

Specificity and 6-Month Durability of Immune Responses Induced by DNA and Recombinant Modified Vaccinia Ankara Vaccines Expressing HIV-1 Virus-Like Particles

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Background. Clade B DNA and recombinant modified vaccinia Ankara (MVA) vaccines producing virus-like particles displaying trimeric membrane-bound envelope glycoprotein (Env) were tested in a phase 2a trial in human immunodeficiency virus (HIV)–uninfected adults for safety, immunogenicity, and 6-month durability of immune responses.

Methods. A total of 299 individuals received 2 doses of JS7 DNA vaccine and 2 doses of MVA/HIV62B at 0, 2, 4, and 6 months, respectively (the DDMM regimen); 3 doses of MVA/HIV62B at 0, 2, and 6 months (the MMM regimen); or placebo injections.

Results. At peak response, 93.2% of the DDMM group and 98.4% of the MMM group had binding antibodies for Env. These binding antibodies were more frequent and of higher magnitude for the transmembrane subunit (gp41) than the receptor-binding subunit (gp120) of Env. For both regimens, response rates were higher for CD4⁺ T cells (66.4% in the DDMM group and 43.1% in the MMM group) than for CD8⁺ T cells (21.8% in the DDMM group and 14.9% in the MMM group). Responding CD4⁺ and CD8⁺ T cells were biased toward Gag, and >70% produced 2 or 3 of the 4 cytokines evaluated (ie, interferon γ , interleukin 2, tumor necrosis factor α , and granzyme B). Six months after vaccination, the magnitudes of antibodies and T-cell responses had decreased by <3-fold.

Conclusions. DDMM and MMM vaccinations with virus-like particle–expressing immunogens elicited durable antibody and T-cell responses.

Keywords. HIV/AIDS; vaccines; clinical trial; T cells; antibodies; DNA; recombinant MVA.

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A human immunodeficiency virus (HIV) vaccine faces the challenge of eliciting immune responses that can prevent the acquisition of virus and the establishment of latency. Vaccine-induced antibodies (Abs) can block infection by directly neutralizing virus [1] and by binding to virus and virus-infected cells to tag them for destruction by the innate immune response [2, 3]. Elicited cytotoxic (ie, CD8⁺) T cells can modulate the severity of infection and slow disease progression by recognizing and killing infected cells.

The one vaccine to achieve at least partial prevention of infection (efficacy, 31.2%) was tested in Thailand in the RV144 trial [4]. This vaccine regimen consisted of 2 recombinant canarypox primes (ALVAC-HIV vCP1521), followed by 2 canarypox plus bivalent gp120 protein in alum boosts (AIDSVAX B/E) [5]. The elicited Abs had limited ability to neutralize tier 1 HIV isolates, which are easy to neutralize, and no detectable neutralizing activity for tier 2 viruses, which are more difficult to neutralize and characteristic of most currently circulating viruses [6]. In the correlates of risk analysis, the primary immune response that reduced the risk of infection was the binding of Abs to variable loops 1 and 2 of HIV envelope glycoprotein (Env) [7–9]. Provocatively, certain circulating Env-specific immunoglobulin A (IgA) appeared to decrease vaccine efficacy by competing with immunoglobulin G (IgG) binding and Fcγ-initiated mechanisms of protection, such as Ab-dependent cellular cytotoxicity [7, 10, 11]. Nonneutralizing Ab, induced by the RV144 vaccine regimen, captured infectious virions [12]. The binding and neutralizing Ab responses elicited in RV144 rapidly waned, falling by ≥ 10 -fold in the first 6 months [6, 13]. Efficacy also fell with time, from an estimated 60% at peak vaccine response to $< 30\%$ by 2.5 years [14].

The DNA and recombinant modified vaccinia Ankara (MVA62B) vaccines tested in this phase 2a study produce virus-like particles that display membrane-bound trimeric forms of Env [15–17]. Simian immunodeficiency virus (SIV) prototypes of these vaccines elicited 61%–64% reductions in the per-challenge risk of intrarectal infection and prevented infection in 25% of the animals receiving 12 weekly rectal administrations of the heterologous SIVE660 [18]. Phase 1 testing of these vaccines revealed that both the DDMM regimen, involving 2 doses of DNA vaccine followed by 2 doses of MVA62B vaccine, and the MMM regimen, involving 3 doses of MVA62B vaccine, were well tolerated and replicated the overall patterns of immunogenicity observed for analogous SIV immunogens in macaques [19, 20]. The current phase 2a trial expanded testing of the DDMM and MMM regimens and extended analyses of both T-cell and Ab responses to include the durability of elicited responses at 6 months after vaccination.

SUBJECTS, MATERIALS, AND METHODS

Vaccines

The GeoVax vaccine, GOVX-B11 comprises a DNA prime and an MVA boost. The DNA component, pGA2/JS7 DNA, expresses Gag, protease, reverse transcriptase, Tat, Rev, and Vpu from clade B HIV-HXB-2/BH10 sequences and Env from HIV ADA sequences (gp41/120 cleavage site intact) from a single transcript by subgenomic splicing [16]. The vaccine is rendered noninfectious by gene deletions and inactivating point mutations [15, 16]. The MVA component, MVA62B, encodes HIV-1 Gag, protease, reverse transcriptase, and Env from the same sequences and also produces immature noninfectious virus-like particles

[17]. In MVA62B, the ADA Env gene is truncated for the 115 C-terminal amino acids of the endodomain of gp41 to enhance stability of the vaccine insert during manufacture [21].

Study Design

HIV Vaccine Trials Network (HVTN) protocol 205 was a randomized, double-blind, placebo-controlled trial conducted at clinical sites in the United States and Peru among participants who were considered to be at lower risk for HIV infection (clinical trials registration NCT00820846). The institutional review boards or ethics committees for each site provided initial and ongoing approvals and review of the research. Adults aged 18–50 years who were deemed healthy on the basis of medical history, physical examination findings, laboratory test results, troponin levels, and electrocardiogram (ECG) findings were enrolled. In part A of the study, 180 participants were enrolled, of whom 120 were vaccinated with 3 mg of DNA at months 0 and 2, followed by 10^8 median tissue culture infective doses of MVA62B at months 4 and 6 (the DDMM regimen); 60 additional participants were enrolled and received normal saline as placebo injections. In part B, 29 enrolled participants received DDMM, and 75 enrolled participants received MVA62B at months 0, 2, and 6 (the MMM regimen); 15 additional enrolled subjects received normal saline as placebo injections.

