Safety and Immunogenicity of Adenovirus-Vectored Near-Consensus HIV Type 1 Clade B *gag* Vaccines in Healthy Adults

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Abstract

Vaccines inducing pathogen-specific cell-mediated immunity are being developed using attenuated adenoviral (Ad) vectors. We report the results of two independent Phase I trials of similar replication-deficient Ad5 vaccines containing a near-consensus HIV-1 clade B *gag* transgene. Healthy HIV-uninfected adults were enrolled in two separate, multicenter, dose-escalating, blinded, placebo-controlled studies to assess the safety and immunogenicity of a three-dose homologous regimen of Ad5 and MRKAd5 HIV-1 *gag* vaccines given on day 1, week 4, and week 26. Adverse events were collected for 29 days following each intradeltoid injection. The primary immunogenicity endpoint was the proportion of subjects with a positive unfractionated Gag-specific IFN- γ ELISPOT response measured 4 weeks after the last dose (week 30). Analyses were performed after combining data for each dose group from both protocols, stratifying by baseline Ad5 titers. Overall, 252 subjects were randomized to receive either vaccine or placebo, including 229 subjects (91%) who completed the study through week 30. Tolerability and immunogenicity did not appear to differ between the Ad5 and MRKAd5 vaccines. The frequency of injection-site reactions was dose dependent. Systemic adverse events were also dose dependent and more frequent in subjects with baseline Ad5 titers <200 versus $\geq$ 200, especially after the first dose. The percent of ELISPOT responders and the ELISPOT geometric means overall were significantly higher for all four vaccine doses studied compared to placebo, and were generally higher in vaccine recipients with baseline Ad5 titers <200 versus $\geq$ 200. Ad5 titers increased after vaccination in a dose-dependent fashion. Both Ad5-vectored HIV-1 vaccines were generally well tolerated and induced cell-mediated immune responses against HIV Gag-peptides in the majority of healthy adults with baseline Ad5 titers <200. Preexistent and/or vaccine-induced immunity to the Ad5 vector may dampen the CMI response to HIV Gag.

Introduction

THE HUMAN IMMUNODEFICIENCY VIRUS (HIV) pandemic continues to inflict devastating morbidity and mortality in many areas of the world.^{1,2} The global spread of multidrug-resistant HIV increasingly threatens the utility of currently available antiretroviral agents.^{3–10} In this context, developing an effective HIV vaccine remains an urgent, albeit formidable priority.^{11,12} Early studies in chimpanzees suggested that induction of neutralizing antibodies might provide protection

against HIV infection in humans.¹³ Consequently, initial prophylactic HIV-1 vaccine trials in humans focused on inducing neutralizing antibody to envelope proteins. However, this approach has not yet proven to be effective.^{14–17} More recent efforts have concentrated on developing vaccines to elicit potent and sustained cell-mediated immunity (CMI) against HIV.^{14,18–20}

Virus-specific T lymphocytes appear to be critical for the control of simian immunodeficiency virus and HIV infections.^{21–23} Various modalities to induce HIV-1-specific CMI

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have been evaluated in animals and humans, including DNA plasmids containing partial or whole copies of HIV genes and expression of HIV genes via attenuated viral and bacterial vectors. After comparing several vector-based approaches in primate challenge model systems, Merck Research Laboratories initiated clinical development of HIV-1 vaccines using replication-defective adenovirus serotype 5 (Ad5) vectors expressing a near-consensus clade B *gag* transgene. The current report summarizes immunogenicity, safety, and tolerability data from the first two dose-ranging studies evaluating these candidate vaccines in healthy human volunteers.

Subsequent to completion of these Phase I studies, vaccination in a proof-of-concept Phase II trial using a similar trivalent *gag/pol/nef* vaccine to prevent or modulate HIV-1 infection was suspended because a planned interim analysis indicated lack of efficacy.^{24,25} Subjects with high titers of neutralizing antibodies to Ad5 at baseline developed numerically more HIV infections postvaccination than those with lower Ad5 titers. Despite the Phase II efficacy results, data from the Phase I program provide valuable information pertinent to the safety and immunogenicity of HIV and other vaccines using adenoviral vectors.

Materials and Methods

Objectives

The primary objectives of dose-ranging V520 Protocol 007 and Protocol 012 were to assess the safety, tolerability, and immunogenicity of a three-dose regimen of Ad5-vectored HIV-1 vaccine candidates. The primary immunogenicity endpoint was specified per protocol as the proportion of subjects with a positive unfractionated Gag-specific interferon (IFN)- γ ELISPOT response to 15-mer peptides using peripheral blood mononuclear cells (PMBC) obtained at week 30 (4 weeks after the last dose).

