MAJOR ARTICLE

Safety and Immunogenicity of Combinations of Recombinant Subtype E and B Human Immunodeficiency Virus Type 1 Envelope Glycoprotein 120 Vaccines in Healthy Thai Adults

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Safety and immunogenicity of 2 recombinant human immunodeficiency virus (HIV) type 1 envelope glycoprotein (gp) 120 vaccines derived from SF2 (subtype B) and CM235 (CRF01_AE, Thai E) were evaluated in 370 Thai adults at low risk of HIV infection. Various doses of CM235 (25, 50, or 100 μg) and SF2 (0, 25, or 50 μg) gp120 were used. Eighty volunteers received placebo. There were no serious adverse events related to vaccination. Binding antibody developed in all vaccine recipients. There was no dose response to CM235 gp120, but a dose response to gp120 SF2 was present. Neutralizing antibodies to subtype E HIV-1 NPO3 and subtype B HIV-1 SF2 developed in 84% and 82% of vaccine recipients, respectively. Lymphoproliferative responses were detected in >95% of vaccine recipients. There was no evidence of antigenic interference in HIV-specific humoral or cellular responses. The gp120 Thai E and SF2 vaccines were safe and immunogenic in combination and could be advanced into phase 3 testing.

AIDS has emerged as an uncontrolled, worldwide public health problem and is associated with high morbidity and mortality. From the beginning of the pandemic until the end of 2001, >40 million people worldwide were infected with human immunodeficiency virus (HIV) [1]. The prevalence of adult HIV infection in Thailand is estimated to be 2.15%; ~1 million persons have been infected [1], despite an effective condom campaign [2, 3]. An HIV preventive vaccine is urgently needed as a supplementary public health tool against this pandemic.

One potential obstacle to the development of an effective HIV vaccine is genetic variation, particularly in the envelope protein [4]. HIV genetic sequences (genotypes) form genetic clusters or subtypes, some of which are geographically limited. Envelope gp160 amino acid sequence variation between genetic subtypes is ~20%–30% [5, 6]. Given this degree of intersubtype variation, matching the genetic subtype of the vaccine with the predominant circulating genotype has been one approach to selection of candidate vaccines for eventual phase 3 trials. In Thailand, the principal

Received 2 October 2002; accepted 19 February 2003; electronically published 3 July 2003.

Presented in part: XIII International AIDS Conference, Durban, South Africa, 9–14 July 2000 (abstract WePe4470).

Financial support: US Army Medical Research and Materiel Command and its Cooperative Agreement (DAMD17-98-2-7007) with the Henry M. Jackson Foundation for the Advancement of Military Medicine.

The opinions expressed here are those of the authors and do not purport to represent the official position of the US or Royal Thai Armies.

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The Journal of Infectious Diseases 2003;188:219–27

0022-1899/2003/18802-0005$15.00

variant of HIV is a recombinant of subtypes A and E, designated CRF01_AE (referred to subsequently as subtype E) [5–9].

A number of HIV-1 envelope (gp120/gp160) vaccines have been tested in phase 1 and 2 trials in HIV-1–seronegative volunteers [10–19], and 2 subtype B gp120 subunit vaccines (MN- and SF2-based) have been tested in Thailand [20, 21]. The HIV gp120 subunit vaccines evaluated in human clinical trials in Thailand were derived from T cell line–adapted (TCLA) strains of HIV-1 that use the CXCR4 (X4) coreceptor. Despite the induction of high titers of neutralizing antibody (NAb) against homologous and some heterologous TCLA strains, primary X4 and CCR5 (R5) HIV-1 strains were not neutralized [22, 23]. The failure of TCLA gp120 vaccines to generate primary isolate NAb has been attributed to the lack of appropriate quaternary structure of the gp120, the lack of induction of conformation-dependent antibody, masking of immune epitopes within the gp120 of primary isolates, and/or the immunodominance of variable linear epitopes such as the V3 loop [24–26].

Animal studies using the subtype E, CM235 gp120 vaccine with MF59 adjuvant showed high-titer, HIV-specific antibody that preferentially recognized conformationally intact epitopes. Although the NAb response to CM235 gp120 was capable of neutralizing heterologous, subtype E TCLA HIV-1 strains, there was little neutralization of homologous CM235 in peripheral blood mononuclear cells (PBMCs), similar to the strain-specific neutralization seen in nonhuman primates [27]. Vaccination of rabbits with a subtype E, R5-using HIV-1 gp120 (CM244) resulted in high-titer CM244-specific antibody that neutralized CM244 but not heterologous subtype E strains [28]. Given the predominance of subtype E infection in the region, Thai national authorities approved human clinical trials of 2 different subtype E vaccines, CM235 gp120 and CM244 gp120. Here, we describe a phase 2 trial of the safety and immunogenicity of subtype E CM235 gp120, alone and in combination with subtype BSF2 gp120, in low-risk, HIV-seronegative Thai adults.

SUBJECTS AND METHODS

Study Design

Following an open-label, dose-escalation study of 12 volunteers (J.K., unpublished data), a randomized, double-blind, placebo-controlled, multicenter trial (phase 2) was conducted by the Thai AIDS Vaccine Evaluation Group (TAVEG) at 4 collaborating centers: Thai and US Components of the Armed Forces Research Institute of Medical Sciences (Bangkok); Faculty of Medicine, Siriraj Hospital, Mahidol University (Bangkok); Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University (Bangkok); and Research Institute for Health Sciences, Chiang Mai University (Chiang Mai). Volunteers in the phase 2 trial were healthy HIV-seronegative adults (20–50 years old, of both sexes) from the community, who denied engaging in high-risk behavior for HIV exposure. All volunteers were counseled regarding HIV risk behavior and were tested for HIV before enrollment and 5 times during the trial. HIV status was based on screening with an HIV-specific EIA (Vironostika HIV-Uni-Form II plus O; Organon Teknika), and EIA-positive results were further evaluated with an HIV-specific Western blot assay (New LAV blot I; Bio-Rad Laboratories).