Vaccines were delivered intramuscularly by needle injection at a final volume of 1 mL into the deltoid region. Safety evaluations included physical examinations, standard clinical chemistry and hematological tests, and cardiac troponin analysis. Postvaccination chest symptoms were evaluated with a 12-lead ECG, and findings were interpreted by a central ECG laboratory. Local reactogenicity (ie, injection site pain, tenderness, redness, erythema, and induration) and systemic reactogenicity (ie, malaise, headache, fever, chills, myalgias, arthralgias, nausea, vomiting, and fatigue) were assessed for 3 days following each vaccination or until resolution. Adverse events were recorded for 12 months after the first vaccination for each participant and were graded as mild, moderate, or severe according to standard criteria (available at: <http://rcc.tech-res.com/safetyandpharmacovigilance/>). Social impact assessments were obtained at each study visit following the first vaccination and consisted of 10 targeted questions about potential discrimination due to study participation.

Immune Response Assays

Ab Responses

Validated binding Ab multiplex assays [22] for IgG and IgA were performed according to a prespecified assay study plan and good clinical laboratory practices guidelines. HIV-specific anti-IgG Abs were detected with mouse anti-human IgG (Southern Biotech, Birmingham, AL). Anti-HIV IgA responses in serum were detected with goat anti-human IgA (Jackson ImmunoResearch, West Grove, PA) in specimens depleted of IgG by use of protein G high-performance MultiTrap plates (GE

Healthcare Life Sciences, Pittsburgh, PA) according to the manufacturer's instructions, with minor modifications. Antibody measurements were performed using a Bio-Plex 200 instrument (Bio-Rad, Hercules CA), and results are expressed as mean fluorescence intensity. The preset criteria for inclusion of samples in data analysis were a coefficient of variation of $\leq 15\%$ for duplicate measurements and the presence of >100 beads counted per sample. Positive controls included anti-HIV immunoglobulin and monoclonal IgA Ab carrying the b12 region (kindly provided by Drs Dennis Burton and Ann Hessel). Negative controls were blank beads, HIV-1-negative normal human serum (Sigma Aldrich, St. Louis, MO), and serum samples obtained before vaccination. The consensus antigens ConS gp140 and Con6 gp120 were kindly provided by Drs Larry Liao and Barton Haynes (Duke Human Vaccine Institute, Durham, NC). Recombinant MN gp41 (ImmunoDiagnostics, Woburn, MA), ADA gp120 (MyBioSource, San Diego, CA), and p24 (BD Biosciences, San Jose, CA) proteins were purchased. To evaluate vaccine-induced seroreactivity, we performed enzyme-linked immunosorbent assays (Abbot Laboratories, Abbot Park, IL) and Western blot testing (Bio-Rad) on specimens obtained after the final vaccination [23].

Neutralizing Abs were measured as a reduction in Tat-regulated luciferase reporter gene expression in either TZM-bl or A3R5 cells, as described elsewhere [6]. The TZM-bl assay measured neutralization titers against a panel of heterologous Env-pseudotyped viruses that exhibit either a tier 1A (MN.3, SF162.LS, Bal.26, W61D-TCLA.71, and MW965.26), a tier 1B (SS1196.1), or tier 2 (CAAN5342.A2, REJO4541.67, SC422661.8, and TRO.11) neutralization phenotype in TZM-bl cells. The A3R5 assay measured neutralization titers against infectious molecular clones that exhibit either a tier 1A (9020.A13) or tier 2 (CH77, RHPA, SC22.3C2) neutralization phenotype in A3R5 cells. Virus stocks were produced by transfection in 293 T cells. All viruses are clade B, except for MW965.26, which is clade C.

T-Cell Responses

Peripheral blood mononuclear cells (PBMCs) were processed from whole-blood specimens and cryopreserved at the HVTN clinical site laboratories within 8 hours of venipuncture [24, 25]. Blood specimens for PBMC processing were obtained 2 weeks after each vaccination and 3 and 6 months after the last vaccination. HIV-specific T-cell responses were measured using intracellular cytokine staining as previously described [26, 27]. Global potential T-cell epitope peptide pools representing HIV Env (3 pools), Gag (2 pools), or Pol (3 pools) were used at a final concentration of 1 μg per mL for each peptide [28]. Cells were first stained with the Aqua Live/Dead Fixable Dead Cell Stain (Invitrogen) and then fixed, permeabilized, and stained with CD3 PE-TR (Beckman-Coulter), CD4 APC-Cy7, CD8 PerCP-Cy5.5, interferon γ (IFN- γ) phycoerythrin (PE)-Cy7,

interleukin 2 (IL-2)-PE, tumor necrosis factor α (TNF- α) fluorescein isothiocyanate, perforin Alexa 647, granzyme B Alexa 700, and CD57 Alexa 405 (BD Biosciences for all except CD3 PE-Texas red). Positive responses were identified using 1-sided Fisher exact test and the numbers of CD4 $^{+}$ or CD8 $^{+}$ T cells producing IFN- γ and/or IL-2 in response to peptide stimulation.

Selection of Samples for Longevity Assays

Longevity studies were conducted at 24 weeks after the last vaccination on samples from participants who had positive assay results 2 weeks after their last vaccination.

Statistical Analyses

For safety, the number and percentage of participants experiencing each reactogenicity symptom were tabulated by severity and vaccination regimen by visit and overall. Each participant's reactogenicity was counted once under the maximum severity

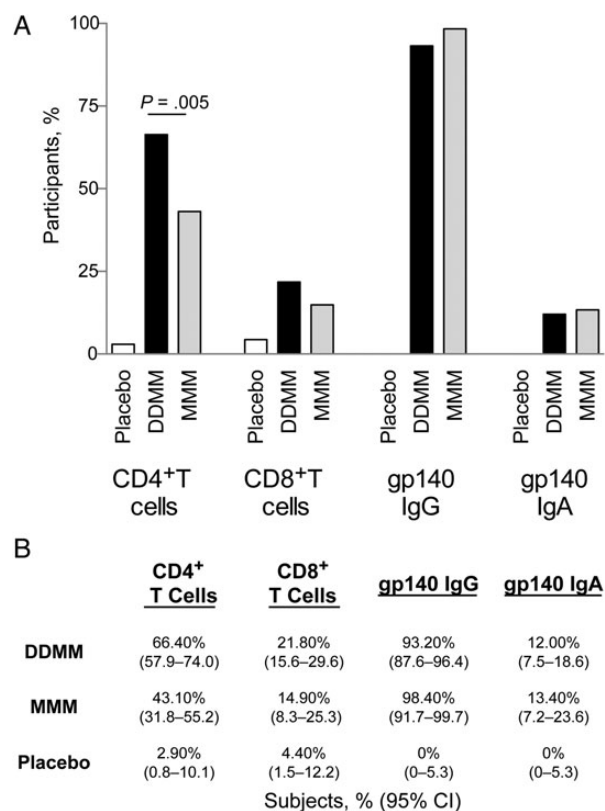


Figure 1. Response rates for DDMM- and MMM-elicited T cells and antibody; see Subjects, Materials, and Methods for a description of each regimen. **A**, The percentage of participants with human immunodeficiency virus (HIV)-specific CD4 $^{+}$ and CD8 $^{+}$ T-cell responses to any protein (Gag, Env, or Pol) and immunoglobulin G (IgG) and immunoglobulin A (IgA) responses to consensus gp140. Data are for samples collected at 2 weeks following the final vaccination for all participants for whom measurements could be obtained. **B**, Summary of response rates shown in panel A. Abbreviation: CI, confidence interval.