Vector construction and vaccine composition

Two similar adenoviral vectors were studied. Each vector was based on a replication-defective adenovirus type 5 backbone in which the deleted E1 gene region had been replaced with an expression cassette for the *gag* gene from the CAM-1 strain of HIV-1 (GenBank Locus BAA00992). This HIV strain was chosen because its *gag* gene closely resembles the clade B consensus amino acid sequence. The *gag* construct was "optimized" using nucleotide base substitution to improve protein expression in mammalian cells. The vaccine was formulated in a sterile solution of 10 mM TRIS buffer with 10 mM histidine, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.1 mM EDTA, 0.5% ethanol, and 0.02% polysorbate 80, pH 7.4. Placebo consisted of an identical vehicle without the adenovirus component.

The first vector studied was simply designated Ad5 HIV-1 *gag*. In this vector, the adenovirus E3 gene region had also been deleted. In preclinical studies, this vaccine appeared to be immunogenic and to have a safety profile that was acceptable for moving into human trials, culminating in Protocol 007 to study this vector in humans. After the initiation of Protocol 007, studies designed to support large-scale manufacture of the Ad5 HIV-1 *gag* vaccine revealed a degree of genetic instability in later passages of the vector that would make the vector unsuitable for large-scale production.

In an attempt to improve the genetic stability of the vector, the same *gag* transgene was incorporated into a related Ad5 vector with a modified backbone designated MRKAd5 HIV-1 *gag*.²⁶ The key differences between the original and the modified vector included the following: (1) the orientation of the HIV-1 transgene was changed from antiparallel to parallel, (2) intron A of the CMV promoter was removed, (3) the 5' adenovirus packaging region was modified, and (4) the adenoviral E3 region was reinserted into this backbone. The MRKAd5 HIV-1 *gag* vaccine was used in Protocol 012. This modified vector was subsequently chosen for further development because no significant genetic instability could be demonstrated.

Study design

Both studies were Phase 1, placebo-controlled, multicenter, double-blind, randomized, dose-escalating trials. Eligible subjects were healthy HIV-seronegative adults aged 18–50 years who denied engaging in behaviors that would put them at high risk of exposure to HIV. Subjects had to be in good health as determined by medical history and physical examination, and laboratory tests of blood chemistry, hematology, liver function, renal function, and clotting function had to be within normal limits. Pregnant or breastfeeding women were excluded. Female subjects of childbearing potential were required to have a negative urine pregnancy test immediately prior to each injection and were counseled to avoid pregnancy during trial participation. Ongoing HIV risk assessment and prevention counseling was offered to the study participants during the trials. Each protocol was approved by the institutional review board at the participating sites. Written informed consent was obtained from all subjects.

The studies enrolled volunteers in successive dose-escalating stages such that clinical and laboratory safety data for 12 subjects (nine vaccine and three placebo recipients) at each dose level were reviewed and found to be acceptable by a Safety Evaluation Committee prior to enrollment of subjects in the next higher dose group. After the highest dose level had been satisfactorily tested, additional subjects were randomized across all dose levels. In Protocol 007, the dose levels of the Ad5 HIV-1 *gag* vaccine tested were 1×10^8 , 1×10^9 , 1×10^{10} , or 1×10^{11} vp/dose. In Protocol 012, the dose levels of the MRKAd5 HIV-1 *gag* vaccine tested were 1×10^9 , 1×10^{10} , or 1×10^{11} vp/dose. Randomization was not stratified by pre-existing Ad5 immunity, except for the final stage in Protocol 012 when subjects were randomized across all dose levels.

Enrolled subjects were randomized to receive 1.0-ml injections of placebo or vaccine into the deltoid muscle. Allocation schedules were generated by computer. Doses were to be given at day 1, week 4, and week 26. Investigators, subjects, clinical safety monitors, and laboratory personnel performing the biological assays were blinded to treatment assignments. The studies were designed to have a total duration of 5 years, but subjects could discontinue from the parent study after 1 year to participate in another Phase I HIV vaccine trial.

