The Journal of Infectious Diseases

MAJOR ARTICLE

# First-in-Human Randomized, Controlled Trial of Mosaic HIV-1 Immunogens Delivered via a Modified Vaccinia Ankara Vector

**Lindsey R. Baden,[1,2,4,a] Stephen R. Walsh,[1,2,3,a] Michael S. Seaman,[2,3] Yehuda Z. Cohen,[2,3,b] Jennifer A. Johnson,[1,2] J. Humberto Licona,[1,2] Rachel D. Filter,[1,b] Jane A. Kleinjan,[1] Jon A. Gothing,[1] Julia Jennings,[3,b] Lauren Peter,[3,b] Joseph Nkolola,[3] Peter Abbink,[3] Erica N. Borducchi,[3] Marinela Kirilova,[3] Kathryn E. Stephenson,[2,3,4] Poonam Pegu,[5,6] Michael A. Eller,[5,6] Hung V. Trinh,[5,6] Mangala Rao,[5] Julie A. Ake,[5] Michal Sarnecki,[10] Steven Nijs,[11] Katleen Callewaert,[11] Hanneke Schuitemaker,[11] Jenny Hendriks,[11] Maria G. Pau,[11] Frank Tomaka,[8] Bette T. Korber,[9] Galit Alter,[2,4] Raphael Dolin,[2,3,4] Patricia L. Earl,[7] Bernard Moss,[7] Nelson L. Michael,[5] Merlin L. Robb,[5,6] and Dan H. Barouch,[2,3,4]; for the IPCAVD006/RV380/HIV-V-A002 Study Group[c]**

[1]Division of Infectious Diseases, Brigham and Women's Hospital, [2]Harvard Medical School, and [3]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, and [4]Ragon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts, [5]US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, and [6]Henry M. Jackson Foundation for the Advancement of Military Medicine, and [7]Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, [8]Janssen Pharmaceutical Research and Development, Titusville, New Jersey, [9]Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, New Mexico, [10]Janssen Vaccines, Bern, Switzerland, and [11]Crucell Holland, Janssen, Johnson & Johnson, Leiden, the Netherlands

***Background.*** Mosaic immunogens are bioinformatically engineered human immunodeficiency virus type 1 (HIV-1) sequences designed to elicit clade-independent coverage against globally circulating HIV-1 strains.

***Methods.*** This phase 1, double-blinded, randomized, placebo-controlled trial enrolled healthy HIV-uninfected adults who received 2 doses of a modified vaccinia Ankara (MVA)–vectored HIV-1 bivalent mosaic immunogen vaccine or placebo on days 0 and 84. Two groups were enrolled: those who were HIV-1 vaccine naive (n = 15) and those who had received an HIV-1 vaccine (Ad26.ENVA.01) 4–6 years earlier (n = 10). We performed prespecified blinded cellular and humoral immunogenicity analyses at days 0, 14, 28, 84, 98, 112, 168, 270, and 365.

***Results.*** All 50 planned vaccinations were administered. Vaccination was safe and generally well tolerated. No vaccine-related serious adverse events occurred. Both cellular and humoral cross-clade immune responses were elicited after 1 or 2 vaccinations in all participants in the HIV-1 vaccine–naive group. Env-specific responses were induced after a single immunization in nearly all subjects who had previously received the prototype Ad26.ENVA.01 vaccine.

***Conclusions.*** No safety concerns were identified, and multiclade HIV-1–specific immune responses were elicited.

***Clinical Trials Registration.*** NCT02218125.

**Keywords.** HIV vaccine; mosaic immunogens; safety; immunogenicity; modified vaccinia Ankara.

Development of a human immunodeficiency virus type 1 (HIV-1) vaccine remains a global priority. Only 4 vaccine concepts have completed evaluation in field trials [1–5], and to date only 1 study has shown a reduction in the risk of HIV-1 infection among vaccine recipients [4]. Vaccine-elicited T-cell responses have not been effective in preventing HIV-1 infection or reducing viremia [1, 3, 5], perhaps because they targeted an insufficient number of epitopes [6] or because they targeted highly variable regions of the viral genome [7]. However, vaccine-elicited HIV-1–specific T-cell responses have been found to exert selective pressure on infecting viruses [8], and an increased breadth of vaccine-induced Gag-specific T cells has been associated with a reduced viral load following infection [9]. Furthermore, in chronic HIV-1 infection, control of viremia correlates with the breadth of $CD8^+$ T-cell recognition of specific Gag epitopes [10–12].

Mosaic immunogens are in silico–derived, full-length recombinant proteins optimized to maximize potential T-cell epitopes and are designed to allow natural expression and antigen processing and presentation [13]. Studies of complementary bivalent or trivalent mosaic HIV-1 immunogens in macaques have shown that mosaic sequences increase the breadth and depth of cellular immune responses, compared with either consensus or natural sequences [14, 15]. This increased magnitude and quality of cellular immune responses could potentially improve the coverage of circulating viruses and help control viremia

Received 31 January 2018; editorial decision 5 April 2018; accepted 10 April 2018; published online April 18, 2018.

Presented in part: HIV Research for Prevention 2016, Chicago, Illinois, 17–21 October 2016. Abstract OA11.01.

[a]L. R. B. and S. R. W. contributed equally to this report.

[b]Present affiliations: Rockefeller University (Y. Z. C.) and Columbia University (R. D. F.), New York, New York; and University of Massachusetts, Worcester (J. J.).

[c]Members of the study group are listed at the end of the text.

Correspondence: S. R. Walsh, MDCM, Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Three Blackfan Cir, Boston, MA 02115 (srwalsh@bidmc.harvard.edu).

**The Journal of Infectious Diseases® 2018;218:633–44**



DOI: 10.1093/infdis/jiy212

by preventing the in vivo evolution of variant escape viruses. Recent evidence from the nonhuman primate/simian immunodeficiency virus (SIV) model supports the association of an increased magnitude and breadth of vaccine-elicited cellular responses with viremic control, using conventional SIV immunogens [16]. Furthermore, macaques vaccinated with HIV mosaic Gag/Pol/Env were able to inhibit acquisition of infection in a macaque SIV/HIV (SHIV) heterologous challenge study, and the vaccinated animals had increased survival rates and reduced viral loads [17].

In this study, we report the first-in-human safety and immunogenicity evaluation of a mosaic HIV-1 vaccine that uses modified vaccinia Ankara (MVA) as a delivery system and evaluate the capacity of this novel immunogen to induce cross-clade immune responses.

## METHODS

### Vaccine

The vaccine used in this study was an MVA-vectored HIV-1 bivalent mosaic immunogen vaccine (MVA.mos1 and MVA.mos2; MVA Mosaic), which delivered 2 different but complementary HIV-1 *gag/pol/env* inserts [14] and was administered at a total dose of $1\times10^8$ plaque-forming units. The MVA mosaic vaccine was provided by the US Military HIV Research Program and Janssen was responsible for the investigational new drug application. The placebo was 0.9% sodium chloride, USP.

### Participants and Study Design

This study was a single center, randomized, double-blinded, placebo-controlled trial to evaluate safety and immunogenicity of a single dose regimen of MVA Mosaic vaccine administered at a total dose of $1\times10^8$ plaque-forming units/mL at weeks 0 and 12, an interval that has been used in other HIV-1 vaccine studies [18, 19]. Study subjects were healthy volunteers at low risk for acquiring HIV, based on standard criteria [20]. The protocol was approved by the institutional review board and biosafety committee, and written informed consent was obtained from each subject. The study was registered at ClinicalTrials.gov (NCT02218125).