for that injection visit. Adverse events were classified by the maximum severity or the strongest recorded causal relationship to treatment, using Medical Dictionary for Regulatory Activities–preferred terms. Immunogenicity response rates and magnitudes among positive responders were compared between groups using Fisher exact or χ^2 tests and Wilcoxon rank sum tests. Response magnitudes for studies on longevity were compared for all participants (not just positive responders), using Wilcoxon signed rank tests. *P* values were not adjusted for

multiple comparisons. Wilson score intervals were reported for binomial proportions [29].

RESULTS

Participant Accrual, Demographic Characteristics, Tolerability, and Safety

The median age of the enrolled participants was 25 years, and 59% were male. The majority of participants were white (59%);

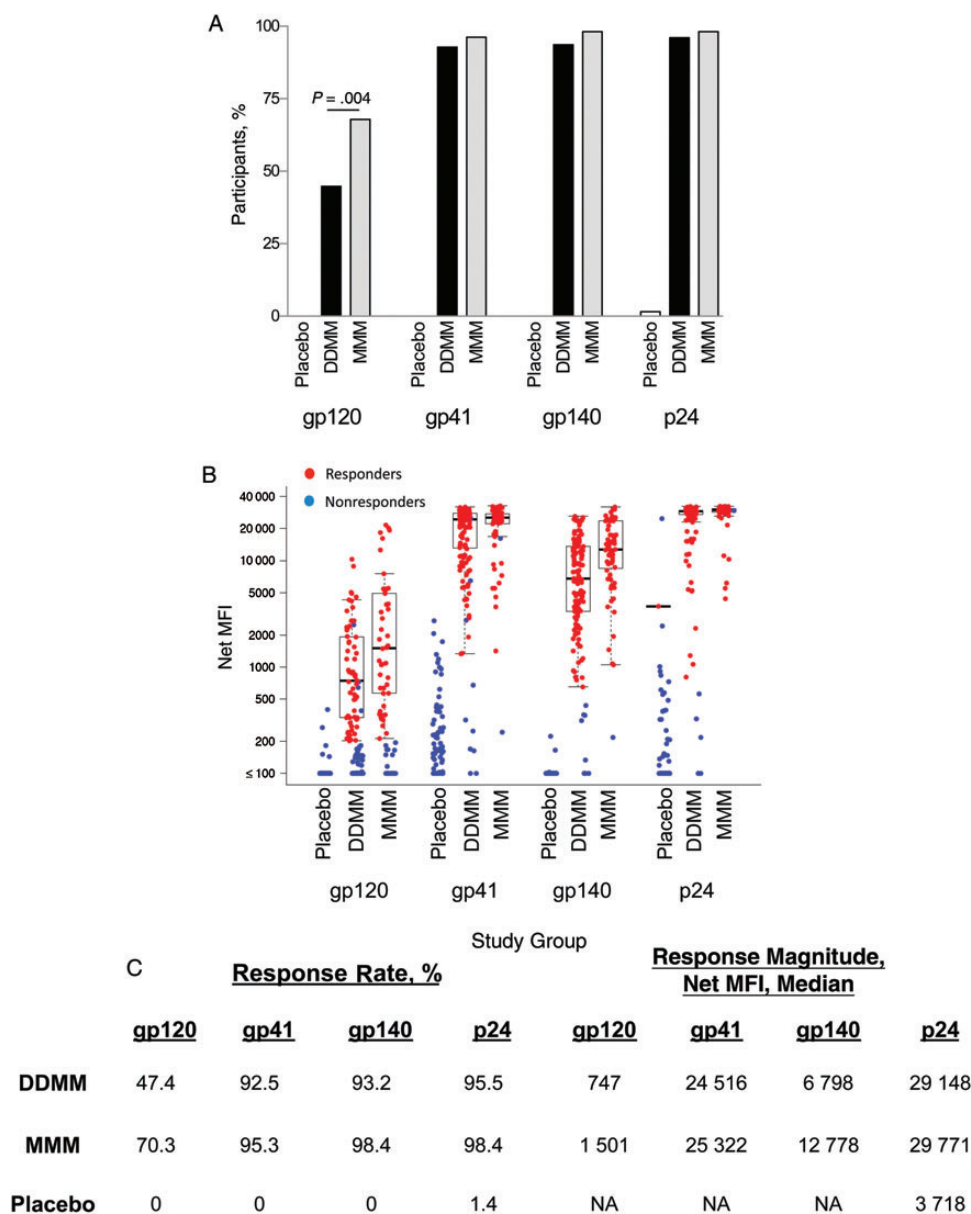


Figure 2. The proportion of responders and specificity of vaccine-induced binding antibody responses. *A–C*, The percentage of participants with detectable antibody responses to the indicated proteins (*A*) is given along with response magnitudes (*B*) and a response summary for the subset of individuals in the DDMM group, MMM group, or placebo group (*C*); see Subjects, Materials, and Methods for a description of each regimen. Response magnitudes for gp41 and p24 represent low estimates because responses of >23 000 are above the range of the assay. Median titers are in fluorescence units and are for positive responses. Box plots show median values and interquartile ranges (IQRs); the whiskers indicate the lowest datum still within 1.5 IQR of the lower quartile and the highest datum still within 1.5 IQR of the upper quartile. Abbreviations: MFI, mean fluorescence intensity, NA, not applicable.

13% were African American, and 23% were Hispanic. All participants received their initial vaccination, and 274 (91%) received all doses.