Safety and immunogenicity assessments

Clinic visits for safety evaluations were scheduled 1 and 2 weeks after each dose. For 5 days after each injection, subjects were to record the largest diameter of induration or erythema at the injection site with a standard ruler. Injection site pain

and tenderness were to be rated as “none,” “mild,” “moderate,” or “severe.” Subjects were to complete a vaccine report card tracking daily temperatures and physical complaints for 29 days after each dose. All adverse events were graded according to prespecified severity scales. Fever was defined as a temperature $>100^{\circ}\text{F}$ at any time within the 29-day period following each injection. Routine laboratory tests including a complete blood count, urinalysis, and serum levels of creatinine, amylase, prothrombin and partial thromboplastin times, C-reactive protein, creatine phosphokinase, phosphorus, and liver function tests were to be performed at each safety follow-up visit. Surveillance for shedding of the vaccine Ad5 strain was to be performed during the 2-week period following each injection by collecting pharyngeal adenovirus culture from subjects developing symptoms compatible with a viral respiratory infection or influenza-like illness (manifested as fever, chills, fatigue, and/or myalgia) and by collecting urine cultures from subjects experiencing symptoms of urinary tract infection or with asymptomatic hematuria or pyuria on routine urinalysis. Ad5 isolates were compared to the vaccine strain.²⁷

CMI responses were measured by an unfractionated HIV-1 Gag-specific IFN- γ ELISPOT assay.^{28,29} A positive ELISPOT response required both ≥ 55 spot-forming cells (SFCs)/ 10^6 peripheral blood mononuclear cells (PBMCs) and ≥ 4 -fold increase over the nonantigen control result.^{29,30} Serum Ad5 titers were measured at baseline and ~ 4 weeks after each dose in a quantitative neutralization assay using a recombinant adenovirus type 5.³¹

Statistical analyses

Combining data from protocols 007 and 012. Because the vaccine vector was the only meaningful difference between the protocols and was not expected to differentially impact the safety or immunogenicity results, it was decided after completion of the studies to perform analyses on the combined dataset. No formal test of homogeneity across studies was performed.

Safety. All subjects receiving one or more doses were included in the safety analysis. The proportions of subjects

FIG. 1. Subject accounting for protocols 007 and 012 combined. *Primary immunogenicity analysis was performed at week 30 (4 weeks after the third dose). An individual subject could be excluded for multiple reasons.

TABLE 1A. BASELINE CHARACTERISTICS BY TREATMENT GROUP FOR BOTH PROTOCOLS COMBINED

	Placebo groups (N = 49)	Vaccine groups ^a				Total (N = 252)
		1×10^8 vp/d ^a (N = 24) ^b	1×10^9 vp/d (N = 49)	1×10^{10} vp/d (N = 65)	1×10^{11} vp/d (N = 65)	
Gender, <i>n</i> ^c (%)						
Female	28 (57.1)	13 (54.2)	21 (42.9)	30 (46.2)	34 (52.3)	126 (50.0)
Male	21 (42.9)	11 (45.8)	28 (57.1)	35 (53.8)	31 (47.7)	126 (50.0)
Age, years						
Mean	34.3	35.5	35.6	32.3	33.7	34.0
SD	10.1	8.9	10.0	8.8	9.5	9.5
Median	35.0	37.0	36.0	31.0	33.0	34.0
Range	18 to 51	20 to 50	19 to 50	18 to 49	19 to 50	18 to 51
Race/ethnicity, <i>n</i> (%)						
Asian/Pacific	0 (0)	1 (4.2)	0 (0)	1 (1.5)	2 (3.1)	4 (1.6)
Black	6 (12.2)	4 (16.7)	5 (10.2)	5 (7.7)	4 (6.2)	24 (9.5)
White	43 (87.8)	19 (79.2)	42 (85.7)	54 (83.1)	56 (86.2)	214 (84.9)
Hispanic	0 (0)	0 (0)	2 (4.1)	5 (7.7)	1 (1.5)	8 (3.2)
Native American	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.5)	1 (0.4)
Other	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.5)	1 (0.4)
Baseline Ad5 titer, <i>n</i> (%)						
≤18	15 (30.6)	6 (25.0)	22 (44.9)	23 (35.4)	28 (43.1)	94 (37.3)
19–200	16 (32.7)	9 (37.5)	11 (22.4)	14 (21.5)	17 (26.2)	67 (26.6)
>200	17 (34.7)	8 (33.3)	16 (32.7)	26 (40.0)	17 (26.2)	84 (33.3)
Unknown	1 (2.0)	1 (4.2)	0 (0)	2 (3.1)	3 (4.6)	7 (2.8)

^avp/d, viral particles per dose.^bN, number of subjects randomized.^c*n*, number of subjects with the specified characteristic.

