env, there was no correlation of virus capture with binding breadth, ADCC, or neutralization. Taken together, these data indicate that the measurement of HIV-1 infectious virion capture of different HIV-1 strains is not represented by the analysis of anti-Env protein binding alone or other functional assays (i.e., neutralization or ADCC).

TABLE 1 Infectious virion capture is distinct from Env binding, ADCC, and neutralization

MAb	Antibody specificity (reference)	HIV-1 AE.CM244 gp120 binding[a]	HIV-1 AE.CM244 IgG-virion IC (RLU)[b]	ADCC activity (EC [μg/ml])[c]	CM244 virus neutralization titer (IC_{50} [μg/ml]) (reference)[d]
CH21	gp120 conformational epitope (3)	–	781 (negative)		>50 (3)
CH22	V3 linear epitope	+	3,080		>50 (3)
CH40	C1 conformational antibody; A32-blockable conformational epitope (34)	+	18,482	0.039	>50
CH81	A32-blockable conformational epitope (34)	–	1,213	0.125	>50
CH90	A32-blockable conformational epitope (34, 36)	–	—	1.29	>50
HG107	V1/V2 (37)	++++	—	ND	>50

[a] The Env binding response, expressed as the MFI, was determined using an binding antibody multiplex assay: +, 10^2 to 10^3; ++, 10^3 to 10^4; +++, 10^4 to 2×10^4; and ++++, $>2 \times 10^4$ at 20 or 5 μg/ml.

[b] IgG-virion IC (microplate infectious virus capture). The RLU cutoff is 1,000. —, The infectious viral capture was measured by protein G capture.

[c] ADCC activity against HIV-1 AE.CM235-infected cells, reported as the endpoint concentration (EC) (34). ND, not done. However, HG107 MAb did mediate ADCC against gp120-coated CD4 target cells (37).

[d] The neutralizing activity in TZM-bl cells against HIV-1 A/E CM244 is presented as the IC_{50} (37).

TABLE 2 IgG virion capture measurements overlap but are distinct from other humoral immune responses[a]

Measurement	IgG virion capture					
	MN ($n = 42$)		CM244 ($n = 23$)		NL4-3 ($n = 42$)	
	r	P	r	P	r	P
MN neutralization	**0.54**	<0.001	0.37	0.09	0.144	0.377
Binding breadth	**0.35**	<0.05	**0.43**	<0.05	0.236	0.137
ADCC	**0.52**	<0.001	0.37	0.08	–0.047	0.78
MN Env gp120 binding magnitude	**0.52**	<0.001	0.24	0.27	0.192	0.222
A244 Env gp120 binding magnitude	0.181	0.252	0.34	0.115	0.274	0.079
Env ConS gp140 binding magnitude	**0.4**	<0.01	–0.197	0.369	0.17	0.281
MN Env gp120 avidity score	0.3	0.104	0.365	0.095	–0.27	0.09
Env ConS gp140 avidity score	**0.37**	<0.05	**0.44**	<0.05	0.096	0.55
A244 Env gp120 avidity score	0.125	0.43	0.4	0.066	0.297	0.059

[a] Spearman correlations (r) are shown between HIV-1 Env IgG binding, HIV-1 Env IgG avidity, ADCC or neutralization, and HIV-1 virion capture results. Significant correlations ($P < 0.05$) are indicated in boldface.

Virion capture is a distinct measurement from Env binding. To directly examine whether there was concordance between envelope binding and virion capture, we tested V1/V2 MAbs generated from RV144, i.e., HG107 (37), and broadly neutralizing antibodies that target the variable region V1/V2, i.e., CH01 (36), PG9, and PG16 for infectious virion capture (Fig. 3). PG16 MAb efficiently captured all four different viruses tested—B MN, A/E CM244, 92TH023, and T/F A/E 427299 infectious virions (>90% input)—despite the lack of binding to the matched Env gp120. Another conformational antibody, PG9, showed virion capture similar to that of PG16 but also demonstrated weak gp120 protein binding. CH01 MAb did not bind gp120 MN, 92TH023, and 427299 envelope proteins but demonstrated robust virion capture of MN (75%), 92TH023 (52%), and 427299 (98%) infectious virions. However, CH01 MAb bound both HIV-1 A/E CM244 gp120 protein and captured CM244 virions, demonstrating that there were differences among viruses in the epitope specificities that are exposed on virions compared to Env protein. HG107 MAb, a V2 specific antibody from RV144, bound CM244 gp120 but did not capture CM244 virions. These results indicate that infectious virion capture is a unique property from HIV-1 envelope binding assays.