Both the DDMM and MMM vaccine regimens were safe and well tolerated. Participants had similar local (injection site) reactogenicity after placebo and JS7 DNA administrations, reporting no or mild local symptoms [19]. However, MVA62B was associated with an increased number of participants experiencing mild (up to 68% of participants) or moderate (up to 27% of participants) local reactogenicity (Supplementary Figure 1A). Most of the local reactogenicity was pain at the injection site. A majority of participants reported no or mild systemic symptoms, with a few moderate reactions, similar to placebo recipients (Supplementary Figure 1B).

There were no serious or life-threatening adverse events related to the vaccinations (Supplementary Table 1). However, 1 participant experienced an allergic reaction ≤ 15 minutes after the second MVA62B vaccination that was considered definitely related to the MVA62B inoculation (Supplementary Table 1). The symptoms resolved within 2 hours, and no further vaccinations were given. Fewer than 7% of the participants reported a social problem, and the majority felt that the problem represented a minimal impact.

Immunogenicity

HIV-1-Specific Ab Responses

The initial screen for Ab responses was conducted using gp140, an antigen that represents the gp120 receptor binding subunit of Env and the extracellular region of the gp41 transmembrane domain (Figure 1). The DDMM and MMM regimens elicited IgG Abs to gp140 in 93.2% and 98.4% of vaccine recipients, respectively. Testing of the specificity of the gp140 IgG response revealed that it was strongly biased toward gp41, to which 92.5% of subjects in the DDMM group and 95.3% in the MMM group responded (Figure 2A). In contrast, responses to Con6 gp120 were detected in 47% in the DDMM group and 70% in the MMM group. Response rates and titers to the matched ADA gp120 did not differ significantly from those to the consensus gp120. Titers of gp41-specific IgG were much higher than titers of gp120-specific IgG (>33 -fold higher for the DDMM group and >17 -fold higher for the MMM group; Figure 2B and 2C).

To assess the durability of the responses, Ab titers were assessed 2 and 24 weeks following vaccination. During this period, the proportion of responders for gp140 decreased by 15.6% for the DDMM group but did not decline in the MMM group (Figure 3). Over the same period, the

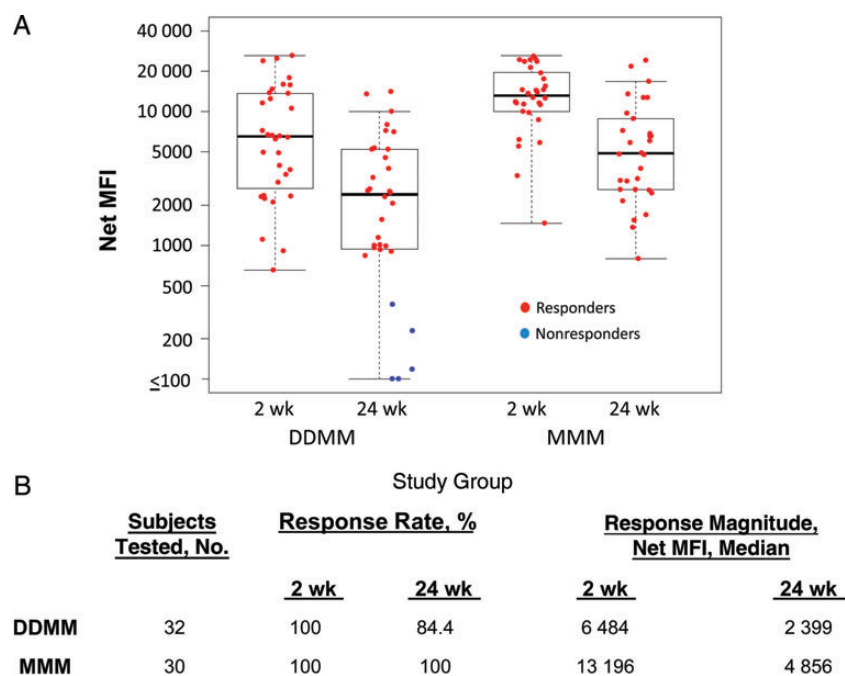


Figure 3. The durability of binding antibody responses in the DDMM and MMM groups; see Subjects, Materials, and Methods for a description of each regimen. *A*, The durability of positive antibody responses targeting gp140, by time after receipt of last vaccination. Box plots show median values and interquartile ranges (IQRs); the whiskers indicate the lowest datum still within 1.5 IQR of the lower quartile and the highest datum still within 1.5 IQR of the upper quartile. *B*, Summary of the number of participants and response rates and titers for antibody responses, by time after receipt of last vaccination. Throughout, the response rates and magnitudes are for the subset of individuals chosen for durability studies. Response titers are fluorescence units. All participants selected for this durability analysis had positive responses at 2 weeks. Responses for a few became negative at 24 weeks; summary statistics include both positive and negative responses. Abbreviation: MFI, mean fluorescence intensity.

magnitudes of Ab responses for both groups declined by 2.7-fold (Figure 3).

Two weeks after the last vaccination, the serum IgA response rates for gp140 were similar in the DDMM and MMM groups (12% vs 13%; Figure 1). The IgA response was also biased toward gp41, with IgA being detected for gp120 in only 2 participants. In the 11%–12% of participants with both IgG and IgA responses, the median ratio of IgG to IgA for gp140 was 17 for the DDMM group and 34 for the MMM group (data not shown).

Both vaccine regimens induced neutralizing Abs, with 64.4% in the MMM group and 30.4% in the DDMM group having detectable serum neutralizing responses against ≥ 1 tier 1 isolate (Figure 4A). Tier 2 isolates were neutralized by sera from 37% of participants in the MMM group and 15% in the DDMM

group (Figure 4C). Titers for 2 tier 1 isolates and 1 tier 2 isolate were higher for the MMM recipients (Figure 4B and 4D).

Vaccine-induced seroreactivity was noted in 84% of the individuals in the DDMM group and 87.7% in the MMM group (Supplementary Table 2). The majority of participants who developed vaccine-induced seroreactivity had a positive Western blot result, with reactivity to Gag and Env, the 2 major diagnostic determinants of HIV infection.