Volunteers were randomly assigned to 1 of 10 groups receiving various dose combinations of CM235 gp120 antigen, SF2 gp120 antigen, and placebo. After passing a test of understanding during a screening protocol before enrollment in this study, all volunteers gave written informed consent. Human experimentation guidelines of authors' institutions were followed in the conduct of the clinical research reported here. Female volunteers were tested for pregnancy before each immunization. There were no pregnancies detected during the study period.

Investigational Vaccines

Two investigational vaccines (CM235 gp120 and SF2 gp120) were produced in genetically engineered CHO cells at Chiron Vaccines (Emeryville, CA). The 2 vaccines were used with the adjuvant MF59, which is also a component of a licensed influenza vaccine. On the basis of prior experiments with monovalent SF2/MF59, doses of 25, 50, and 100 μg were chosen for CM235/MF59. Doses of each antigen (CM235 and SF2) were assessed in a matrix to identify the optimal combination. The response of Thai adults to SF2 gp120 alone has been reported elsewhere [20]. The 4 collaborating centers evaluated CM235 gp120/MF59 vaccine at 25, 50, and 100 μg alone, or combined with the SF2 gp120 vaccine at doses of 25 or 50 μg. Ninety-two volunteers at each center were randomly assigned to receive 1 of the vaccine combinations or placebo; 8 volunteers each were randomly assigned to 1 of 9 vaccine groups ($n = 72$) or to placebo ($n = 20$). The placebo contained MF59 adjuvant without immunogen. Volunteers were immunized at study months 0, 1, and 6. Study personnel and volunteers were blinded to the assignment of active agent or placebo.

Evaluation of Volunteers and Follow-Up

All volunteers were observed for local and systemic reactions in the 30 min immediately after immunization and were monitored for the next 7 days by use of self-reported diary cards. Each volunteer was contacted 24–48 h after immunization by a study nurse. Clinical evaluation, hematologic studies, serum chemistry measurements, liver and renal function tests, serum for antibody assays, and cells for lymphoproliferation studies were obtained during the course of the study.

Cells and Viruses

T cell lines. The $CD4^+CXCR4^+$ T cell line H9 was used for all screening and titration NAb assays using the NP03 (X4-tropic, subtype E) and SF2 (X4, subtype B) TCLA HIV strains, as reported elsewhere [20, 22, 29]. The $CD4^+CXCR4^+CCR5^+$ T cell line A3R5-6 was used for assays in which the CM235M4-C4.6 virus (R5-tropic, subtype E) was tested. A3R5-6 is derived from A3.01 cells and is the result of transfection of an expression plasmid containing CCR5-neo into A3.01 with serial selection by fluorescence-activated cell sorting and geneticin for high expression of CD4 and CCR5. Levels of CD4, CXCR4, and CCR5 expression are consistent with levels seen in phytohemagglutinin (PHA)/interleukin (IL)–2–activated PBMCs (J.K., unpublished data). PHA/IL-2–stimulated PBMCs were used for NAb experiments involving primary CM235 virus (R5, subtype E).

HIV-1 isolates. As described elsewhere [27], NP03 is a TCLA subtype E virus (X4, E) derived from a participant in the Thai Natural History Study. SF2 is a TCLA subtype B (X4, B) virus similar to the strain used in the gp120 bivalent vaccine. CM235M4-C4.6 is a subtype E, CCR5-tropic virus that initially had been grown in MOLT-4 cells and was further adapted for growth in the A3R5-6 T cell line. It is completely identical in V3 sequence to CM235. It will not infect $CCR5^{-/-}$ PBMCs and is relatively resistant to neutralization by soluble CD4 (J.K., unpublished data). CM235 is a PBMC-derived, early passage, primary HIV-1 isolate and is the parent strain of the subtype E gp120 envelope in the bivalent vaccine tested in this study.

Immunological Assessments

Binding antibody (BAb) assays. Anti-gp120 BAb titers were determined by ELISA for each volunteer. Serum samples were tested for BAbs at 4 time points: baseline and at 1 month after the first, second, and third immunizations. Serial 3-fold dilutions of serum samples, ranging from 1:100 to 1:218,700, were added to microtiter plates precoated with recombinant gp120/CM235 or gp120/SF2 produced in CHO cells (Chiron Vaccines) at a concentration of 0.2 μg/well. Plates were incubated for 1 h at 37°C and washed, and anti-gp120 antibodies were detected using peroxidase-conjugated goat anti–human IgG (Biosource International), followed by reaction with tetramethyl benzidine substrate. All serum samples were tested on duplicate plates. The optical density was determined at 450 nm and 650 nm (background), and the result was calculated as the difference. Anti-gp120 BAb titer was defined as the reciprocal of the serum dilution that yielded an OD of 1.0, as determined by a third-order polynomial curve fit. Validity of the assay was monitored using a 2-fold dilution of a standard HIV-positive serum, which had to give a titer of 50% of the undiluted standard, and a difference between duplicate samples, which had to be <2-fold. Data are expressed as the geometric mean titer (GMT) of the reciprocal serum dilution. Serum samples were considered to be positive for HIV-specific BAb if the reciprocal titer was $\geq$100. Nonreactive serum samples were assigned a titer of 50 (half the reciprocal titer of the lower limit of detection of the assay).