Two groups of subjects were enrolled: those who were naive to a prior HIV-1 vaccine (n = 15) and those who had received 2 or 3 doses of a prototype HIV-1 vaccine (Ad26.ENVA.01) containing only an EnvA insert (clinical trials registration NCT00618605) [21] between 4 and 6 years earlier (n = 10). Within each group, subjects were randomized to receive MVA Mosaic vaccine or placebo at a 4:1 ratio. Therefore, among naive subjects, 12 received MVA Mosaic vaccine (group 1), and 3 received placebo; and among past recipients of Ad26.ENVA.01, 8 received MVA Mosaic vaccine (group 2), and 2 received placebo. Injections were given on days 0 and 84. All vaccines were given by needle and syringe (0.5 mL) in the deltoid muscle, preferentially in the subjects' nondominant arm. Systematic safety assessments were conducted and are described in the Supplementary Materials.

### Immunogenicity Studies

Immunogenicity assessments were performed on samples collected on days 0, 14, 28, 84, 98, 112, 168, 270, and 365. All immunogenicity assays were performed in a blinded fashion according to good clinical laboratory practices and are described in detail in the Supplementary Materials. Interferon γ (IFN-γ) enzyme-linked immunospot (ELISPOT) assays were performed to assess HIV-specific cellular immune responses, using pools of overlapping peptides [22] derived from Gag, Pol, and Env and corresponding to the Mosaic 1 and Mosaic 2 sequences, as well as the potential T-cell epitope Gag/Pol/Env sets [23] and EnvA. Intracellular cytokine staining for IFN-γ and interleukin 2 (IL-2) was performed as described elsewhere [24, 25], following stimulation with peptide pools corresponding to Gag (Mos1 and Mos2) and Env (potential T-cell epitope). Direct enzyme-linked immunosorbent assays (ELISAs) were performed with sera to assess HIV-specific binding antibodies against clade A (92UG037), clade B (UK7LN), clade C (C97ZA.012), and Mos1 gp140 trimers [22]. The HIV-1 Env–specific neutralizing antibody titer was quantified by the TZM-bl assay [26]. Antibodies binding to Env V2 and V3 loops were determined by surface plasmon resonance in quadruplicate as described previously [27], with modifications. Antibody-dependent cellular phagocytosis was measured with beads coated with Env from HIV-1 clades A, B, and C, using the THP-1 phagocytosis assay as described previously [28, 29]. Luciferase-based MVA neutralization assays were performed to assess vector-specific neutralizing antibodies as previously described [30]. Ad26-specific neutralizing antibodies were quantified in participants who had previously received Ad26.ENVA.01, using a luciferase-based assay as described elsewhere [31].

Criteria for positive MVA neutralizing antibody responses were titers of >20 and those for Env ELISA responses were titers of ≥100; antibody responses below the lower limit of quantitation of the assay were imputed to half the numeric value of the lower limit of quantitation. Criteria for ELISPOT assay positivity were ≥55 spot-forming cells (SFCs) per million peripheral blood mononuclear cells (PBMCs) and at least 3 times greater than the background value; ELISPOT responses that were <10 following subtraction of medium-only responses were considered baseline values and imputed as a response of 10.

### Statistical Methods

All analyses are based on the intent-to-treat principle; all subjects are evaluated as members of the group to which they were randomized. Summaries of responses are presented as median titers, for the MVA neutralizing antibody test and HIV-1 ELISA data, and as median numbers of SFCs, for the HIV-1 ELISPOT data. Differences in proportions were tested with 2-sided

Fisher exact tests. Kruskal-Wallis nonparametric analysis of variance was used to test for differences among the groups. When a significant overall difference in the result yielded by the Kruskal-Wallis test was identified at a given time point, pairwise tests of all possible treatment pairs were performed using the Mann-Whitney-Wilcoxon nonparametric test. Paired data were assessed using the Wilcoxon signed rank test. Tests with a 2-sided *P* value of <.05 were considered statistically significant. As this was a phase 1 study, no adjustments were made for multiple comparisons.

## RESULTS

### Subject Characteristics

Of the 25 subjects enrolled (Supplementary Figure 1), the sex of 20 (80%) was female, the median age was 24 years (range, 19–49 years), and the race/ethnicity of 1 (4%) was African American, of 4 (16%) was Asian, and of 19 (76%) was non-Hispanic white. Overall retention was 99% (296 of 300 planned visits completed), and 50 of 50 planned vaccinations (100%) were given.

### Safety and Tolerability

The MVA Mosaic vaccine was safe and generally very well tolerated (Figure 1). Two serious adverse events (SAEs) occurred during the study, and neither was deemed related to the vaccine. Three subjects had a greater than moderately severe AE, all of which were considered unrelated to vaccination: dental infection, an SAE, occurred on day 355 in the placebo group; thyroid cancer, an SAE, was detected on day 166 in group 1; and urinary tract infection, an AE, was detected on day 80 in group 1. No significant abnormal laboratory findings were observed, and no cardiac events [32] were noted. All grade ≥2 unsolicited AEs were deemed probably not or not related to study vaccination.

The mild-to-moderate systemic reactogenicity pattern noted with the first dose generally occurred within the first 24–48 hours following vaccination and typically resolved spontaneously within another 1–2 days. The mild-to-moderate local reactogenicity symptoms occurred within 1–2 days of vaccination and resolved over 4–7 days. There were no significant differences in reactogenicity among the groups. Seventeen of 20 vaccinees had vaccine-induced seropositivity [33] when tested at the end-of-study visit by means of commonly used HIV diagnostic tests.

### HIV-1–Specific Cellular Immune Responses

Five subjects from group 2 had detectable IFN-γ ELISPOT responses to at least 1 Env protein pool at baseline, likely reflecting their exposure to Ad26.ENVA.01. Figure 2 shows the kinetics of the responses by vaccination group. Env-specific responses were detected in 8 group 1 subjects after the first vaccine dose and in 10 after the second dose. By comparison, all 8 group 2 subjects had detectable responses to at least 1 Env peptide pool after the first injection (Figure 2 and Supplementary Figure 2). Most group 2 subjects had a further increase in the magnitude of ELISPOT responses to the Env pools after the second inoculation. Median ELISPOT responses on days 0, 14, 98, and 365 to potential T-cell epitope Env peptides (Figure 2A) were 11, 99, 207, and 120 SFCs/$10^6$ PBMCs, respectively, in group 1 subjects and 74, 611, 647, and 475 SFCs/$10^6$ PBMCs, respectively, in group 2 subjects ($P$ = .0008 and $P$ = .0028 for the difference between groups on days 14 and 98, respectively). These responses were significantly different from the baseline responses in group 1 subjects ($P$ < .003 at all time points) and group 2 subjects ($P$ < .016 at all time points). Median responses to EnvA peptides (Supplementary Figure 2*A*) were 10, 40, 77, and 69 SFCs/$10^6$ PBMCs, respectively, among group 1 subjects and 36, 503, 452, and 375 SFCs/$10^6$ PBMCs, respectively, among group 2 subjects ($P$ < .0001 and $P$ = .0001 for days 14 and 98, respectively) and were significantly different than baseline values