with injection-site reactions, systemic adverse events, and laboratory adverse events within 29 days after each dose were summarized by treatment group separately for the two baseline Ad5 antibody strata as well as for the two strata combined. For the combined strata, summary statistics were calculated using a weighted average of the observed stratum-specific percentages, with weights proportional to the overall observed stratum sizes. The frequencies of specific types of injection-site reactions, systemic adverse events, and laboratory adverse events in the vaccine dose groups were compared to the corresponding frequency in the placebo group by the Cochran–Armitage trend test, starting with all the vaccine

groups and sequentially excluding the highest remaining vaccine dose group as long as the difference versus the placebo group remained significant as judged by a one-tailed *p*-value <0.025. Since multiple adverse events were being compared separately, *p*-values were deemed statistically significant only if they remained <0.025 after applying a multiplicity adjustment.³⁰ Small sample sizes precluded comparisons between treatment groups within baseline Ad5 strata.

Immunogenicity. Only subjects receiving all three scheduled doses of vaccine or placebo and without major protocol violations were included in the analysis of immunogenicity.

TABLE 2. SUMMARY OF PERTINENT DATA FOR THE THREE SUBJECTS DIAGNOSED WITH HIV INFECTION DURING THE STUDIES

HIV-infected patients	Protocol/treatment group ^a	Doses received (n)	Baseline Ad5 titer	Study week of diagnosis ^b	Plasma HIV RNA level at diagnosis ^c	Week 30 ELISPOT ^d reactivity	Week 30 Gag-ELISPOT ^d responses ^e
No. 1	007/ 1×10^{10}	3	846	104	53500	Positive	400
No. 2	007/placebo	3	18	34 (unscheduled visit)	29591	Positive	370
No. 3	012/ 1×10^9	3	18	42	>75000	Positive	173

^aFor vaccine recipients, the vaccine dose is given as viral particles per dose.

^bDiagnosis was based on positive EIA and reflexive Western blot results confirmed by PCR demonstration of viremia. Per protocol, EIA was to be performed at screening and week 78 but could also be done at the investigator's discretion. Time of infection cannot be precisely determined because EIA was not periodically repeated during the study (and the expected but variable delay between infection and seroconversion).

^cUltrasensitive HIV RNA levels were measured by the HIV-1 Amplicor Monitor test (Roche Diagnostics, Branchburg, NJ) and are reported as copies/ml. The dynamic range for this assay is 50 to 75,000 copies/ml. This study was not designed to assess virologic endpoints. Since serologic and PCR testing was not done systematically throughout the study and treatment histories were unavailable, the HIV RNA levels at the time of diagnosis may not reflect the steady-state viral "set point."

^dELISPOT results are presented for the primary week 30 immunogenicity time point. A positive ELISPOT required both ≥ 55 SFC/ 10^6 PBMC and ≥ 4 -fold increase over the nonantigen control.

^eResponses are reported as SFC/ 10^6 PBMC.

TABLE 3. FREQUENCIES OF COMMON^a SYSTEMIC ADVERSE EVENTS DURING THE 29 DAYS AFTER EACH DOSE IN THE COMBINED STUDIES FOR THE THREE HIGHEST DOSE LEVELS BY BASELINE Ad5 TITER^b