HIV-1 Specific T-Cell Responses

T-cell responses were less frequent than Ab responses (Figure 1). Two weeks following the final vaccination, 66% of participants in the DDMM group had detectable CD4⁺ T-cell responses, compared with 43% in the MMM group ($P = .005$). Vaccine-induced HIV-1-specific CD8⁺ T cells were detected in 22% in

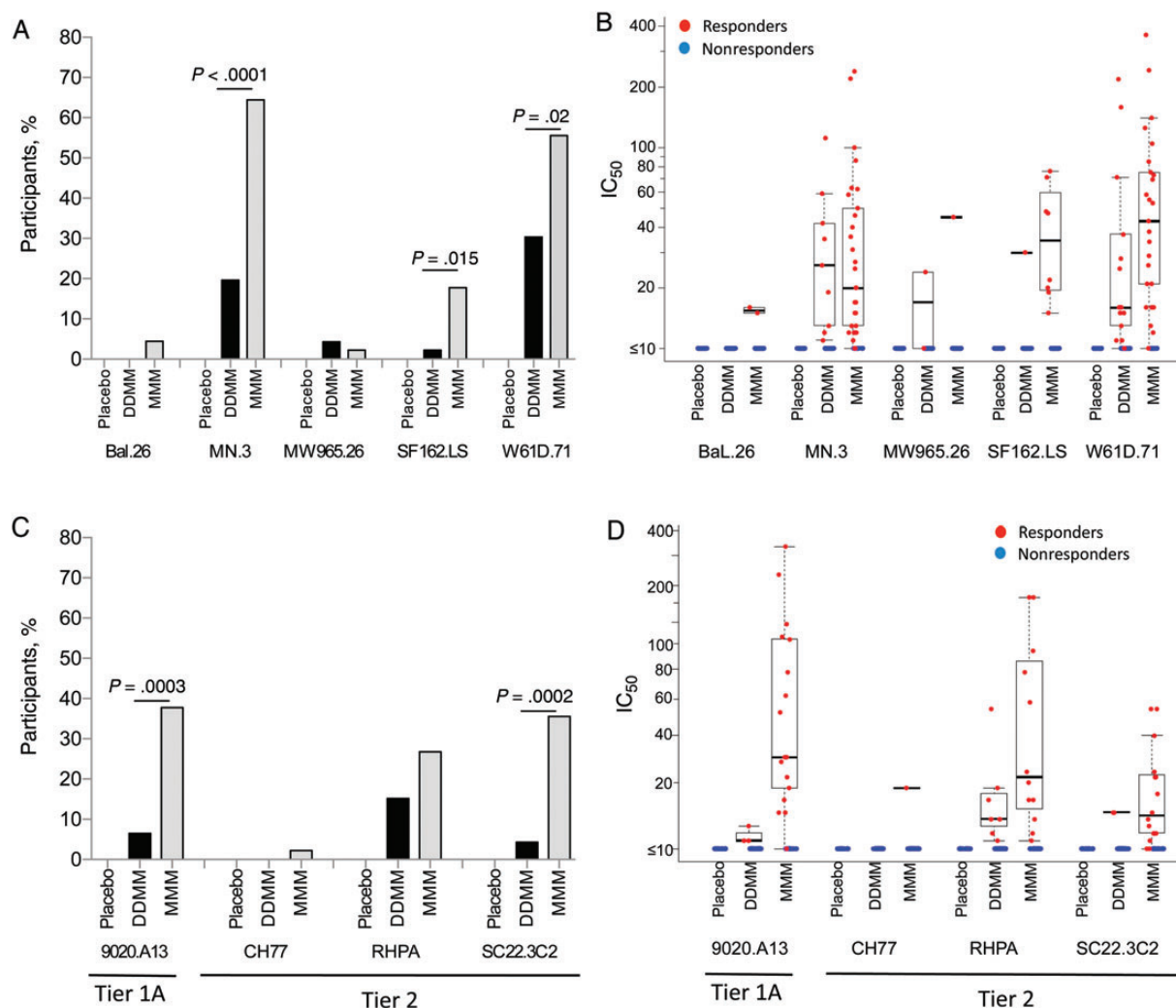


Figure 4. Vaccine-induced neutralizing antibody responses. *A* and *C*, The percentage of participants with human immunodeficiency virus–neutralizing antibodies. *B* and *D*, Magnitude of these responses 2 weeks after the final vaccination (both regimens). Panels *A* and *B* represent tier 1 viral isolates tested using the TZMBCL cell line, and panels *C* and *D* represent a tier 1A and 3 tier 2 viruses analyzed with the A3R5 cell line. See Subjects, Materials, and Methods for a description of each regimen and for further details. Abbreviation: IC₅₀, half maximal inhibitory concentration.