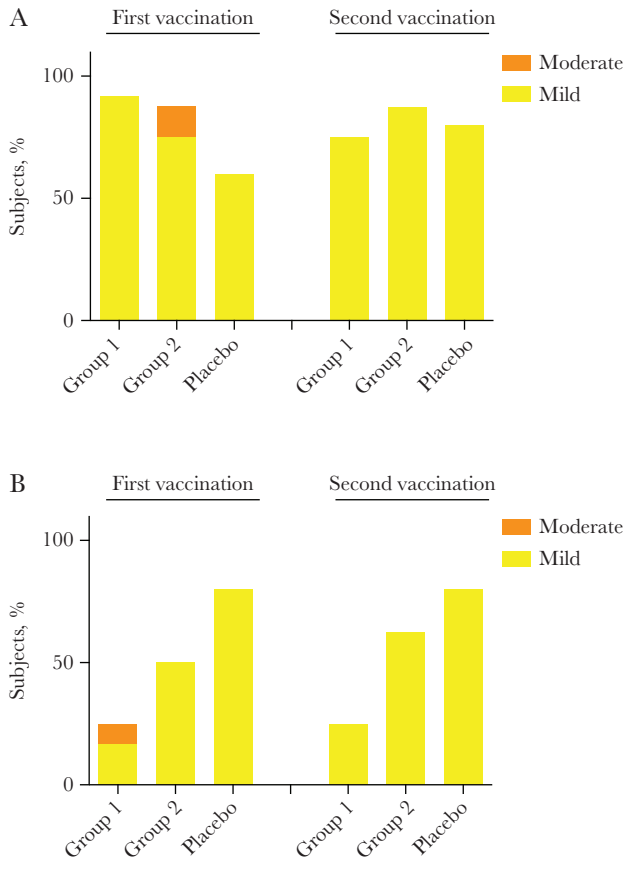


**Figure 1.** Local and systemic reactogenicity. Solicited adverse events (AEs) were collected for 8 days following each vaccination. Shown are the proportions of vaccinees experiencing local reactogenicity (including pain and/or tenderness, erythema, pruritus, warmth, swelling, or induration; *A*) or systemic symptoms (fatigue, myalgia, headache, chills, nausea, or vomiting; *B*) following the first or second vaccination by dose group. See Methods for descriptions of group 1, group 2, and placebo recipients.

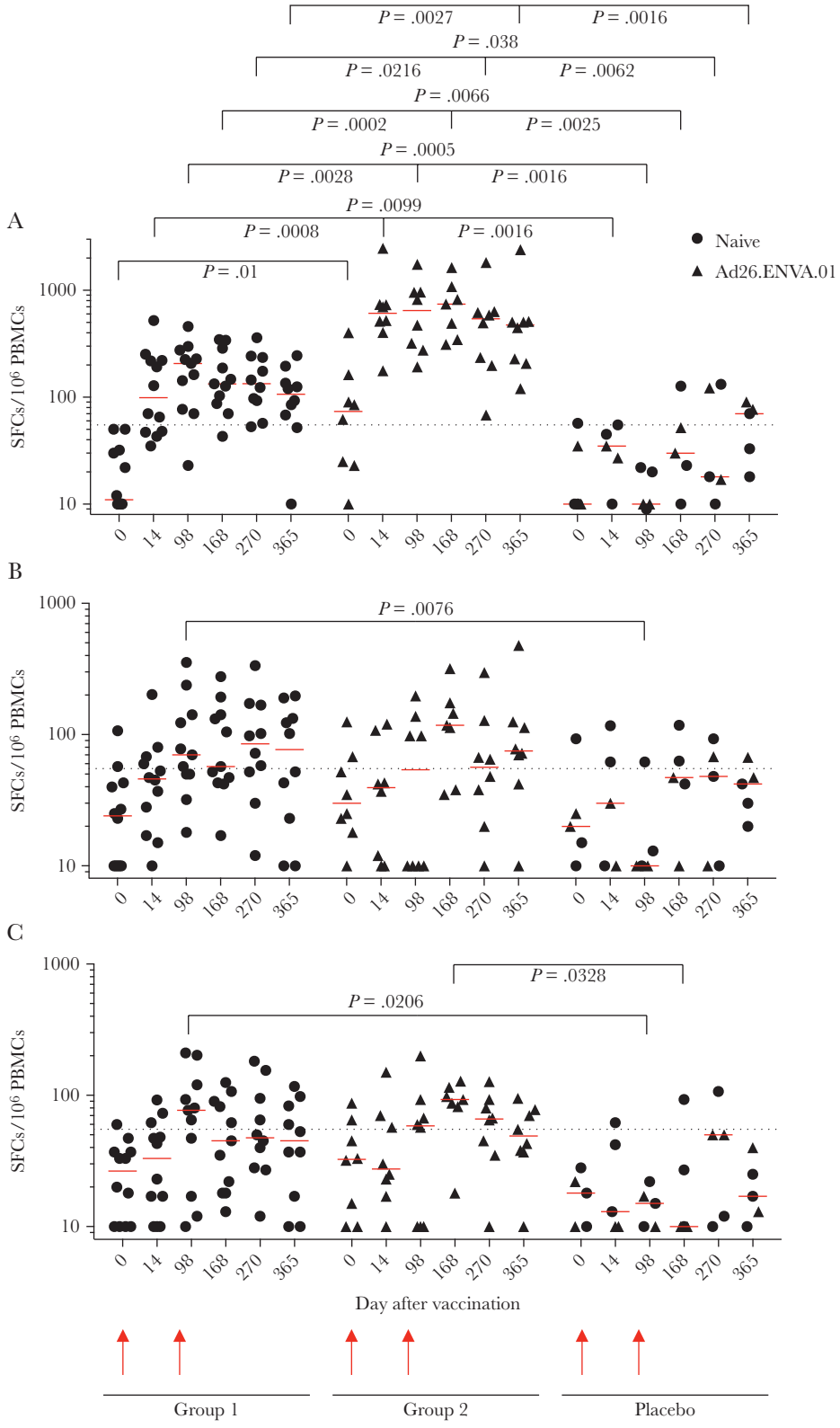


**Figure 2.** Interferon γ enzyme-linked immunospot responses measured using peptide pools corresponding to the potential T-cell epitope Env (*A*), Gag (*B*), and Pol (*C*) sets. Median responses at each time point are indicated with a solid line, and the dashed line indicates the positive threshold of 55 spot-forming cells (SFCs) per $10^6$ peripheral blood mononuclear cells (PBMCs). Arrows indicate the injection time points. Statistical significance was determined by the Mann-Whitney-Wilcoxon test. All subjects were either naive to a prior human immunodeficiency virus type 1 (HIV-1) vaccine or had received 2 or 3 doses of a prototype HIV-1 vaccine (Ad26.ENVA.01) containing only an EnvA insert. See Methods for descriptions of group 1, group 2, and placebo recipients.

at all time points ($P < .014$ and $P < .016$ for group 1 and group 2 subjects, respectively). Among placebo recipients, ELISPOT responses against potential T-cell epitope Env or EnvA peptide pools were not significantly different from baseline values at any time point. Compared with placebo recipients, ELISPOT responses were higher among group 1 subjects at all postvaccination time points until day 365 ($P < .04$) and higher among group 2 subjects at all postvaccination time points ($P < .007$).

Baseline responses to any Gag or Pol peptide pool did not differ among the groups and remained at background levels in placebo recipients throughout the study (Figure 2B and 2C and Supplementary Figure 3). After the first injection, rates of ELISPOT responses to the potential T-cell epitope Gag pool (Figure 2B) did not differ between group 1 and group 2. After the second injection of MVA Mosaic, 7 subjects in group 1 and 4 subjects in group 2 had responses to at least 1 Gag pool. Median ELISPOT responses on days 0, 14, 98, and 365 to potential T-cell epitope Gag peptides were 24, 46, 70, and 77 SFCs/$10^6$ PBMCs, respectively, among group 1 subjects and 30, 40, 54, and 75 SFCs/$10^6$ PBMCs, respectively, among group 2 subjects ($P$ = not significant at all time points, compared with baseline). Compared with Gag-specific ELISPOT responses among placebo recipients, those among group 1 subjects were only significantly different at day 98 ($P = .0076$), and those among group 2 subjects were not significantly different at any time point.

Three group 1 subjects and 3 group 2 subjects had responses to at least 1 Pol pool after the first injection, while 7 group 1 subjects and 5 group 2 subjects had responses to one of the Pol pools following the second vaccination (Figure 2C and Supplementary Figure 3). After the first injection, rates of ELISPOT responses to the potential T-cell epitope Pol pool did not differ between group 1 and group 2. Median ELISPOT responses on days 0, 14, 98, and 365 to Pol peptides (Figure 2C were 27, 33, 77, and 45 SFCs/$10^6$ PBMCs, respectively, among group 1 subjects and 33, 28, 59, and 49 SFCs/$10^6$ PBMCs, respectively, among group 2 subjects ($P$ = not significant at all time points, compared with baseline). Compared with Pol-specific ELISPOT responses among placebo recipients, responses were significantly different only on day 98 among group 1 subjects ($P = .0206$) and only on day 168 among group 2 subjects ($P = .0328$).