	Baseline Ad5 titer ≤200				Baseline Ad5 titer >200				Combined Ad5 strata ^c			
	1×10 ⁹		1×10 ¹⁰		1×10 ⁹		1×10 ¹⁰		Pbo		1×10 ⁹	
	(N = 31)	(N = 31)	(N = 37)	(N = 45)	(N = 16)	(N = 16)	(N = 25)	(N = 17)	(N = 47)	(N = 47)	(N = 62)	(N = 62)
A. Postdose 1												
Headache	32%	45%	49%	49%	31%	25%	20%	35%	32%	38%	39%	44%
Chills	0%	0%	14%	44%	0%	0%	0%	6%	0%	0%	9% ^d	31% ^{d,e}
Myalgia	7%	10%	16%	36%	0%	6%	0%	18%	4%	9%	11%	29% ^{d,e}
Fatigue	7%	10%	30%	24%	13%	6%	8%	18%	9%	9%	22%	22%
Pyrexia	3%	3%	3%	31%	6%	0%	0%	0%	4%	2%	2%	20% ^{d,e}
Pain	0%	10%	16%	24%	0%	0%	4%	0%	0%	6%	12% ^d	16% ^{d,e}
Pharyngolaryngeal pain	7%	7%	5%	11%	0%	0%	0%	24%	4%	4%	4%	15%
Nausea	10%	10%	11%	20%	6%	0%	12%	6%	9%	6%	11%	15%
Nasal congestion	3%	7%	5%	9%	0%	0%	0%	24%	2%	4%	4%	14% ^d
Back pain	3%	0%	11%	13%	0%	6%	12%	12%	2%	2%	11%	13% ^d
Diarrhea	13%	10%	8%	13%	13%	25%	4%	12%	13%	15%	7%	13%
Body temperature increased	0%	0%	0%	13%	6%	0%	4%	0%	2%	0%	1%	9%
Arthralgia	0%	0%	8%	7%	0%	0%	0%	12%	0%	0%	5%	8% ^d
Dizziness	0%	7%	0%	9%	6%	19%	0%	6%	2%	11%	0%	8%
Cough	3%	10%	5%	7%	6%	0%	0%	6%	4%	6%	4%	6%
Pain in extremity	3%	7%	3%	7%	0%	6%	0%	6%	2%	2%	5%	6%
Vomiting	0%	0%	0%	2%	0%	0%	0%	12%	0%	0%	0%	6%
Herpes simplex	3%	10%	3%	2%	6%	0%	4%	12%	4%	6%	3%	6%
Rhinorrhea	0%	0%	0%	4%	6%	0%	0%	0%	6%	0%	0%	3%
Abdominal pain upper	7%	0%	5%	5%	6%	0%	0%	0%	6%	0%	4%	4%
Nasopharyngitis	0%	7%	5%	0%	0%	0%	8%	6%	0%	4%	6%	2%
Muscle spasms	3%	0%	5%	0%	0%	0%	8%	0%	2%	0%	6%	0%

(continued)

TABLE 3. (CONTINUED)

	Baseline Ad5 titer ≤200				Baseline Ad5 titer >200				Combined Ad5 strata ^c			
	Pbo (N = 31)	1 × 10 ⁹ (N = 31)	1 × 10 ¹⁰ (N = 37)	1 × 10 ¹¹ (N = 45)	Pbo (N = 16)	1 × 10 ⁹ (N = 16)	1 × 10 ¹⁰ (N = 25)	1 × 10 ¹¹ (N = 17)	Pbo (N = 47)	1 × 10 ⁹ (N = 47)	1 × 10 ¹⁰ (N = 62)	1 × 10 ¹¹ (N = 62)
B. Postdose 2												
Headache	30%	32%	24%	27%	19%	6%	42%	24%	26%	23%	30%	26%
Fatigue	10%	7%	8%	16%	0%	0%	13%	29%	7%	4%	10%	20% ^d
Myalgia	10%	7%	3%	13%	6%	0%	4%	12%	9%	4%	3%	13%
Nausea	17%	3%	0%	11%	6%	0%	4%	12%	13%	2%	1%	11%
Pharyngolaryngeal pain	13%	13%	16%	11%	0%	13%	8%	12%	9%	13%	14%	11%
Cough	10%	13%	0%	4%	6%	0%	13%	18%	9%	9%	4%	9%
Nasal congestion	7%	3%	8%	7%	6%	6%	0%	12%	7%	4%	5%	8%
Diarrhea	3%	3%	0%	4%	6%	13%	0%	12%	4%	6%	0%	7%
Chills	0%	7%	3%	9%	0%	0%	4%	0%	0%	4%	3%	6%
Neck pain	3%	3%	0%	9%	6%	6%	0%	0%	4%	4%	0%	6%
Pain	7%	7%	8%	9%	6%	0%	8%	0%	7%	4%	8%	6%
Vomiting	10%	3%	0%	0%	6%	0%	0%	12%	9%	2%	0%	4%
Rhinorrhea	0%	13%	3%	2%	6%	6%	8%	6%	2%	11%	5%	4%
Sinus congestion	0%	13%	5%	4%	0%	0%	8%	0%	0%	9%	6%	3%
Dysmenorrhea	0%	3%	8%	0%	0%	6%	4%	0%	0%	4%	7%	0%
Sneezing	3%	13%	3%	0%	0%	0%	0%	0%	2%	9%	2%	0%
C. Postdose 3												
Headache	17%	16%	21%	13%	29%	29%	21%	18%	21%	20%	21%	15%
Fatigue	3%	0%	12%	8%	21%	14%	8%	24%	10%	5%	11%	13%
Pain	0%	7%	9%	10%	0%	7%	0%	18%	0%	7%	6%	13% ^d
Pharyngolaryngeal pain	10%	7%	15%	10%	14%	21%	13%	12%	12%	12%	14%	11%
Diarrhea	0%	3%	3%	8%	29%	21%	8%	12%	10%	10%	5%	9%
Myalgia	7%	10%	9%	13%	14%	7%	0%	0%	9%	9%	6%	8%
Pain in extremity	0%	0%	0%	3%	0%	7%	0%	12%	0%	2%	0%	6%
Back pain	3%	10%	3%	5%	7%	7%	8%	6%	5%	9%	5%	5%
Body temperature increased	0%	0%	3%	5%	0%	7%	4%	6%	0%	2%	3%	5%
Nausea	3%	13%	3%	5%	0%	0%	4%	6%	2%	9%	3%	5%
Pyrexia	0%	3%	3%	5%	7%	0%	8%	6%	2%	2%	5%	5%
Sinus congestion	0%	3%	0%	5%	7%	14%	0%	6%	2%	7%	0%	5%
Nasal congestion	7%	0%	9%	3%	0%	0%	0%	0%	5%	0%	6%	2%