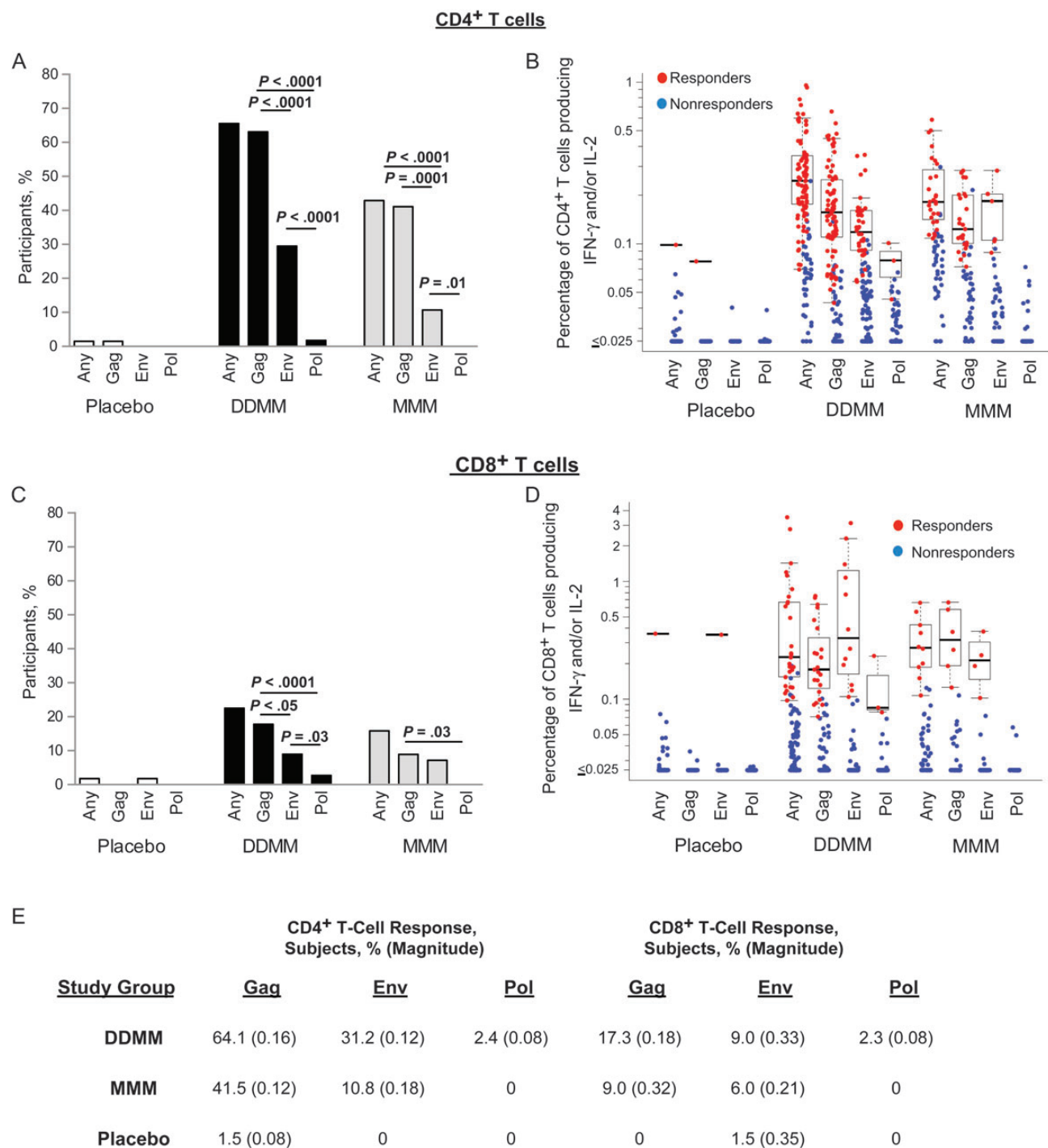


Figure 5. The proportion of responders and magnitude of vaccine-induced T-cell responses. *A* and *C*, The percentage of participants demonstrating CD4⁺ (*A*) and CD8⁺ (*C*) T-cell responses directed against any protein, Gag, Env, or Pol. *B* and *D*, The magnitude of responses directed against any protein, Gag, Env, or Pol among CD4⁺ and CD8⁺ T cells, shown as a percentage of the total CD4⁺ or CD8⁺ T cells. Box plots show median values and interquartile ranges (IQRs); the whiskers indicate the lowest datum still within 1.5 IQR of the lower quartile and the highest datum still within 1.5 IQR of the upper quartile. *E*, Median percentage of participants with CD4⁺ and CD8⁺ T-cell responses for Gag, Env, and Pol. The median magnitude of interferon γ (IFN- γ) and/or interleukin 2 (IL-2) production as a percentage of the total CD4⁺ or CD8⁺ T cells is shown in parentheses.

the DDMM group and 15% in the MMM group. The magnitudes of positive CD4⁺ and CD8⁺ T-cell responses were similar between groups (Figure 5*B*, 5*D*, and 5*E*).

In the DDMM group, both CD4⁺ and CD8⁺ T-cell responses targeted Gag more frequently than Env (Figure 5*A*, 5*C*, and 5*E*). For CD4⁺ T cells, 64.1% of the participants