Two weeks after the second vaccination, modest $CD4^+$ T-cell responses to the potential T-cell epitope Env peptide pool were detected by intracellular cytokine staining, without clear differences between group 1 and group 2 (Figure 3A). Modest $CD8^+$ T-cell responses were detected to Env as well, although predominantly in group 2 (Figure 3B). A minority of subjects had detectable $CD4^+$ or $CD8^+$ T-cell responses to Gag (Mos1 or Mos2) pools (Figure 3C–F). Individual cytokine responses are shown in Supplementary Figures 5 (for IL-2) and 6 (for IFN-γ).

Taken together, these data show that MVA Mosaic consistently induced HIV-specific cellular immune responses against Env peptides and that these responses could be maintained for 1 year. Responses against Gag and Pol were modest but were detectable in some vaccinees.

#### HIV-1 Env–Specific Antibody Responses

All subjects in group 1 had negative results of EnvA-specific ELISA binding antibody tests at baseline, and results for all naive subjects in the placebo group remained negative throughout the study. Of subjects in group 2, only 1 had detectable ELISA responses to EnvA at baseline. No subjects had detectable responses to EnvB, EnvC, or Mos1 gp140 at baseline. Figure 4 shows the kinetics of the binding antibody responses by vaccination group. After the first injection of MVA Mosaic, 5 subjects (63%) in group 2 had positive responses to at least 1 Env antigen assayed, while all group 1 subjects remained seronegative ($P = .0036$). At day 112, after the second injection, 10 group 1 subjects (88%) had positive responses to at least 1 Env antigen; response rates and titers were highest against the clade B antigen. Among group 2 subjects, 7 (88%) responded to at least 2 Env antigens, with an increase in titer against all 4 antigens assayed.

At days 0, 28, 112, and 365 median clade A (92UG037.9) ELISA titers were 25, 25, 225, and 25, respectively, in group 1 and 25, 150, 225, and 75, respectively, in group 2; median clade B (UK7LN) ELISA titers were 25, 25, 450, and 50, respectively, in group 1 and 25, 225, 450, and 75, respectively, in group 2; median clade C (C97ZA.012) ELISA titers were 25, 25, 150, and 13, respectively, in group 1 and 25, 75, 225, and 25, respectively, in group 2; and median Mos1 titers were 25, 25, 450, and 50, respectively, in group 1 and 25, 150, 450, and 75, respectively, in group 2. The responses in group 2 were highly statistically significant as compared to those in group 1 at day 28 ($P < .0008$) but not at days 112 or 365 for all 4 Env antigens assayed.

All subjects had negative results of neutralizing antibody tests at baseline (Figure 5), and results for all subjects in the placebo group remained negative throughout the study. After both injections of MVA Mosaic, 9 group 1 subjects had neutralizing antibody responses to at least 1 tier 1 virus assayed at day 112, while 5 group 2 subjects had neutralizing antibody responses ($P$ = not significant). Median responses did not differ between the groups.

Because the binding antibody titer against the V1V2 loop of Env was found to be a correlate of protection in the Thai RV144 trial [34], we analyzed binding antibodies against several cyclic peptide constructs, using surface plasmon resonance (Figure 6). After a single dose of MVA Mosaic, antibody levels did not significantly rise in either group 1 or group 2 subjects when assayed with cyclic V2 or V3 loop peptides derived from a clade AE isolate (92TH023). However, modest increases were detected after the second vaccination, without differences between groups.

We also assessed functional nonneutralizing antibody responses by assaying antibody-dependent cellular phagocytosis (Figure 7). After 2 doses of MVA Mosaic, subjects in

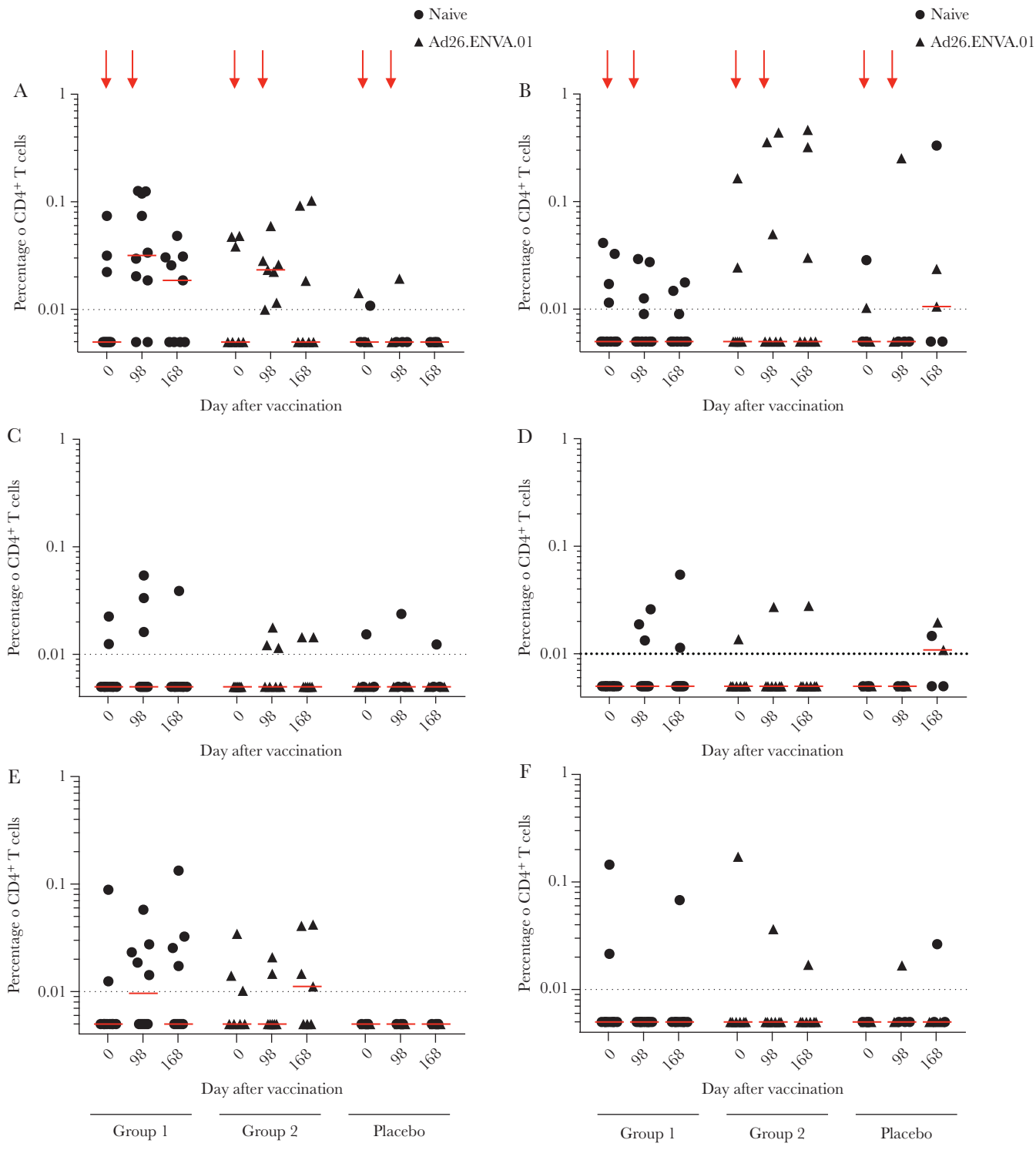


**Figure 3.** Cellular immune responses measured by intracellular cytokine staining. The percentage of CD4+ T cells (*A*, *C*, and *E*) and CD8+ T cells (*B*, *D*, and *F*) producing interleukin 2 and or interferon γ in response to stimulation with potential T-cell epitope Env (*A* and *B*), Gag Mos1 (*C* and *D*), and Gag Mos2 (*E* and *F*) peptide pools are shown. Median responses at each time point are indicated with a solid line, and the dashed line indicates the lower limit of detection of the assay. Arrows indicate the injection time points. All subjects were either naive to a prior human immunodeficiency virus type 1 (HIV-1) vaccine or had received 2 or 3 doses of a prototype HIV-1 vaccine (Ad26.ENVA.01) containing only an EnvA insert. See Methods for descriptions of group 1, group 2, and placebo recipients.