^aAdverse events with combined incidence $\geq 5\%$ for at least one of the active dose levels are displayed. Table entries are sorted by the combined incidence for the highest dose level.^bDose units, viral particles per dose; Pbo, Placebo; N, number of subjects with follow-up.^cCombined across baseline Ad5 strata using a weighted average, with weights proportional to the observed total stratum sizes. Small sample sizes precluded comparisons *within* baseline Ad5 strata.^dOne-tailed p -value < 0.025 before a multiplicity adjustment.^eOne-tailed p -value < 0.025 after a multiplicity adjustment.

Three patients who acquired HIV infection were removed from the analyses. ELISPOT results were summarized by treatment arm at each time point in a manner analogous to that used for the safety analyses. Summary statistics included the proportions of ELISPOT responders and the geometric means of the quantitative ELISPOT responses. Differences in the frequencies of postprime (week 8) and postboost (week 30) ELISPOT responders between a given vaccine dose group and the placebo group were analyzed using the Cochran-Armitage trend test as detailed above. One-tailed *p*-values <0.025 were considered statistically significant; a multiplicity adjustment was not required because a given dose was formally compared to placebo only if the higher doses statistically differed from placebo.³³ An analogous sequential trend testing approach was used for comparison of geometric mean ELISPOT values between each vaccine dose and placebo. The effect of baseline Ad5 titers on ELISPOT responses was explored using standard linear regression techniques.

Results

Subject accounting and baseline characteristics

For Protocol 007, 302 subjects were screened for eligibility from January 2001 through May 2003; for Protocol 012, 137 subjects were screened for eligibility from February 2002 through January 2003 (Fig. 1). Overall, 252 subjects were randomized to receive either vaccine or placebo. In Protocol 007, 112 subjects entered the trial during the open enrollment stage; in Protocol 012, 56 subjects entered the trial during the open enrollment stage. Tolerability and immunogenicity did not appear to differ between the Ad5 and MRKAd5 vaccines. Because no notable differences were observed in results for the two studies, the data were combined to increase the power of the analyses.

A total of 230 subjects received all three doses, including 217 subjects who completed the study through the primary

immunogenicity time point at week 30. The placebo and vaccine groups did not materially differ in terms of age, race, or gender, but the vaccine groups overall had a modestly higher proportion of Hispanics and Asians than the placebo groups (Table 1). There were no discontinuations for vaccine-related adverse events. As of April 1, 2008, three subjects had been diagnosed with HIV infection during the studies (Table 2). These subjects were excluded from all the immunogenicity analyses. In retrospect, the three HIV-infected subjects reported high-risk sexual behavior during the study period.

As of April 1, 2008, seven pregnancies had been reported in five participants during the studies. The two pregnancies in two vaccine recipients resulted in the delivery of a healthy full-term infant at study week 88 and an elective first-trimester abortion during study week 16.