NAb assays. T cell line–based and PBMC-based neutralization assays were performed as described elsewhere [20, 22, 29]. First, screening assays for NAb to the heterologous TCLA subtype E strain NPO3 (X4) and the homologous TCLA subtype B strain SF2 (X4) were performed. Subsequently, NAb responses of a subgroup of volunteers who received 25, 50, or 100 μg of Thai E plus 50 μg of SF2 ($n = 95$) were analyzed in greater detail. In this subgroup, all volunteers were tested for NAb against homologous CM235 and against CM235M4-C4.6 in A3R5-6 cells. Finally, postvaccination serum samples from this subgroup were serially diluted, and NAb titers against NP03 were determined.

Screening neutralization assay. A screening neutralization assay was performed by incubating a fixed dilution (1:10) of pre- and postvaccination serum samples with virus in triplicate wells for 30 min at 37°C. For TCLA isolates, 1×10^5 H9 or A3R5-6 cells were added and incubated overnight. For primary CM235, 1.5×10^6 PHA/IL-2–stimulated PBMCs were added and incubated overnight. The next day, the cells were washed thrice with complete medium. On the day when regular medium rows were >10 ng/mL, supernatant was harvested, and p24 analyses were performed. The percentage of neutralization was calculated as follows: $100 \times [1 - (V_{post}/V_{pre})]$, where V_{post} is the p24 level in cultures incubated with serum samples taken after the third vaccination, and V_{pre} is the p24 level in cultures incubated with prevaccination serum samples. Neutralization $\geq$50% was considered to be a positive NAb response in the assay [20]. For determination of NAb, the samples were assayed twice, when feasible, and the average percentage of neutralization of the 2 assays was calculated. Eighty-five percent of samples had 2 separate determinations, with priority being given to subtype E NP03 assays (nearly 100% were run twice).

Determination of neutralization titer. H9 cells were suspended in complete medium at 3.0×10^6 cells/mL. A frozen aliquot of test serum was thawed quickly at 37°C in a water bath. Serum dilutions were set up in a 25-μL volume. Pretitered HIV stock was diluted to 100 $TCID_{50}$/25 μL of complete medium. Twenty-five microliters of diluted virus stock was added into each of 3 wells of a 96-well box. The virus and serum were allowed to incubate at 37°C for 30 min. H9 cells (1×10^5) were added and incubated overnight. The next day, the cells were washed thrice with complete medium. After the final wash, 200 μL of complete medium was added to each well, and the entire mix was transferred to a round-bottom, 96-well plate. One hundred microliters was removed for analysis and replaced with 100 μL of complete medium on sequential days, starting at day 3. At the point when 10 ng of p24 was detected in pooled,

regular-medium control wells, 100 μL of supernatant was removed from each well for p24 analysis. Supernatant from triplicate experimental wells (V_n) was pooled and compared with pooled p24 expression in 6 wells containing complete medium only (V_o). In addition, a coefficient of variation (C_v) was calculated for the individual p24 expression of the 6 control wells; if the C_v was >50, the experiment was repeated. The ratio V_n: V_o was plotted against the reciprocal dilution, and 50%, 80%, and 90% neutralization titers were calculated by performing a quadratic regression and extrapolating the titers against the *X*-axis (reciprocal serum dilution). A reciprocal serum dilution ⩾10 was considered to be indicative of HIV-specific NAb.

Lymphoproliferation assays (LPAs). LPAs were performed using cryopreserved PBMCs obtained from subjects who received any dose of Thai E antigen (25, 50, or 100 μg) plus the high dose (50 μg) of SF2 antigen at 2 time points: preimmunization and 1 month after the third immunization. The proliferative responses of volunteer PBMCs to antigens and mitogens were measured by incubating 1×10^5 cells/well in 96-well U-bottom plates (Corning) with serial concentrations of reduced carboxymethylated (rcm) gp120 SF2 (10, 5, and 1 μg/mL) and rcm gp120 CM235 (8, 4, and 1 μg/mL). Tetanus toxoid was used at a 5-μg/mL dilution; in separate plates, PHA was used at a 2-μg/mL dilution. After 3 days of incubation with PHA and 6 days with the antigens, cells were pulsed with 1.56 μCi/well of ^{3}[H]-thymidine for 6 h, harvested using the Tomtec Mach 3M (EG & G Wallac), and counted in a 1450 Microbeta Trilux (EG & G Wallac).

The data were expressed as lymphocyte stimulation indices (LSIs), calculated as counts per minute of PBMCs with antigen or mitogen divided by counts per minute of PBMCs in medium alone, to define antigen specificity. Individual responses were defined as positive if the LSI was ⩾5 [20, 30].

Statistical Analysis

Qualitative demographic data are summarized as percentages and were compared by χ^2 test; for marital status, "widowed" and "separated/divorced" were combined. Mean ages of different groups were compared by analysis of variance (ANOVA). For reactogenicity and adverse events, assessments were categorized as mild, moderate, or severe and as local or systemic. Differences between vaccine groups, with respect to proportion of subjects exhibiting moderate or severe reactions or adverse events, were analyzed by χ^2 test.