group 1 and group 2 had significantly increased antibody-dependent cellular phagocytosis scores, compared with baseline, when assayed with a clade A Env ($P$ = .0269 and $P$ = .0078, respectively), a clade B Env ($P$ = .0210 and $P$ = .0078, respectively), and 1 of 2 clade C Envs ($P$ = .0342 and $P$ = .0078, respectively, for 1086C and $P$ = not significant in either group for ZA).

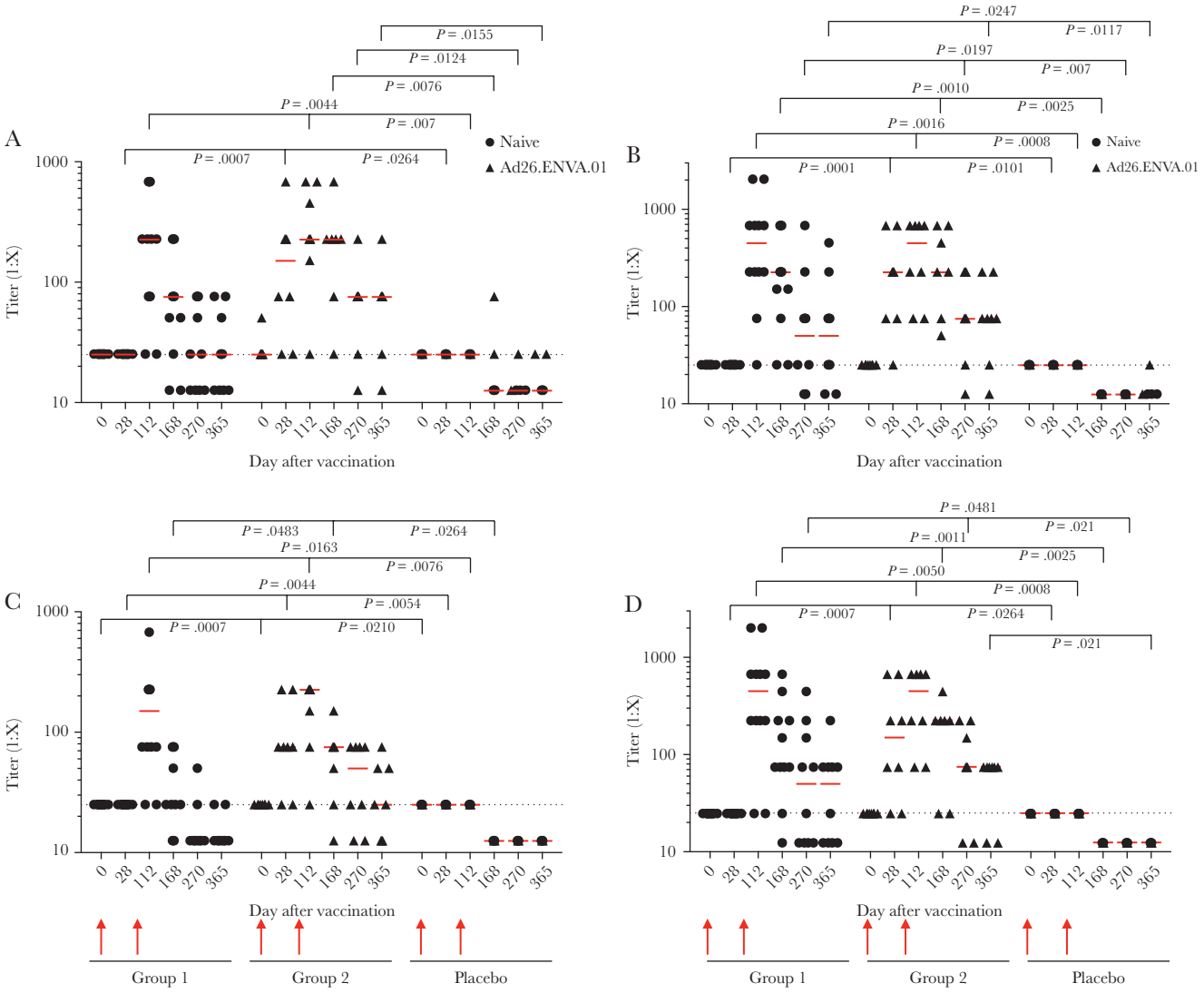


**Figure 4.** Humoral immune responses measured against EnvA (*A*), EnvB (*B*), EnvC (*C*), and Mos1 Env (*D*) by an enzyme-linked immunosorbent assay. Median responses at each time point are indicated with a solid line and the dashed line indicates the lower limit of detection of the assay. Arrows indicate the injection time points. Statistical significance was determined by the Mann-Whitney-Wilcoxon test. All subjects were either naive to a prior human immunodeficiency virus type 1 (HIV-1) vaccine or had received 2 or 3 doses of a prototype HIV-1 vaccine (Ad26.ENVA.01) containing only an EnvA insert. See Methods for descriptions of group 1, group 2, and placebo recipients.

These data show that MVA Mosaic consistently induced Env-specific binding antibody responses and that these responses were cross-reactive against Env proteins derived from at least 3 clades.

Antivector neutralizing antibody responses are presented in the Supplementary Materials.

## DISCUSSION

These data provide the first assessment of a mosaic immunogen in humans. The novel recombinant MVA mosaic vaccine candidate was generally safe, well tolerated, and immunogenic in this first-in-human evaluation of the mosaic immunogen concept. In group 1, both cellular and humoral cross-clade immune responses were elicited after 1 or 2 vaccinations in all participants. Env-specific responses were induced after a single immunization in nearly all group 2 subjects. All group 2 groups developed both vector-specific and insert-specific humoral immune responses that generally persisted for 1 year. These findings are consistent with data from a nonhuman primate model in which MVA mosaic vectors elicited humoral and cellular responses and provided partial protection against SHIV challenges in a heterologous prime-boost vaccine regimen [17].