DISCUSSION

The ALVAC prime, gp120 protein boost vaccine elicited an HIV-1 Env IgG response capable of binding infectious virions, including the vaccine strain virus (AE.CM244) and a virus expressing clade B transmitted/founder Env (from B.WITO.c). The ability of antibodies to capture infectious virions may have multiple consequences, such as direct neutralization, immobilization of virions leading to virus decay, virion aggregation, or binding to virions with targeting to Fc receptors on phagocytes. Our results indicate that the ability of HIV-1 vaccine-elicited antibodies to capture virions is nonredundant with other antibody functions (i.e., ADCC, neutralizing antibodies, and binding breadth); thus, virion capture represents a unique immunological space for vaccine evaluation. Our conclusions are consistent with other studies that have examined correlations between envelope protein binding,

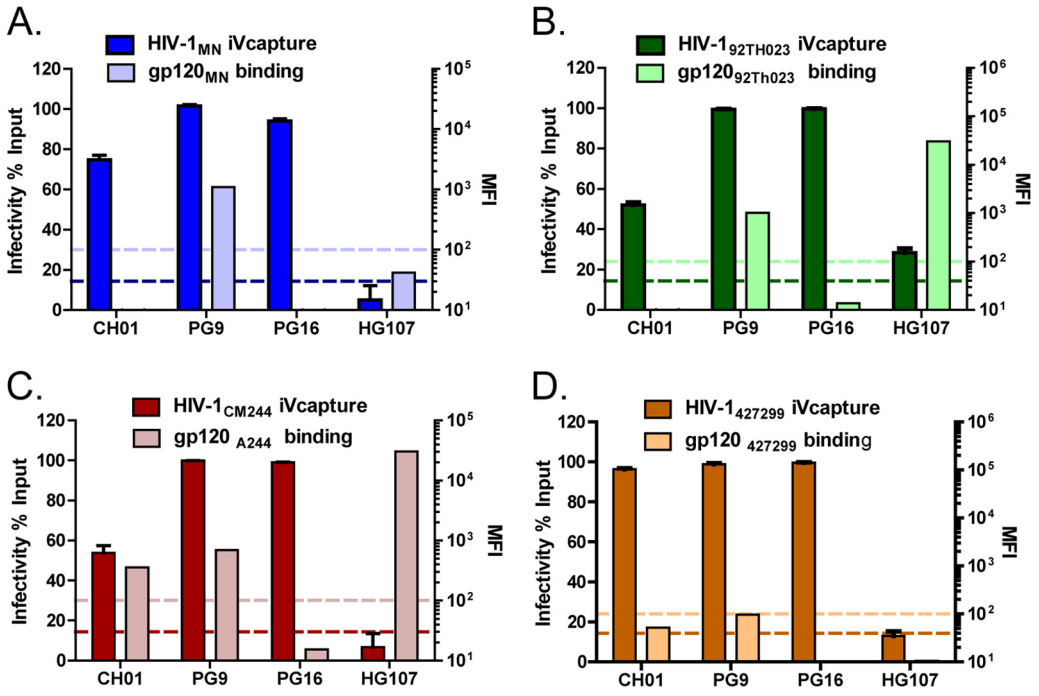

FIG 3 Infectious virion capture is distinct from Env protein binding. HIV-1 virion-IgG immune complexes were absorbed by the protein G column, and the infectivity of the flowthrough fraction was measured (iVcapture) for the vaccine strain boost MN (A), vaccine strain prime 92TH023 (B), vaccine strain boost CM244 (C), and virus and Env generated from an infected RV144 placebo, 427299 (D). HIV-1 Env gp120 binding was determined by an HIV-1 binding antibody multiplex assay. The dashed solid and shaded lines indicate positivity criteria for iVcapture and Env gp120 binding, respectively. The data for each MAb and each virus represent two independent experiments and are reported as the mean + the SEM from triplicate assay measurements.