Immunogenicity data were summarized by percentage of detectable responses and by geometric mean titers (GMTs). For samples with undetectable responses, half of the lower limit of detection of the assay was assigned. Differences in percentage response were analyzed by χ^2 test, whereas GMTs were analyzed by 2-way ANOVA linear model. We considered differences to be significant at $P < .05$ (2-sided).

RESULTS

A total of 370 volunteers were enrolled in this phase 2 trial. The median age was 30.9 years, with males predominant. The most common single occupation was Buddhist monk (20%); education levels were equal to or higher than secondary school for 68% of recruited volunteers. There were no significant differences in age, sex distribution, and education levels among the groups.

Safety profile. The immunizations were well tolerated (table 1). All participants, except 2 subjects in the 50-μg E antigen/0-μg B antigen (50/0) group, reported pain on injection. The pain was rated severe by 1 or 2 subjects (3%–6%) in each group except in the placebo group, in which 6 subjects (8%) rated the pain as severe. The most frequently reported local reaction was pain, occurring in 70% (50-μg E antigen/25-μg B antigen [50/25] and 25-μg E antigen/50-μg B antigen [25/50] groups) to 89% (100-μg E antigen/50-μg B antigen [100/50] group) of subjects. Pain on injection did not increase in frequency with subsequent immunizations. Adverse events possibly, probably, or definitely related to vaccination occurred in 11% of subjects receiving placebo, 19% of subjects receiving HIV gp120 E alone, and in 19% of subjects receiving both antigens. Adverse events possibly, probably, or definitely related to vaccination and that occurred in >5 subjects were found only in groups receiving both antigens and included headache (6 subjects [3%]), injection-site edema (6 subjects [3%]), injection-site reaction (11 subjects [6%]), and myalgia (6 subjects [3%]).

One death, caused by suicide, occurred during the trial (placebo group). Eleven other subjects experienced serious adverse events, the majority of which were due to motorcycle accidents. None of these events was considered to be related to the study vaccines, and none resulted in premature withdrawal from the study. There were no significant differences in distribution of reported reactogenicity among the groups. There were no intercurrent HIV infections of volunteers during the study.

Immunological profiles. Among vaccinated volunteers, 15% developed anti-HIV reactivity, as measured by the commercial diagnostic EIA, most frequently detected in the serum samples collected 12 weeks after the third immunization. This EIA reactivity decreased to 1.7% by 6 months after the third immunization. None of these EIA-reactive specimens were confirmed as HIV positive by Western blot.

BAb responses. Vaccination induced BAb to CM235 gp120 or SF2 gp120 or both in all recipients. No placebo recipients had detectable BAb. BAb was observed as early as after the first immunization, increasing to maximum titers after the third immunization. The GMTs after the second and third immunizations were 1449–7764 for CM235 gp120 and 56–2600 for gp120 SF2 (figure 1 and figure 2). No dose response was observed for CM235 gp120 (figure 1). In contrast, there was a clear increase in SF2 gp120 BAb titer with increasing SF2 gp120

Table 1. Subjects ($n = 383$) with postimmunization reactions (within 7 days of any injection) during a phase 1/2 trial of human immunodeficiency virus subtype B and E vaccines in Thailand, by dose group.

Reactions	E antigen dose/B antigen dose, μg									
	25/0 ($n = 35$)	50/0 ($n = 33$)	100/0 ($n = 35$)	25/25 ($n = 35$)	50/25 ($n = 33$)	100/25 ($n = 32$)	25/50 ($n = 33$)	50/50 ($n = 32$)	100/50 ($n = 35$)	0/0 ($n = 80$)
Local										
Pain	77	67	83	77	70	88	70	84	89	79
Warmth	54	64	31	51	64	56	45	44	49	43
Erythema	29	24	37	20	21	38	24	34	26	19
Induration	11	15	11	11	15	25	12	16	26	6
Systemic										
Chills	9	12	9	11	6	9	6	13	9	11
Nausea	23	18	0	9	12	19	9	0	14	10
Malaise	49	36	46	40	39	44	45	38	69	38
Myalgia	40	30	34	31	27	44	30	31	57	39
Rash	6	9	14	11	6	6	9	9	11	10
Arthralgia	14	15	14	20	18	16	9	13	29	14
Headache	34	33	51	34	27	44	42	28	46	34
Temperature ⩾38°C	6	6	9	20	3	6	6	22	17	10
Other										
Stayed home	14	9	9	6	3	9	3	0	11	8
Analgesic or antipyretic	23	33	40	29	27	16	27	31	37	16

NOTE. Data are percentage of subjects. Reactions are summarized as the worst severity following any injection.

dose (figure 2). Combining the SF2 gp120 BAb titers from different doses of CM 235 over each SF2 dose, regardless of the dosage of subtype E vaccination, the GMTs of the BAb were 281, 1623, and 2337 for the doses of SF2 at 0, 25, and 50 μg, respectively. Anti-CM235 gp120 BAb titers in recipients of bivalent (E plus B gp120) and monovalent (E only) groups were similar (figure 1). There was no evidence of antigenic interference. Of interest, immunization with CM235 gp120 (E) alone resulted in BAb that cross-reacted with SF2 gp120 (B) antigen, although the response was augmented by >5-fold when SF2 gp120 was included in the immunization regimen.