HIV-specific T-cell responses were elicited in most vaccinees, but Env-specific responses were higher in frequency and magnitude than Gag- or Pol-specific responses. Group 2 subjects had an amnestic response to Env after receipt of vaccine, in terms of both humoral and cellular immune responses. The recall immune responses appeared more rapidly (after a single

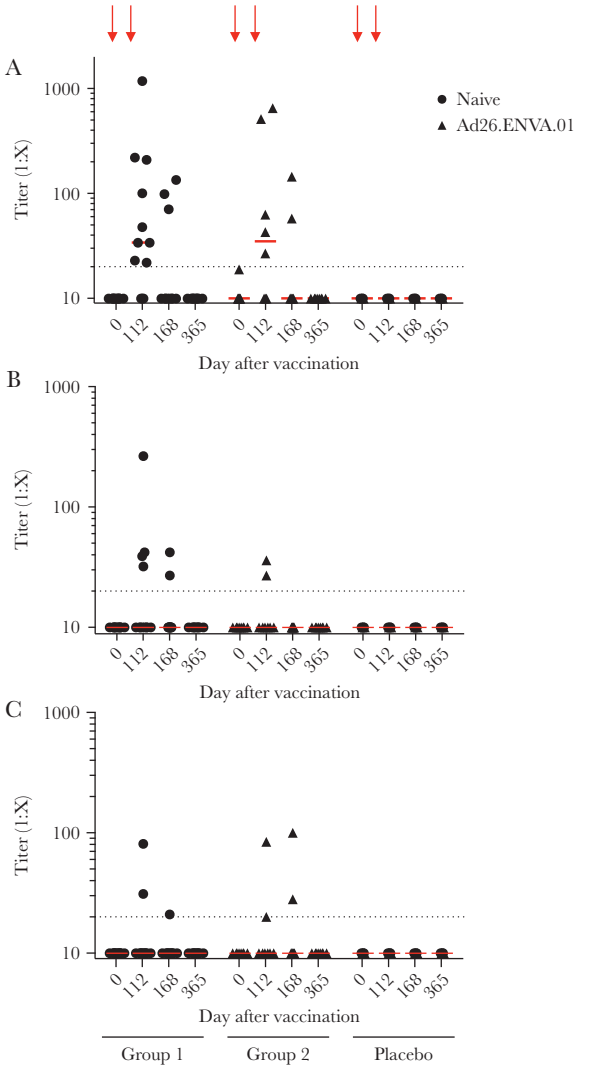


**Figure 5.** Neutralizing antibody activity was measured using pseudoviruses expressing Env derived from tier 1A viruses MN3 (clade B; *A*), SF162 (clade B; *B*), and MW965 (clade C; *C*). Median responses at each time point are indicated with a solid line, and the dashed line indicates the lower limit of detection of the assay. Arrows indicate the injection time points. All subjects were either naive to a prior human immunodeficiency virus type 1 (HIV-1) vaccine or had received 2 or 3 doses of a prototype HIV-1 vaccine (Ad26.ENVA.01) containing only an EnvA insert. See Methods for descriptions of group 1, group 2, and placebo recipients.

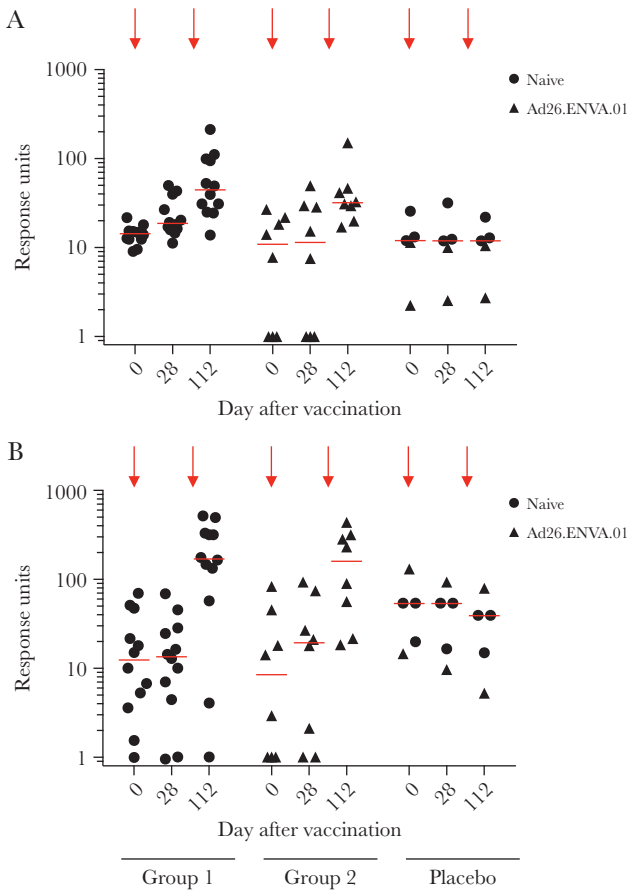


**Figure 6.** Env binding antibodies as measured by surface plasmon resonance. Binding antibodies were measured against the cyclic V2 loop (*A*) and the cyclic V3 loop (*B*) peptides of 92TH023 (clade AE). Median responses at each time point are indicated with a solid line. Arrows indicate the injection time points. All subjects were either naive to a prior human immunodeficiency virus type 1 (HIV-1) vaccine or had received 2 or 3 doses of a prototype HIV-1 vaccine (Ad26.ENVA.01) containing only an EnvA insert. See Methods for descriptions of group 1, group 2, and placebo recipients.

vaccination) and reached a greater magnitude as compared to responses in group 1. In group 1, the increased immune response detected after the second vaccination is consistent with responses seen after receipt of other MVA-vectored HIV-1 vaccines [35, 36]. Group 2 subjects appeared to have slightly lower rates and magnitudes of responses to Gag and Pol, suggesting that recall responses dominate de novo responses, a phenomenon termed "original antigenic sin" [37].

Env-specific binding antibody responses detected by ELISA were elicited in nearly all subjects after the first immunization to the homologous antigen (Mos1) and to at least 3 clades (A, B, and C), and they persisted 1 year after the first vaccination. Interestingly, these responses also suggested an amnestic pattern among group 2 subjects (for whom responses rapidly appeared after the first vaccination and were high in magnitude), compared with group 1 subjects (for whom no responses were detected until after the second vaccination).

Several recombinant MVA-HIV vaccine vectors have completed phase 1 and phase 2 trials in recent years [38]. Overall, these vaccines have been well tolerated, with no vaccine-related SAEs identified [35, 36, 39]. In one study, 69% of MVA-HIV vaccinees developed HIV-specific binding antibodies, but only 43% developed HIV-specific $CD4^+$ T-cell responses, and only 21% developed insert-specific $CD8^+$ T-cell responses [35]. In a more recent study with a different MVA-HIV vaccine, 98% of vaccinees developed Env-specific binding antibodies, while only 43% and 15% of vaccinees developed HIV-specific $CD4^+$ and $CD8^+$ T-cell responses, respectively [40]. These studies suggest that MVA vectors delivering natural HIV-1–derived inserts