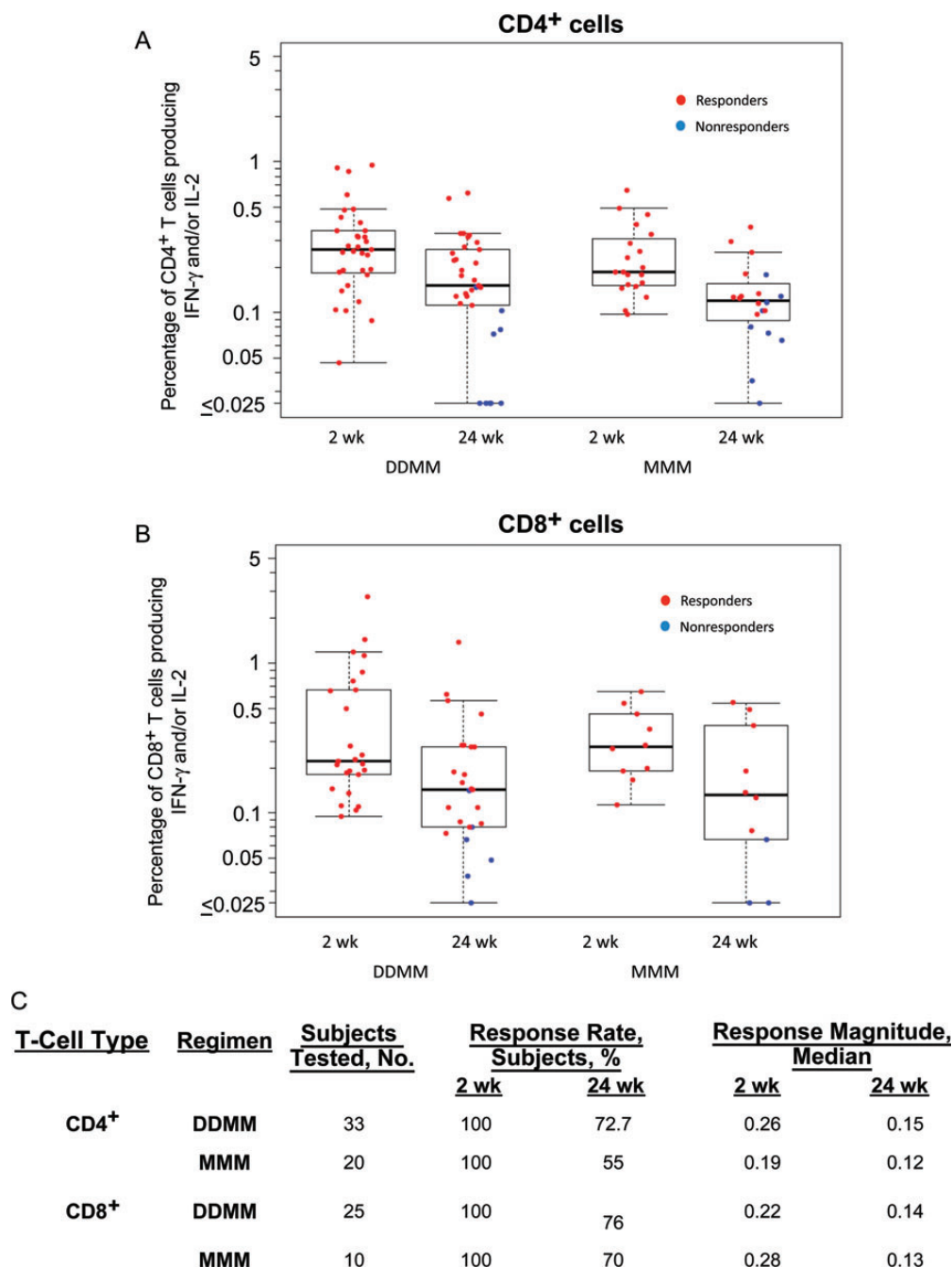


Figure 6. The durability of vaccine-induced T-cell responses for the subset of individuals in the DDMM and MMM groups who were chosen for durability studies; see Subjects, Materials, and Methods for a description of each regimen. *A* and *B*, Magnitude of vaccine-induced T-cell responses to Gag among CD4⁺ (*A*) and CD8⁺ (*B*) T cells obtained 2 and 24 weeks after the final vaccination from subjects, shown as a percentage of the total CD4⁺ or CD8⁺ T cells. Box plots show median values and interquartile ranges (IQRs); the whiskers indicate the lowest datum still within 1.5 IQR of the lower quartile and the highest datum still within 1.5 IQR of the upper quartile. *C*, Summary of the number of participants evaluated and CD4⁺ and CD8⁺ T-cell response rates and magnitudes. Response magnitudes are medians for responding cells as a percentage of the total number of CD4⁺ or CD8⁺ T cells. All participants selected for this durability analysis had positive responses at 2 weeks. A few became negative at 24 weeks; summary statistics include both positive and negative responses.

responded to Gag, whereas 31.2% responded to Env ($P < .0001$). For CD8⁺ T cells, 17.3% responded to Gag and 9% to Env ($P = .07$). In the MMM group, CD4⁺ T-cell response rates also were higher for Gag than for Env

(41.5% vs 10.8%; $P = .0001$), whereas for CD8⁺ T-cell response rates, the greater response rate for Gag was only a trend (Figure 5C and 5E). Pol responses were infrequently seen in both groups.

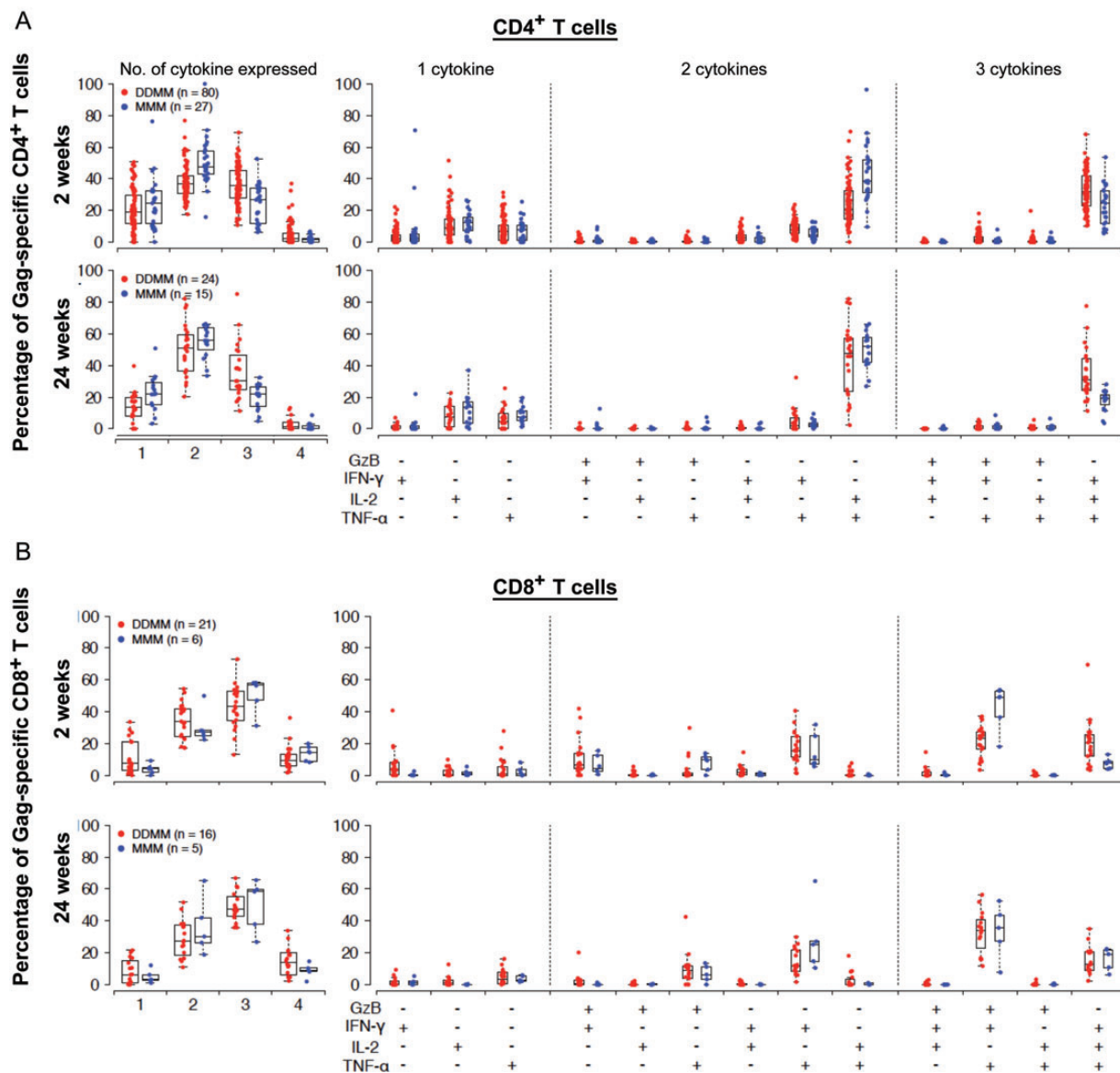


Figure 7. The polyfunctionality of vaccine-induced T-cell responses. *A*, The number of expressed functions and the cytokine production patterns for Gag-responding CD4⁺ (*A*) and CD8⁺ (*B*) T cells at 2 weeks (*A*; upper row) and 24 weeks (*A*; lower row) after the final vaccination. In each row, the left panel summarizes the percentage of Gag-specific cells with 1, 2, 3, or 4 expressed cytokines, and the right panel denotes the percentage of Gag-specific cells expressing each cytokine alone or combinations of 2 and 3 cytokines. Only participants with positive responses as determined for the interferon γ (IFN- γ) and/or interleukin 2 (IL-2) subset are in the analyses (left-most panels). See Subjects, Materials, and Methods for a description of the DDMM and MMM regimens. Box plots present median values and interquartile ranges. Abbreviations: GzB, granzyme B; TNF- α , tumor necrosis factor α .

Studies on the longevity of the T-cell responses to Gag revealed response rates falling by about 25% in the first 6 months after vaccination in the DDMM group and undergoing greater falls of 30% (for CD8⁺ T cells) and 45% (for CD4⁺ T cells) in the MMM group (Figure 6). During this 6-month period, the magnitudes of the CD4⁺ and CD8⁺ T-cell responses to Gag contracted by 1.6–2.1-fold (Figure 6).