NAb response. Previous work has shown that the vaccination of humans with TCLA HIV-1 gp120 results in the generation of NAb against homologous TCLA HIV strains and some additional neutralization of other strains within the same subtype [22]. We first screened for NAb to the heterologous TCLA subtype E strain NP03 (X4) and the homologous TCLA subtype B strain SF2 (X4). Pre- and postvaccination serum samples from 287 vaccine recipients and 80 placebo recipients were studied for neutralization of NP03 and SF2. Serum samples from 242 (84%) of these vaccine recipients neutralized NP03; 235 (82%) samples neutralized SF2. Two of the 80 placebo recipients (2.5%) had ⩾50% neutralization of NP03, whereas 5 (6%) had NAb against SF2. There were no statistically

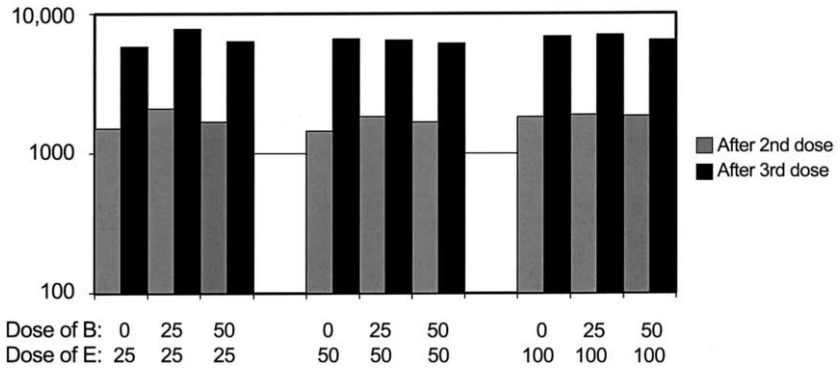

Figure 1. Geometric mean titer of anti-gp120 CM235 after vaccination with various doses (in micrograms) of subtype B and subtype E vaccines. Titers ⩾100 were considered to be positive; nonpositive serum samples were assigned a titer of 50.

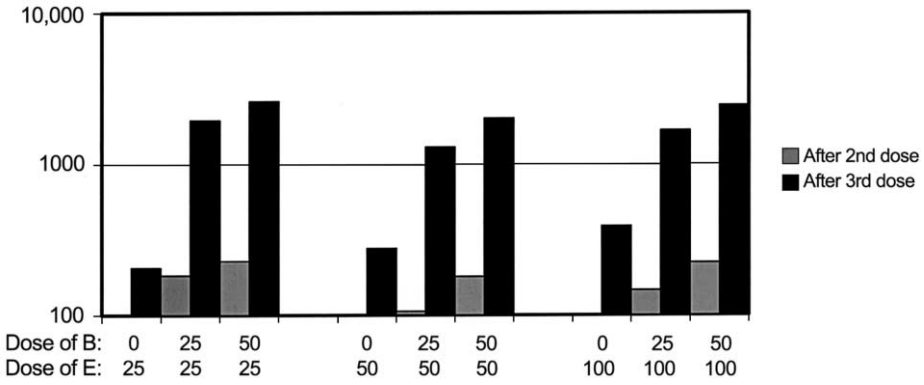

Figure 2. Geometric mean titer of anti-gp120 SF2 after vaccination with various doses (in micrograms) of subtype B and subtype E vaccines. Titers ⩾100 were considered to be positive; nonpositive serum samples were assigned a titer of 50.

significant differences between dose groups among the recipients of various combinations of gp120 CM235 and SF2; only 61% of persons receiving the 25-μg dose of gp120 E alone had NAb against NP03, but the remainder of the groups included 75%–100% responders, with no clear pattern (data not shown). Although the recipients of gp120 CM235 alone did have NAb responses to SF2 (subtype B), these were lower than the responses seen in recipients of bivalent combinations. NAb responses to SF2 were found in 35%, 59%, and 66% of recipients of the 25-, 50-, and 100-μg doses of gp120 CM235, respectively (compared with SF2 responses of 82% overall). NAb responses to subtype E NP03 in recipients of the bivalent combinations were equal to or higher than responses to subtype E gp120 alone. Thus, no evidence of antigenic interference was detected.

The NAb responses of a subgroup of volunteers (recipients of 25, 50, or 100 μg of Thai E plus 50 μg of SF2 ($n = 95$) were analyzed more extensively. In this subgroup, 84% of subjects had NAb responses to NP03, and 96% of subjects had NAb responses to SF2. Only 1 of 95 pre- and postimmunization serum sample pairs showed ⩾50% neutralization using the homologous CM235 (vaccine strain) isolate in PBMCs. The single positive subject had a mean p24 reduction of 52% (2 independent assays). Fifty-six percent of vaccine recipients had ⩾50% neutralization of CM235M4-C4.6.

The dilution of postimmunization serum that induces a 50% or 90% reduction in p24 antigen after infection of target cells with HIV-1 is designated the neutralization titer. The geometric means of the 50% and 90% neutralization titers are displayed in table 2 at each of the indicated doses of CM235 gp120. No dose response was observed between the dose of CM235 gp120 and neutralization of NP03 (table 2). This lack of a dose response between the CM235 immunogen and NAb against NP03 is similar to that observed in the whole study group. V3 loop differences of NP03 from the CRF01_AE consensus (J.K., P.P., C.K., T.C., J. Mascola, S. Frankel, M.d.S., V.P., R. McLinden, A. Sambor, A.E.B., B. Phonrat, K. Rungruengthanakit, A.-M.D., M. L. Robb, J.G.M., D.L.B., and the TAVEG, unpublished data) may explain the lack of an apparent relationship between the dose of CM235 gp120 and the induction of NAb detected by the NP03 virus.