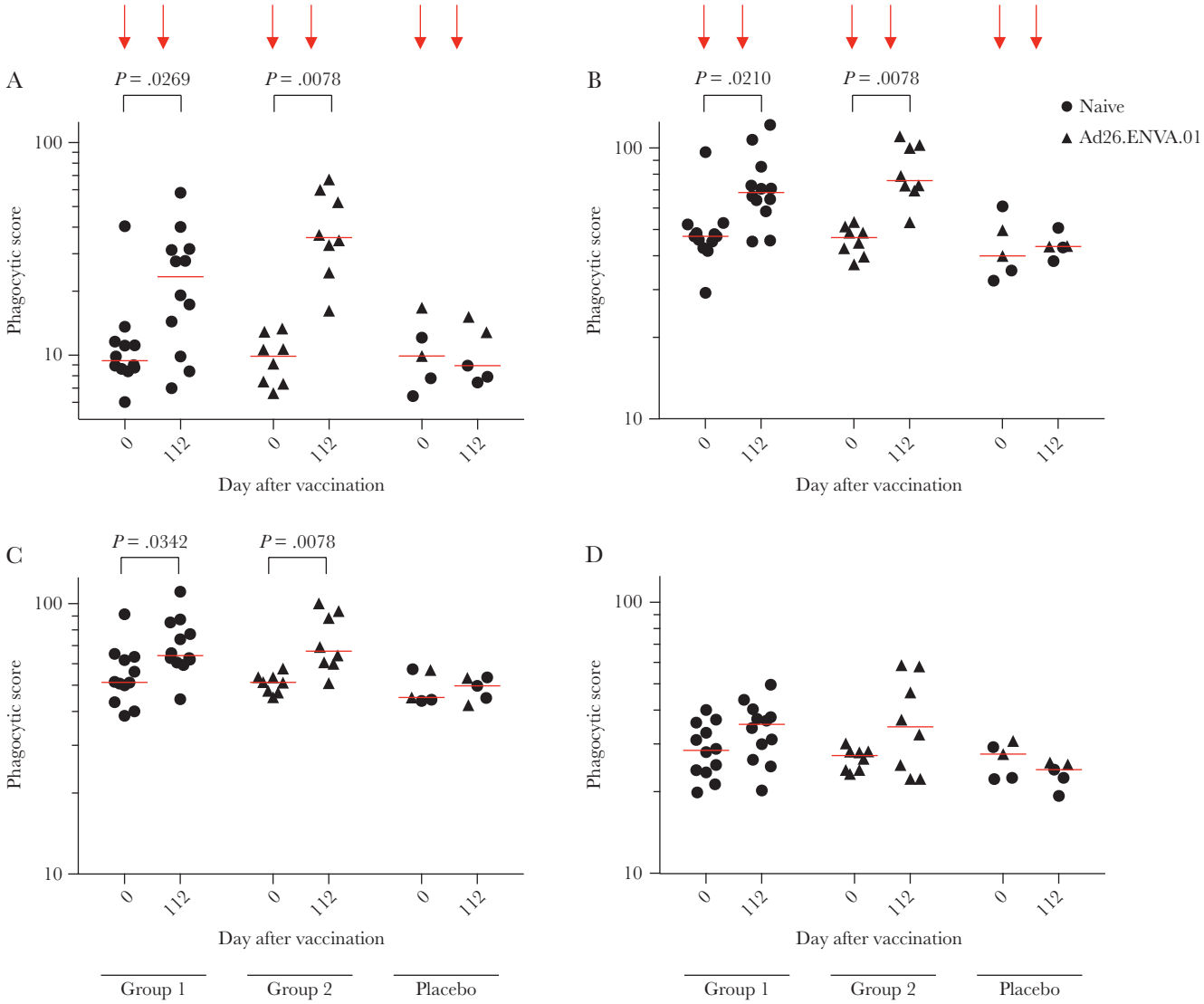


**Figure 7.** Antibody-dependent cellular phagocytosis responses measured using beads coated with Env from clade A (A1.con; *A*), clade B (SF162; *B*), or clade C (1086C [*C*] or ZA [*D*]). Median responses at each time point are indicated with a solid line. Arrows indicate the injection time points. Statistical significance was determined by the Wilcoxon signed rank test. All subjects were either naive to a prior human immunodeficiency virus type 1 (HIV-1) vaccine or had received 2 or 3 doses of a prototype HIV-1 vaccine (Ad26.ENVA.01) containing only an EnvA insert. See Methods for descriptions of group 1, group 2, and placebo recipients.

are immunogenic in humans and may have a role eliciting humoral and $CD4^+$ T-cell responses.

Despite the induction of robust MVA vector-specific neutralizing antibody responses, EnvA insert–specific binding antibody responses were increased following a second vaccination with the homologous vector, and we found no evidence that MVA vector-specific neutralizing antibodies inhibited insert-specific cellular or humoral immune responses. Remarkably, in group 2 subjects, Ad26 neutralizing antibodies were detectable at baseline, 4–6 years after their last vaccination in the previous study, and HIV-1–specific immune responses were clearly boosted by MVA mosaic, demonstrating that Ad26 vaccine–induced HIV-1 specific immune responses may be long lived. Furthermore, our results are consistent with findings from studies of other candidate HIV-1 vaccines. Joachim et al found that a substantial proportion of subjects who had received a DNA-prime and MVA-boost heterologous regimen still had detectable immune responses 3 years later; these responses were further boosted by an additional dose of the candidate MVA-HIV vaccine [41]. Similarly, although immune responses to an MVA-vectored clade B HIV-1 vaccine were found to have decreased over 4 years, an additional dose also boosted HIV-1–specific immune responses, particularly the antibody response [42].

These data are the first to demonstrate the safety and immunogenicity of the novel recombinant MVA bivalent mosaic vaccine in humans. Importantly, HIV-specific humoral and cellular immune responses were consistently elicited with minimal reactogenicity. Interestingly, prior receipt of a different

HIV-1 vaccine (Ad26.ENVA.01) induced a robust Env-specific immune response after a single MVA mosaic vaccination, compared with the response after 2 vaccinations in group 1. This first-in-human study did not include a direct comparison of mosaic and natural sequence immunogens, in order to first verify the immunogenicity of the synthetic mosaic sequences. The novel MVA mosaic vector and the mosaic immunogen concept warrant additional investigation as a vaccine vector for HIV. Future studies will evaluate whether the breadth or magnitude of cellular and humoral immune responses with mosaic immunogens is greater than that with natural sequence immunogens. Based in part on these data, further development of the bivalent mosaic concept is underway, with additional studies using an Ad26 vector as part of a heterologous prime-boost regimen (NCT02315703, NCT02788045, and NCT02935686) and a proof-of-concept trial (NCT03060629) recently initiated.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

***Acknowledgments.*** We thank the safety monitoring committee (Drs Peter Wright (Chair), Michael Keefer, Victor De Gruttola, and Paul Goepfert); the National Institutes of Health (NIH) Vaccine Research Center, for the EnvA vaccine antigen, protein, and peptides; staff from the National Institute Allergy and Infectious Diseases, NIH (Michael Pensiero, Edith Swann, Alan Fix, Elizabeth Adams, and Mary Anne Luzar); Christopher Hamlin, for technical assistance with the Biacore measurements; Patricia Giglio, Benjamin Lee, Mayuri Shetty, Crystal Cabral, Jessica Jimenez, and George Neubauer, for technical assistance, reagents, and advice; and our volunteers, for their generous participation in this study.

Additional members of the IPCAVD006/RV380/HIV-V-A002 Study Group are as follows: Alka Patel, Kevin Zinchuk, Alexis Liakos, Brian Engelson, Sarah Ganley, Chun Su Mei, Mark J. Iampietro, Ann Cheung, Kara Brandariz, Annalena LaPorte, Anna G. McNally, Jennifer Shields, Kelly A. Stanley, Rebecca Dilan, Faye Stephens, Robyn Hamel, Madeline Bayne, Katherine E. Yanosick, Alexander Robles, Marshall Zingg, David J. Dominguez, Christy L. Lavine, Jetta Garrity, Michael Rist, Fadi Ghantous, Nicholas Fredette, Karen Buleza, Raphaele Roten, Olive Yuan, Gitta Huskens, Heidi Muller, Zelda Euler, Caroline Hodin, Lorenz Scheppler, Makoto Wajima, Soniya Gadre, James Nichols, Amy Kinney, and Mo Weijtens.

***Disclaimer.*** The views expressed are those of the authors and should not be construed to represent the positions of the US Army or US Department of Defense.

***Financial support.*** This work was supported by the National Institutes of Health (NIH; grants AI100645, AI096040, AI085181, TR000170, and TR001102); the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, NIH; the Henry M. Jackson Foundation for the Advancement of Military Medicine and the US Department of Defense (cooperative agreement W81XWH-11-2-0174); and the Ragon Institute of MGH, MIT, and Harvard

***Potential conflicts of interest.*** M. S., S. N., K. C., H. S., J. H., M. G. P., and F. T. are employees of Janssen Vaccines and Prevention or affiliated companies. Patents for the mosaic immunogens are held in part by Beth Israel Deaconess Medical Center and Los Alamos National Laboratory; no royalties or income are associated with these patents. B. M. is a named inventor on patents owned by the US government concerning poxvirus recombinant technology. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

## References

1. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet **2008**; 372:1881–93.
2. Flynn NM, Forthal DN, Harro CD, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis **2005**; 191:654–65.
3. McElrath MJ, De Rosa SC, Moodie Z, et al. HIV-1 vaccine-induced immunity in the test-of-concept step study: a case-cohort analysis. Lancet **2008**; 372:1894–905.
4. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med **2009**; 361:2209–20.
5. Hammer SM, Sobieszczyk ME, Janes H, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med **2013**; 369:2083–92.
6. Janes H, Frahm N, DeCamp A, et al. MRKAd5 HIV-1 Gag/Pol/Nef vaccine-induced T-cell responses inadequately predict distance of breakthrough HIV-1 sequences to the vaccine or viral load. PLoS One **2012**; 7:e43396.
7. Hertz T, Ahmed H, Friedrich DP, et al. HIV-1 vaccine-induced T-cell responses cluster in epitope hotspots that differ from those induced in natural infection with HIV-1. PLoS Pathog **2013**; 9:e1003404.
8. Rolland M, Tovanabutra S, deCamp AC, et al. Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. Nat Med **2011**; 17:366–71.
9. Janes H, Friedrich DP, Krambrink A, et al. Vaccine-induced gag-specific T cells are associated with reduced viremia after HIV-1 infection. J Infect Dis **2013**; 208:1231–9.