Local and systemic tolerability

A total of 244 subjects (197 vaccine and 47 placebo recipients) were included in the combined safety analysis (Table 3). Frequencies of adverse events for the lower vaccine dose groups were generally similar to placebo. Both systemic and injection-site reactions occurred more often in the higher vaccine dose groups than in the placebo group. The most common clinical adverse experiences were injection-site reactions, consisting of local pain and erythema. Injection-site reactions were comparably frequent after each of the three injections in the series and did not differ appreciably between subjects with low or high baseline Ad5 titers. In contrast, systemic adverse experiences were most common after the first dose and less frequent with subsequent doses. Especially following the initial dose, subjects with low baseline Ad5 titers reported higher rates of systemic adverse experiences than subjects with high baseline Ad5 titers. The majority of systemic adverse events were categorized as mild or moderate

TABLE 4. SUMMARY OF HIV-1 GAG-SPECIFIC ELISPOT RESPONSES: PERCENTAGE OF RESPONDERS AND GEOMETRIC RESPONSES BY BASELINE Ad5 TITER AND TREATMENT GROUP AT STUDY WEEKS 8 AND 30^{a,b}

Dose (vp/d)	Baseline Ad5 titer ≤200			Baseline Ad5 Titer >200			Combined Ad5 strata ^c		
	N	% Responders ^c	Geometric mean ^d	N	% Responders ^c	Geometric mean ^d	N	% Responders ^c	Geometric mean ^d
Week 8									
Placebo	27	3.70%	28	16	0.0%	28	43	2.4%	28
1×10 ⁸	15	60.0%	175	8	0.0%	28	23	39.4% ^f	94 ^f
1×10 ⁹	30	66.7%	153	16	25.0%	50	46	52.4% ^f	104 ^f
1×10 ¹⁰	36	69.4%	141	23	34.8%	117	59	57.6% ^f	132 ^f
1×10 ¹¹	44	75.0%	183	17	52.9%	124	61	67.4% ^f	160 ^f
Week 30									
Placebo	26	7.7%	28	14	0.0%	27	40	5.1%	28
1×10 ⁸	14	50.0%	156	6	0.0%	46	20	32.9% ^f	103 ^f
1×10 ⁹	29	69.0%	150	14	28.6%	58	43	55.1% ^f	109 ^f
1×10 ¹⁰	32	53.1%	149	23	26.1%	112	55	43.9% ^f	135 ^f
1×10 ¹¹	36	55.6%	126	17	29.4%	73	53	46.6% ^f	104 ^f

^aVaccinations at day 1, week 4, and week 26. ELISPOTs were performed 4 weeks after each dose. The primary immunogenicity time point was prespecified per protocol as week 30. Small sample sizes precluded comparisons within baseline Ad5 strata.

^bvp/d, viral particles per dose; N, number of subjects with evaluable immunogenicity data.

^c% Responders, percentage of subjects with a positive ELISPOT response (defined as ≥55 SFC/10⁶PBMC and ≥4-fold over nonantigen control).

^dGeometric mean ELISPOT reported as SFCs/10⁶ PBMC.

^eCombined across baseline Ad5 strata using a weighted average with weights proportional to the observed total stratum sizes.

^fTwo-tailed *p*-value vs. placebo <0.05.

FIG. 2. Geometric mean ELISPOT responses versus baseline Ad5 titer at weeks 8 (A) and 30 (B) by dose level. Vaccinations were administered at day 1, week 4, and week 26. Week 30 was prespecified as the primary immunogenicity time point per protocol.

and resolved within 5 days without clinical intervention. Most febrile reactions were $<102.0^{\circ}\text{F}$. One participant in the 1×10^{11} vp/dose group developed chills and fatigue after the first dose reported by the investigator as a vaccine-related serious adverse experience resulting in the loss of a work day, but remained in the study and received all three doses. There were no significant differences in laboratory abnormalities between vaccine and placebo recipients. Of 219 adenoviral cultures obtained from 119 vaccine subjects, one culture grew Ad5 subsequently identified as wild type.

Immunogenicity

Immune responses to HIV. Both the proportions of ELISPOT responders and the ELISPOT geometric mean responses combined across baseline Ad5 strata were significantly higher than placebo for all four vaccine doses studied (Table 4). A dose-response was evident at week 8 (particularly in the high Ad5 stratum) but was not apparent at week 30. ELISPOT responses generally persisted for at least 1 year. Overall, ELISPOT response rates were attenuated in subjects

with high compared to low baseline Ad5 titers at all dose levels. ELISPOT responses varied inversely with the titer of neutralizing antibody to Ad5 at baseline in a roughly linear fashion (Fig. 2). At week 8, the slope of the regression line plotting geometric mean ELISPOT response versus baseline Ad5 titer became flatter with increasing vaccine dose, suggesting that higher doses of the vaccine could be used to enhance the priming response in the presence of preexisting Ad5 immunity. On the other hand, the second and third doses overall had a modest and transient impact on the ELISPOT response rate and geometric mean response compared to the responses after the first dose regardless of baseline Ad5 titers.