neutralizing antibodies, and virion capture antibodies (46, 47). In addition to finding that purified IgG from plasma could mediate virion capture, we have shown that gp120 specific MAbs of multiple specificities derived from RV144 vaccinees also could capture virions with a breadth similar to that of plasma antibodies. Thus, the capacity of different HIV-1 vaccine regimens to elicit antibodies that can capture/bind to infectious circulating strains will be important to determine in addition to the evaluation of antibody specificity, neutralization, and ADCC/ADCVI.

The binding antibody repertoire in RV144 consisted of multiple HIV-1 specificities directed to the gp120 envelope (1, 4). By using peptide microarrays (48) to epitope map the gp120 specific responses, four predominant epitope specificities were identified (C1, V2, V3, and C5), with V2 IgG antibodies found to be significantly correlated with decreased risk of infection. We also previously reported (4) that the RV144 vaccine elicited antibodies to conformational epitopes in gp120, specifically for CD4 binding site (CD4BS) binding antibodies and CD4i antibodies as measured using paired gp120 mutant proteins. Moreover, conformational antibodies to the C1 region, as indicated by A32 blocking assays, were reported (4, 34). Thus, diverse linear and conformational specificities of anti-gp120 antibodies were elicited by RV144, in addition to those identified (i.e., V1/V2) as a correlate of infection risk in the correlates analysis. Our results demonstrate that multiple antibody specificities elicited from RV144 vaccination can capture virions. This is consistent with previous studies (46, 49) that concluded that antibodies with different epitope specificities (i.e., V2, V3, and C5 gp120 and gp41) could bind virions, albeit at various levels. Further studies are needed to determine the vaccine-elicited antibody specificities that allow for the greatest magnitude of virion capture antibodies that can target a diverse panel of infectious circulating transmitted/founder virions.

This is the first report characterizing vaccine-elicited virion capture antibodies as part of an HIV-1 vaccine efficacy trial. Although we have previously found that candidate HIV-1 vaccines can elicit Env-specific IgG antibodies that can bind and/or capture infectious virions (50), the epitope specificity of the plasma IgG antibodies that mediate virion capture likely comprise epitopes that can either be expressed just on the surface of infectious virions or also expressed on the surface of infected cells, since some ADCC-mediating antibodies can also capture virions.

A passive infusion study utilizing a non-neutralizing F240 MAb (22) demonstrated that F240 captured infectious virions and hypothesized that this may have played a role in the limited protection that was observed. Whether mucosal antibodies that can capture HIV-1 virions were elicited in RV144 is unknown, since mucosal samples were not collected. However, follow-up studies that have collected mucosal samples (i.e., RV305 and RV306) will be able to address this question.

We have previously reported that antibodies that capture virions during the initial phase of acute HIV-1 infection are gp41 specific (25, 33) and that gp120 specific antibodies that can mediate virion capture arise later in infection (25). Thus, eventually in HIV-1 infection, the antibody repertoire expands beyond non-neutralizing gp41 antibodies (33, 51) to include a polyclonal mix of gp120 plasma antibodies that can mediate ADCC (52) and autologous neutralization (53, 54). RV144 vaccination elicited gp120 antibodies that were most similar to this later stage in acute infection when plasma antibodies with a myriad of functional properties emerge. These early gp120 and gp41 antibody responses in acute infection have typically been described as easy to elicit, unlike broadly neutralizing antibodies that can take 2 to 3 years to develop (55–57). Although the appearance of gp120 antibodies in acute infection is too late to mitigate infection, it is currently unknown whether the presence of vaccine-elicited preexisting gp120-specific virion capture antibodies can impact HIV-1 acquisition. Detailed examination of all of the functional properties of the RV144 vaccine-elicited antibody response may enable a better understanding of how to increase the level of vaccine efficacy.

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