10. Walker BD, Yu XG. Unravelling the mechanisms of durable control of HIV-1. Nat Rev Immunol **2013**; 13:487–98.
11. Kunwar P, Hawkins N, Dinges WL, et al. Superior control of HIV-1 replication by CD8+ T cells targeting conserved epitopes: implications for HIV vaccine design. PLoS One **2013**; 8:e64405.
12. Kiepiela P, Ngumbela K, Thobakgale C, et al. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med **2007**; 13:46–53.
13. Korber B, Gnanakaran S. The implications of patterns in HIV diversity for neutralizing antibody induction and susceptibility. Curr Opin HIV AIDS **2009**; 4:408–17.
14. Barouch DH, O'Brien KL, Simmons NL, et al. Mosaic HIV-1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. Nat Med **2010**; 16:319–23.
15. Santra S, Muldoon M, Watson S, et al. Breadth of cellular and humoral immune responses elicited in rhesus monkeys by multi-valent mosaic and consensus immunogens. Virology **2012**; 428:121–7.
16. Borducchi EN, Cabral C, Stephenson KE, et al. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. Nature **2016**; 540:284–7.
17. Barouch DH, Stephenson KE, Borducchi EN, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell **2013**; 155:531–9.
18. Baden LR, Karita E, Mutua G, et al. Assessment of the safety and immunogenicity of 2 novel vaccine platforms for HIV-1 prevention: a randomized trial. Ann Intern Med **2016**; 164:313–22.
19. Walsh SR, Moodie Z, Fiore-Gartland AJ, et al. Vaccination with heterologous HIV-1 envelope sequences and heterologous adenovirus vectors increases T-cell responses to conserved regions: HVTN 083. J Infect Dis **2016**; 213:541–50.
20. Andrasik MP, Karuna ST, Nebergall M, Koblin BA, Kublin JG, HVTN. Behavioral risk assessment in HIV Vaccine Trials Network (HVTN) clinical trials: a qualitative study exploring HVTN staff perspectives. Vaccine **2013**; 31:4398–405.
21. Baden LR, Walsh SR, Seaman MS, et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). J Infect Dis **2013**; 207:240–7.
22. Barouch DH, Liu J, Li H, et al. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. Nature **2012**; 482:89–93.
23. Li F, Malhotra U, Gilbert PB, et al. Peptide selection for human immunodeficiency virus type 1 CTL-based vaccine evaluation. Vaccine **2006**; 24:6893–904.
24. Liu J, Ewald BA, Lynch DM, et al. Magnitude and phenotype of cellular immune responses elicited by recombinant adenovirus vectors and heterologous prime-boost regimens in rhesus monkeys. J Virol **2008**; 82:4844–52.
25. Rerks-Ngarm S, Pitisuttithum P, Excler JL, et al. Randomized, double-blind evaluation of late boost strategies for HIV-uninfected vaccine recipients in the RV144 HIV vaccine efficacy trial. J Infect Dis **2017**; 215:1255–63.
26. Sarzotti-Kelsoe M, Bailer RT, Turk E, et al. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. J Immunol Methods **2014**; 409:131–46.
27. Karasavvas N, Billings E, Rao M, et al. The Thai Phase III HIV Type 1 Vaccine trial (RV144) regimen induces antibodies that target conserved regions within the V2 loop of gp120. AIDS Res Hum Retroviruses **2012**; 28:1444–57.
28. Dugast AS, Chan Y, Hoffner M, et al. Lack of protection following passive transfer of polyclonal highly functional low-dose non-neutralizing antibodies. PLoS One **2014**; 9:e97229.
29. Ackerman ME, Moldt B, Wyatt RT, et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. J Immunol Methods **2011**; 366:8–19.
30. Wilck MB, Seaman MS, Baden LR, et al. Safety and immunogenicity of modified vaccinia Ankara (ACAM3000): effect of dose and route of administration. J Infect Dis **2010**; 201:1361–70.
31. Baden LR, Liu J, Li H, et al. Induction of HIV-1-specific mucosal immune responses following intramuscular recombinant adenovirus serotype 26 HIV-1 vaccination of humans. J Infect Dis **2015**; 211:518–28.
32. Elizaga ML, Vasan S, Marovich MA, et al. Prospective surveillance for cardiac adverse events in healthy adults receiving modified vaccinia Ankara vaccines: a systematic review. PLoS ONE **2013**; 8:e54407.
33. Cooper CJ, Metch B, Dragavon J, Coombs RW, Baden LR; NIAID HIV Vaccine Trials Network (HVTN) Vaccine-Induced Seropositivity (VISP) Task Force. Vaccine-induced HIV seropositivity/reactivity in noninfected HIV vaccine recipients. JAMA **2010**; 304:275–83.
34. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med **2012**; 366:1275–86.
35. Keefer MC, Frey SE, Elizaga M, et al.; NIAID HIV Vaccine Trials Network. A phase I trial of preventive HIV vaccination with heterologous poxviral-vectors containing matching HIV-1 inserts in healthy HIV-uninfected subjects. Vaccine **2011**; 29:1948–58.
36. Goepfert PA, Elizaga ML, Sato A, et al.; National Institute of Allergy and Infectious Diseases HIV Vaccine Trials Network. Phase 1 safety and immunogenicity testing of DNA and recombinant modified vaccinia Ankara vaccines

expressing HIV-1 virus-like particles. J Infect Dis **2011**; 203:610–9.
37. Morens DM, Burke DS, Halstead SB. The wages of original antigenic sin. Emerg Infect Dis **2010**; 16:1023–4.
38. Walsh SR, Dolin R. Vaccinia viruses: vaccines against smallpox and vectors against infectious diseases and tumors. Expert Rev Vaccines **2011**; 10:1221–40.
39. Harrer E, Bäuerle M, Ferstl B, et al. Therapeutic vaccination of HIV-1-infected patients on HAART with a recombinant HIV-1 nef-expressing MVA: safety, immunogenicity and influence on viral load during treatment interruption. Antivir Ther **2005**; 10:285–300.
40. Goepfert PA, Elizaga ML, Seaton K, et al.; HVTN 205 Study Group; National Institutes of Allergy and Infectious Diseases HIV Vaccines Trials Network. Specificity and 6-month durability of immune responses induced by DNA and recombinant modified vaccinia Ankara vaccines expressing HIV-1 virus-like particles. J Infect Dis **2014**; 210:99–110.
41. Joachim A, Munseri PJ, Nilsson C, et al. Three-year durability of immune responses induced by HIV-DNA and HIV-modified vaccinia virus Ankara and effect of a late HIV-modified vaccinia virus Ankara boost in Tanzanian volunteers. AIDS Res Hum Retroviruses **2017**; 33:880–8.
42. Guardo AC, Gomez CE, Diaz-Brito V, et al. Safety and vaccine-induced HIV-1 immune responses in healthy volunteers following a late MVA-B boost 4 years after the last immunization. PLoS One **2017**; 12:e0186602.