Immune responses to Ad5. For subjects with low titers of neutralizing antibodies to Ad5 at entry, Ad5 titers increased in a dose-dependent way after each vaccination (Fig. 3). Boosting of Ad5 titers occurred to a lesser degree in subjects with baseline Ad5 titers >200 after the first injection of the higher vaccine doses. As Ad5 titers approached or exceeded

FIG. 2. (Continued).

200, there was little incremental ELISPOT response to vaccine except after the initial vaccination at the higher dose levels.

Discussion

In these two similarly designed Phase I trials, both Ad5-vectored HIV-1 vaccines induced CMI against HIV-1 Gag-peptides in the majority of healthy adults with baseline Ad5 titers <200. The vaccines were constructed so that each Ad5 vector contained one HIV-1 *gag* transgene. Higher doses of vaccine therefore provided both more *gag* and more Ad5 in a 1:1 ratio. The direct relationship between ELISPOT response and vaccine dose was apparent at week 8 after two doses, but not at week 30 after three doses. A roughly linear inverse correlation was seen between the ELISPOT response and the baseline Ad5 titer, which was most evident at the lower vaccine doses at week 8. The vaccine induced or augmented immunity against the Ad5 vector. Increasing Ad5 titers likely reduced the effective dose of vaccine, thereby dampening responses to later doses and complicating interpretation of the primary week 30 immunogenicity data after the full three-dose series. Although the second and third doses in the series appeared to confer only a modest and short-lived increase in

the frequency and magnitude of ELISPOT responses under most circumstances, multiple doses presumably contributed to the persistence of the immune response.

Clinical adverse experiences were dose related. The frequency of injection-site reactions was comparable after each dose and did not vary with preexisting Ad5 immunity. On the other hand, systemic adverse events decreased in frequency with successive doses. Rates of systemic adverse experiences varied inversely with baseline Ad5 titers, most prominently after the initial vaccination.

Despite the favorable immunogenicity of the monovalent vaccine candidate and its trivalent derivative,³⁴ vaccination in the proof-of-concept STEP trial using a similar MRKAd5 trivalent vaccine was recently stopped following a preplanned interim review by the Data and Safety Monitoring Board because a futility analysis indicated that the study was unlikely to yield evidence of efficacy.^{24,25} The STEP study was being conducted in areas of the world where clade B HIV-1 predominates. The vaccine reduced neither the incidence of HIV acquisition nor the viral load set point in participants who became infected. The disappointing efficacy results of the Phase II trial despite our promising Phase I immunogenicity data highlight the pitfalls of developing an effective HIV-1 vaccine targeting CMI, especially with regard to the still

FIG. 3. Geometric mean ELISPOT responses and Ad5 titers versus study week by baseline Ad5 titer and treatment group. vp/d, viral particles per dose. ELISPOT responses are presented as SFC/10⁶ PBMC on the y-axis. N, number of subjects with evaluable immunogenicity results at each time point. %Resp., percentage of subjects at each time point with a positive ELISPOT response (defined as ≥ 55 SFC/10⁶ PBMC and ≥ 4 -fold increase over nonantigen control).

unresolved challenge of identifying predictive immune correlates of protection against HIV infection.

Paradoxically in the STEP analysis, participants with greater preexisting immunity to the Ad5 vector developed numerically more HIV infections postvaccination than those with lower Ad5 titers. One speculative explanation for this observation (if it turns out to be more than a chance occurrence) would be that immune activation triggered by the Ad5 vector transiently increased susceptibility to HIV infection in subjects primed by natural Ad5 infection in the past. Hopefully, the data reported here will result in a better understanding of HIV and other multidose vaccines using adenoviral vectors to generate protective CMI responses.

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C.H. has received contract support from Merck related to its influenza, HIV-1, and *Staphylococcus aureus* vaccine programs and has previously received fees for service on Safety Advisory Committees for HIV-1 vaccine trials and review of Clinical Safety Reports for *S. aureus* vaccine trials. M.L. has received grant support from Merck related to its HIV-1 and HPV vaccine programs, and she has received honoraria from Merck for lectures about Merck vaccines. F.P. is employed by the International AIDS Vaccine Initiative and has previously received fees from Merck for serving on Safety Advisory Committees for Phase I HIV-1 vaccine trials.

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