Previous data have demonstrated the feasibility of using a CM235 strain adapted to growth in a CCR5$^+$ T cell line (A3R5) to measure NAb induced by either natural CRF01_AE infection or by vaccination with R5-containing envelopes (J.K., unpublished data). The pre- and postvaccination serum samples from the same subgroup of volunteers were screened against the adapted strain CM235M4/C4.6, which is homologous to the vaccine strain CM235. Fifty-three (56%) vaccine recipients had NAb responses to CM235M4/C4.6. Comparison of the proportion of responders among vaccine recipients at each dose of CM235 gp120 suggests that there is a dose-dependent increase in the induction of NAb to CM235M4/C4.6 (48% of 25 μg, 53% of 50 μg, and 66% of 100 μg CM235 plus 50 μg SF2). The use of homologous, adapted CCR5-tropic strains may facilitate the measurement of functional antibody in trials using R5-based envelopes. These data are consistent with our previous observation (J.K., P.P., C.K., T.C., J. Mascola, S. Frankel, M.d.S.,

Table 2. Neutralizing antibody titers to NP03/H9 in the subgroup of vaccine recipients who received the highest dose of gp120 SF2 ($n = 96$), by dose of Thai E (CM235) gp120 vaccine received.

	Value		
Variable	Group 1	Group 2	Group 3
Dosage of gp120, μg			
CM235	25	50	100
SF2	50	50	50
Neutralization titer, geometric mean[a]			
50% neutralization	147	169	155
90% neutralization	1	5	12

NOTE. A reciprocal serum dilution of ⩾8 was considered to be positive.
[a] No statistically significant differences between dose groups.

Table 3. Lymphocyte stimulation indices (LSIs) of reduced carboxy-methylated gp120 SF2 and CM235 vaccines at baseline (Visit 1) and after the third vaccination (Visit 6) of the subgroup of vaccine recipients who received the high dose of SF2, by the dose of CM235 gp120 received.

Dose of CM235 vaccine, visit	Vaccine	
	SF2	CM235
25 μg		
1	1 (1–7)	1 (1–6)
6	44 (1–193)	21 (1–107)
50 μg		
1	1 (1–9)	2 (1–15)
6	30 (1–242)	11 (1–108)
100 μg		
1	1 (1–14)	1 (1–5)
6	25 (1–148)	11 (1–66)

NOTE. Data are geometric mean LSI (range). The maximum response among the 3 antigen dosages used for LPA stimulation was chosen as the representative LSI.

V.P., R. McLinden, A. Sambor, A.E.B., B. Phonrat, K. Rungruengthanakit, A.-M.D., M. L. Robb, J.G.M., D.L.B., and the TAVEG, unpublished data) that there was a correlation between CM235 gp120 BAb and the neutralization of CM235M4/C4.6, but not NP03 or CM235, in PBMCs.

Lymphoproliferation responses. The lymphoproliferative responses to PHA and tetanus toxoid were equivalent in magnitude and frequency at both time points assayed (data not shown), which demonstrates that the PBMCs were well cryopreserved and retained their functional ability to respond to both mitogen and recall antigen. The percentage of responders to rcm gp120 SF2 in each of the 3 Thai E dose groups was >95% after 3 immunizations, whereas the percentage of responders to rcm gp120 CM235 was the highest (97%) for the combination with the lowest dose of Thai E antigen (25 μg) and lower with the higher dose of Thai E antigen (75% for 50 μg and 78% for 100 μg of CM235). The variability of the responses, as measured by the LSI geometric means, showed a greater range for the rcm gp120 SF2, compared with gp120 CM235 (table 3). The percentage of responders to different concentrations of envelope proteins at day 0 (baseline) was 3%–6% for rcm gp120 SF2 and 6%–16% to rcm gp120 CM235.

DISCUSSION

The early identification and extensive characterization of the HIV epidemic in Thailand have allowed for the testing of several HIV vaccine candidates matched to viral subtypes (E and B) currently circulating in that country. The TAVEG has reported that a subtype B, TCLA gp120 SF2 vaccine (also using the MF59 adjuvant) in Thai adults was safe and induced cross-clade $CD4^+$ lymphoproliferative responses, as well as homologous TCLA NAb [20]. A gp120 subtype B TCLA MN vaccine (with alum adjuvant) showed a similar profile in a phase 1 trial in Thailand [21]. The current trial used a coformulation of the previous SF2 gp120 vaccine with a gp120 derived from the subtype E, R5-tropic primary isolate CM235. This report on the safety and immunogenicity of gp120 CM235 alone and in combination with SF2 gp120 is, to our knowledge, the first description of the outcome of vaccination with an R5 gp120 candidate vaccine in humans.

Similar to the safety profile of other gp120 and gp160 subunit vaccines [31, 32], gp120 CM235 alone or combined with SF2 gp120 (with MF59 adjuvant) was well tolerated. The reported reactogenicity was similar to that of SF2 gp120/MF59 alone in Thai adults [20] and was comparable to that in the control group using adjuvant alone. There were no vaccine-related serious adverse events, and no immunizations had to be withheld because of intolerability.