To assess the polyfunctionality of T cells, responding cells were scored for coproduction of IFN- γ , IL-2, TNF- α , and granzyme B

(Figure 7). Two weeks following the final vaccination, CD4⁺ T cells from the DDMM and MMM groups most commonly expressed 2 or 3 of these cytokines (Figure 7A). For both regimens, the most frequent pattern for dual cytokine production was IFN- γ and TNF- α ; triple cytokine production most frequently added IL-2 (Figure 7A). These patterns of cytokine production were similar 24 weeks after the last vaccination (Figure 7B).

The CD8⁺ T-cell response showed higher polyfunctionality than the CD4⁺ T-cell response. Two weeks after the final

vaccination, CD8⁺ T cells from subjects in the DDMM and MMM groups expressed 3 cytokines slightly more often than they expressed 2 cytokines, with a minority of cells expressing 1 or 4 cytokines. Triple cytokine-producing CD8⁺ T cells induced by DDMM tended to include more IL-2 coproduction, whereas the MMM-induced cells included more granzyme B-coproducing cells (Figure 7C). Six months following the final vaccination, the differences in granzyme B and IL-2 coproduction for the DDMM and MMM CD8⁺ T-cell responses were no longer significant (Figure 7D). This reflected a higher proportion of granzyme B-coproducing cells in the DDMM group. Throughout the study, cells that were granzyme B positive were IL-2 negative (Figure 7). Both CD4⁺ and CD8⁺ T cells responding to Env showed patterns of cytokine coproduction similar to those of cells responding to Gag (data not shown).

DISCUSSION

An important finding for this trial was the durability of the Ab response to gp140, which declined by <3-fold during the first 6 months after vaccination (the latest time point studied). In trials using gp120 protein in alum as a boost for a poxvirus prime (RV144) [4] and gp120 protein in AS02A adjuvant for priming and boosting [30], declines in the magnitudes of binding Abs have been 10-fold in the same period [13, 30]. We hypothesize that the durability of the DDMM- and MMM-elicited Ab response reflects a number of factors that include the gp41 dominance of the response and the MVA vector stimulating the generation of survival signals in responding B cells [31]. The parent for the MVA vector, the smallpox vaccine, is known for its ability to establish long-lived Ab responses [32]. The durability of an Env-specific Ab response is important for an HIV vaccine because Env-specific Abs have the greatest potential to prevent infection.

Interestingly, the vaccines induced Env-specific Ab that was mainly focused on the relatively conserved gp41 subunit of HIV Env. The focus on gp41 is consistent with the virus-like particle vaccine inducing a response that mimics the response to HIV infection, in which emergence of Ab to gp41 precedes emergence of Ab to gp120 [22, 33]. Sequences in gp41 are targets for Ab-mediated virion capture [34]. They also include conserved targets for Ab-dependent cellular cytotoxicity that are exposed during virus fusion (G. Lewis, personal communication) [35]. In preclinical studies using a single high-dose simian/human immunodeficiency virus challenge, the avidity of Abs for the highly conserved immunodominant epitope of gp41 correlated with reductions in peak viremia [2].

Env-specific serum IgA responses were poorly induced in this study. In the RV144 study, titers of Env-specific serum IgA correlated with decreased vaccine efficacy, likely due to IgA competing for binding with IgG and reducing the initiation of Fcγ-mediated mechanisms of protection [7, 11].

Comparison of Ab responses elicited by the DDMM and MMM regimens confirmed that the MMM regimen elicited higher titer gp120 responses and higher titer neutralizing Ab responses, as previously noted in the phase 1 trial, HVTN 065 [19]. The immunogens expressed by the DDMM and MMM regimens differ in that the Env expressed by the DNA prime is a C-terminal complete gp160, whereas the gp150 expressed by the MVA is truncated for the C-terminal endodomain of gp41. The increased immunogenicity for gp120 for the MMM regimen could reflect the gp150-truncated Env having a more open structure than the gp160 expressed by the DNA prime. Similar truncations have opened up the structure of Env, as measured by tests for the binding of Abs to the CD4 binding site and sensitivity to neutralization [36].

The magnitude of the T-cell responses also showed good durability, declining by only 1.6–2.1-fold during the first 6 months after vaccination. These responses were qualitatively different than those induced by recombinant adenovirus type 5 vectors (rAd5), as given in the Step Study [37] and HVTN 505 trials [38], neither of which protected vaccinees against HIV-1 infection. rAd5 induced an immune response biased toward CD8⁺ T cells over CD4⁺ T cells, whereas the opposite was true for MVA62B and other pox vectors in the presence or absence of a DNA prime [39, 40]. Also, the DDMM and MMM regimens induced CD8⁺ T cells that predominantly targeted Gag and not Env, as elicited in the VRC DNA/rAd5 regimen [41], or Pol, as elicited in the Step Study [27]. Targeting of Gag epitopes by CD8⁺ T cells has been demonstrated in multiple studies to correlate with viral control [42–45].

Consistent with prior studies, the DDMM and MMM regimens elicited more highly polyfunctional cells than rAd5 regimens have elicited [27, 41]. The DDMM and MMM vaccinations elicited dual-producing CD4⁺ T cells most frequently, followed by triple-producing CD4⁺ T cells and single-producing CD4⁺ T cells, whereas rAd5 immunizations (in the presence or absence of a DNA prime) elicited frequencies of these populations that were similar to each other. The DDMM and MMM regimens also elicited predominantly triple-producing CD8⁺ T cells, whereas dual-producing CD8⁺ cells have been most frequently elicited by rAd5 regimens. The patterns of T-cell polyfunctionality were remarkably stable with time. An instance in which polyfunctionality changed over 6 months was for more granzyme B coexpression appearing in the DDMM group.

The comparisons of binding and neutralizing Abs between the 2 regimens favor the MMM strategy, whereas comparisons of cellular immunity favor the DDMM approach. Because the Ab responses after the second MVA inoculation in the MMM regimen were similar to those after the second (and final) MVA inoculation in the DDMM regimen [19], a DDMM regimen with a third MVA boost (ie, a DDMMM regimen) will be moved forward into efficacy trials. The goal of the DDMMM regimen is to optimize both Ab and T-cell responses.

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflict of interest. H. L. R. is a stakeholder in GeoVax. M. J. M. works for Emory, which is a stakeholder in GeoVax, but M. J. M. does not have any direct holdings in GeoVax. All other authors report no potential conflicts.

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