Clinical trials of HIV-1 TCLA (X4-using) envelope gp120 and gp160 subunit products (e.g., SF2, IIIB, and MN) have shown the induction of high-titer, antigen-specific BAb [4, 10–19]. In the present trial, the magnitude of the CM235 gp120-induced BAb response was independent of the presence of SF2 gp120 antigen, suggesting that there was no antigenic interference or augmentation between the CM235 and SF2 gp120s. Similar results were obtained using the bivalent gp120 A244/MN antigens (AIDSVAX B/E) (P.P., P. W. Berman, B. Phonrat, P. Suntharasamai, S. Raktham, L. Srisuwanvilai, K. Hirunras, D. Kityaporn, J. Kaewkangwal, S. Migasena, H. W. Sheppard, E. Li, M. Chernow, M. L. Peterson, R. Shibata, W. L. Heyward, and D. P. Francis, unpublished data). The level of anti-CM235 gp120 BAb was independent of the dose of CM235 gp120 received. In 95% of vaccine recipients, 2 doses of vaccine were required before antibody could be detected. For all immunization regimens, the highest BAb titer for both antigens was observed after the third dose. After vaccination with gp120 CM235 only, there was induction of a low level of BAb that cross-recognized subtype B gp120. It is not known whether the induction of cross-reactive antibody is a general feature of immunization with R5 tropic (vs X4) gp120 or a particular feature of immunization with gp120 CM235.

The SF2 and MN gp120 vaccines tested previously in Thailand induced both BAbs and NAbs against homologous SF2 and MN in T cell lines [20, 21]. The vaccination of baboons with CM235 gp120 resulted in the induction of antibody that was capable of neutralizing a number of X4 subtype E viruses [27], but no neutralization of the homologous primary isolate CM235 was found. In the present study, NAbs against TCLA HIV NP03 (subtype E) and SF2 (subtype B) were found in >80% of vaccine recipients. Analogous to previous reports of

NAb from TCLA (X4) gp120, no significant neutralization of the vaccine-strain primary isolate CM235 was found in PBMCs. However, antibody capable of neutralizing the homologous, adapted CM235M4/C4.6 virus was present in 56% of vaccine recipients. The mechanism of increased sensitivity of the CM235M4/C4.6 virus to vaccine-induced NAb is not understood (J.K., P.P., C.K., T.C., J. Mascola, S. Frankel, M.d.S., V.P., R. McLinden, A. Sambor, A.E.B., B. Phonrat, K. Rungruengthanakit, A.-M.D., M. L. Robb, J.G.M., D.L.B., and the TAVEG, unpublished data); it is not mediated by an increase in V3-positive charge.

The subset of volunteers studied (those who received 50 μg of gp120 SF2 in combination with different doses of gp120 CM235) mounted good T helper responses to both immunogens, comparable to the previous phase 1/2 trial with gp120 SF2 alone [20]. Although there was a higher background for the Thai E immunogen, compared with the gp120 SF2, the group that received the lowest dose of gp120 CM235 had the best lymphoproliferative response to both immunogens. There was no evidence that gp120 CM235 adversely affected the proliferative response to gp120 SF2.

Taken together, these data demonstrate that the bivalent combination of gp120 CM235 and SF2 is safe, well-tolerated, and immunogenic in low-risk, HIV-seronegative Thai adults. Further human studies of the combined vaccines, after priming with canarypox-vectored HIV vaccine containing subtype B and E antigens, should be pursued in Thailand. It may provide further information on potential antibody specificity, both breadth and magnitude, that would be useful in the context of the planned prime-boost phase 3 trial.

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Acknowledgments

We thank the nursing staffs of the Thai AIDS Evaluation Group for their dedicated study implementation, and Prasong Srisaenchai, Somchintana Chanthong, and Wisut Lokpichart for assistance in data management and analysis.

References

1. Joint United Nations Programme on HIV/AIDS and World Health Organization. AIDS epidemic update, December 2001. Available at: http://www.unaids.org.
2. Joint United Nations Program on HIV/AIDS and World Health Organization. Epidemiological fact sheet on HIV/AIDS and sexually transmitted infections: Thailand, 2000 update. Available at: http://www.unaids.org.
3. Nelson KE, Celentano DD, Eiumtrakol S, et al. Changes in sexual behavior and a decline in HIV infection among young men in Thailand. N Engl J Med **1996**; 335:297–303.
4. Girard MP, Excler JL. Human immunodeficiency virus. In: Plotkin SA, Orenstein WA, eds. Vaccines. 3rd ed. Philadelphia: WB Saunders, **1999**: 928–67.
5. Kalish ML, Baldwin A, Raktham S, et al. The evolving molecular epidemiology of HIV-1 envelope subtypes in injecting drug users in Bangkok, Thailand: implications for vaccine trials. AIDS **1995**; 9:851–7.
6. Subbarao S, Limpakarnjanarat K, Mastro TD, et al. HIV type 1 in Thailand, 1994–1995: persistence of two subtypes with low genetic diversity. AIDS Res Hum Retroviruses **1998**; 14:319–27.
7. Qu CY, Takebe Y, Luo CC, et al. Wide distribution of two subtypes of HIV-1 in Thailand. AIDS Res Hum Retroviruses **1992**; 8:1471–2.
8. McCutchan FE, Hegerich PA, Brennan TP, et al. Genetic variants of HIV-1 in Thailand. AIDS Res Hum Retroviruses **1992**; 8:1887–95.
9. Limpakarnjanarat K, Ungchusak K, Mastro TD, et al. The epidemiological evolution of HIV-1 subtype B and E among heterosexuals and injecting drug users in Thailand, 1992–1997. AIDS **1998**; 12:1108–9.
10. Berman PW, Matthews TJ, Riddle L, et al. Neutralization of multiple laboratory and clinical isolates of human immunodeficiency virus type 1 (HIV-1) by antisera raised against gp120 from the MN isolate of HIV-1. J Virol **1992**; 66:4464–9.
11. Belshe RB, Clements ML, Dolin R, et al. Safety and immunogenicity of a fully glycosylated recombinant gp160 human immunodeficiency virus type 1 vaccine in subjects at low risk of infection. J Infect Dis **1993**; 168: 1387–95.
12. Belshe RB, Graham BS, Keefer MC, et al. Neutralizing antibodies to HIV-1 in seronegative volunteers immunized with recombinant gp120 from the MN strain of HIV-1. JAMA **1994**; 272:475–80.
13. Gorse GJ, Corey L, Patel GB, et al. HIV-1 MN recombinant glycoprotein 160 vaccine-induced cellular and humoral immunity boosted by HIV-1 MN recombinant glycoprotein 120 vaccine. AIDS Res Hum Retroviruses **1999**; 15:115–32.
14. Gorse GJ, McElrath MJ, Matthews TJ, et al. Modulation of immunologic responses to HIV-1 MN recombinant gp160 vaccine by dose and schedule of administration. Vaccine **1998**; 16:493–506.
15. Gorse GJ, Frey SE, Patel G, Newman FK, Belshe RB. Vaccine-induced antibodies to native and recombinant human immunodeficiency virus type 1 envelope glycoproteins. Vaccine **1994**; 12:912–8.
16. Graham BS, Keefer MC, McElrath MJ, et al. Safety and immunogenicity of a candidate HIV-1 vaccine in healthy adults: recombinant glycoprotein (rgp) 120: a randomized, double-blind trial. Ann Intern Med **1996**; 125:270–9.
17. Keefer MC, Graham BS, Belshe RB, et al. Studies of high doses of a human immunodeficiency virus type 1 recombinant glycoprotein 160 candidate vaccine in HIV type 1–seronegative humans. AIDS Res Hum Retroviruses **1994**; 10:1713–23.
18. Keefer MC, Graham BS, McElrath MJ, et al. Safety and immunogenicity of Env 2–3, a human immunodeficiency virus type 1 candidate vaccine, in combination with a novel adjuvant, MTP-PE/MF59. NIAID AIDS Vaccine Evaluation Group. AIDS Res Human Retroviruses **1996**; 12:683–93.

19. Schwartz DH, Gorse G, Clements ML, et al. Induction of HIV-1–neutralising and syncytium-inhibiting antibodies in uninfected recipients of HIV-1 IIIB rgp120 subunit vaccine. Lancet **1993**; 342:69–73.
20. Nitayaphan S, Khamboonruang C, Sirisophana N, et al. A phase I/II trial of HIV SF2 gp120/MF59 vaccine in seronegative Thais. Vaccine **2000**; 18:1448–55.
21. Migasena S, Suntharasamai P, Pitisuttithum P, et al. AIDSVAX (MN) in Bangkok injecting drug users: a report on safety and immunogenicity, including macrophage-tropic virus neutralization. AIDS Res Hum Retroviruses **2000**; 16:655–63.
22. Mascola JR, Snyder SW, Weislow OS, et al. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. J Infect Dis **1996**; 173:340–8.
23. Zolla-Pazner S, Alving C, Belshe R, et al. Neutralization of a clade B primary isolate by sera from human immunodeficiency virus–uninfected recipients of candidate AIDS vaccines. J Infect Dis **1997**; 175: 764–74.
24. VanCott TC, Bethke FR, Burke DS, Redfield RR, Birx DL. Lack of induction of antibodies specific for conserved, discontinuous epitopes of HIV-1 envelope glycoprotein by candidate AIDS vaccines. J Immunol **1995**; 155:4100–10.
25. Earl PL, Broder CC, Long D, et al. Native oligomeric human immunodeficiency virus type 1 envelope glycoprotein elicits diverse monoclonal antibody reactivities. J Virol **1994**; 68:3015–26.
26. Broder CC, Earl PL, Long D, Abedon ST, Moss B, Doms RW. Antigenic implications of human immunodeficiency virus type 1 envelope quaternary structure: oligomer-specific and -sensitive monoclonal antibodies. Proc Natl Acad Sci USA **1994**; 91:11699–703.
27. VanCott TC, Mascola JR, Loomis-Price LD, et al. Cross-subtype neutralizing antibodies induced in baboons by a subtype E gp120 immunogen based on an R5 primary human immunodeficiency virus type 1 envelope. J Virol **1999**; 73:4640–50.
28. Berman PW, Huang W, Riddle L, et al. Development of bivalent (B/E) vaccines able to neutralize CCR5-dependent viruses from the United States and Thailand. Virology **1999**; 265:1–9.
29. Mascola JR. Neutralization of HIV-1 infection of human peripheral blood mononuclear cells (PBMC): antibody dilution method. In: Michael NL, Kim JH, eds. HIV protocols. Vol. 17. Totowa, NJ: Humana Press, **1999**:309–15.
30. Ratto-Kim S, Sitz KV, Garner RP, et al. Repeated immunization with recombinant gp160 human immunodeficiency virus (HIV) envelope protein in early HIV-1 infection: evaluation of the T cell proliferative response. J Infect Dis **1999**; 179:337–44.
31. Coraham BS, Wright PF. Candidate AIDS vaccines. N Engl J Med **1995**; 333:1331–9.
32. Keefer MC, Wolff M, Gorse GJ, et al. Safety profile of phase I and II preventive HIV type 1 envelope vaccination: experience of the NIAID AIDS Vaccine Evaluation Group. AIDS Res Hum Retroviruses **1997**; 13:1163–77.