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Safety and Immunogenicity of a CTL Multiepitope Peptide Vaccine for HIV with or without GM-CSF in a Phase I Trial

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Abstract

There is an urgent need for a vaccine capable of preventing HIV infection or the development of HIV-related disease. A number of approaches designed to stimulate HIV-specific CD8⁺ cytotoxic T cell responses together with helper responses are presently under evaluation. In this phase 1, multi-center, placebo-controlled trial, we tested the ability of a novel multi-epitope peptide vaccine to elicit HIV-specific immunity. To enhance the immunogenicity of the peptide vaccine, half of the vaccine recipients received recombinant GM-CSF protein as a co-adjuvant. The vaccine was safe; tolerability was moderate, with a number of adverse events related to local injection site reactogenicity. Anti-GM-CSF antibody responses developed in the majority of GM-CSF recipients but were not associated with adverse hematologic events. The vaccine was only minimally immunogenic. Six of 80 volunteers who received vaccine developed HIV-specific responses as measured by interferon-gamma ELISPOT assay, and measurable responses were transient. This study failed to demonstrate that GM-CSF can substantially improve the overall weak immunogenicity of a multiepitope peptide-based HIV vaccine.

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1. Introduction

The HIV epidemic continues to spread globally, with 2.5 million new infections and an estimated 2.1 million adult and child deaths due to HIV in 2007 [1]. While education and behavioral modifications are important facets of controlling the epidemic, there is an urgent need for the development of vaccine to reduce or halt the epidemic. Despite more than 20 years of research into HIV vaccine development, no vaccine has yet been shown to prevent HIV infection or disease progression in human clinical trials. Most experts believe that to be effective, an HIV vaccine should elicit both cellular and humoral immune responses, although the precise correlates of immune protection against HIV remain uncertain [2,3]. Research into effective methods to generate HIV-specific cellular immune responses remains a high priority.

Peptide-based HIV vaccine approaches have the potential to generate a balanced CD4+ and CD8+ cellular immune response. Presentation of peptides outside of the context of a complex antigen or mixture of antigens may allow responses against sub-dominant epitopes to emerge [4,5]. Peptides representing regions of the HIV genome of high epitope density or “hotspots” have been identified; these regions when presented in peptide form have the potential to induce responses restricted by a wide variety of HLA molecules [6,7]. Peptide vaccines designed to elicit HIV-specific cellular responses have been immunogenic in animal models [6,8]. A cocktail of peptides administered to rhesus macaques in Freund's adjuvant elicited HIV-specific CTL responses and resulted in protection from a pathogenic simian-human immunodeficiency virus (SHIV) challenge [9,10]. However, peptide-based HIV vaccines have been only modestly immunogenic thus far in human clinical trials [11-13]. Further investigation to identify optimal adjuvants for HIV peptide-based vaccines is therefore warranted.

Cytokine adjuvants have been employed to enhance the humoral immune responses generated by a C4-V3 peptide-based HIV vaccine in macaques [14]. Granulocyte-macrophage colony stimulating factor (GM-CSF) is a cytokine that enhances immune responses by attracting macrophages and inducing their maturation, thus resulting in increased antigen presentation. GM-CSF has been noted to augment the immunogenicity and therapeutic efficacy of tumor vaccines [15-18]. GM-CSF has also enhanced responses to hepatitis B vaccines and to leishmania antigens in humans [19,20]. GM-CSF has demonstrated a variety of promising adjuvant effects when co-administered with DNA, virus-like particles, or live vector-based HIV vaccines in preclinical testing [21-24], but has not previously been used to augment CTL responses induced by peptide-based HIV vaccines in humans.

In this trial, we evaluated the safety and immunogenicity of a multiepitope peptide vaccine designed to elicit HIV-specific T cell responses in human volunteers when administered in an oil-in-water adjuvant (RC529-SE) with or without GM-CSF coadjuvant. The adjuvanted multiepitope peptide was evaluated for safety and immunogenicity in anticipation of its use in prime-boost combination with other CTL-based immunogens. This was the first study in humans of this candidate HIV vaccine.

2. Materials and Methods

2.1 Study Product

The HIV CTL multi-epitope peptide (MEP) vaccine consisted of a combination of 4 hybrid T-helper-CTL MEPs administered in an oil-in-water adjuvant (RC529-SE) with or without GM-CSF coadjuvant. The HIV CTL MEPs were designed and prepared by Wyeth Vaccines Research (Pearl River, New York). The 4 peptides were designated A, B, C, and J and range in length from 27-47 amino acids. The organization and complete amino acid sequence of each peptide are shown in Figure 1. Each peptide contained 1 of 3 different HIV T helper epitopes

derived from *env* or *gag* in combination with 1 of 4 different CTL “hot spots” derived from *gag* or *nef*. The sequence of each peptide is included in Figure 1. This combination of epitopes is restricted by 19 HLA types. The known CTL epitopes included in the 4 peptides based on data from the Los Alamos National Laboratory HIV Immunology Database and their HLA restriction are provided in Table 1. RC529-SE is a chemically synthesized analog of monophosphoryl lipid A (MPL) formulated as an oil-in-water emulsion that was manufactured by the Corixa Corporation (Seattle, WA). RC529 is a member of the aminoalkylglucosamine phosphate family, and possesses a monoglucosamine backbone with 3 fatty acyl side chains. RC529-SE had not been previously administered to humans, although a Hepatitis B vaccine containing RC529 (not formulated as an emulsion) is approved for use in humans in Argentina [25,26]. Recombinant GM-CSF (Leukine) was obtained from Amgen (Thousand Oaks, CA). Sodium chloride injection USP, 0.9% was used as the control for all study products.

2.2 Study Design

The study was a phase 1, multi-center study performed by the NIH/NIAID-funded HIV Vaccine Trials Network (HVTN) in healthy, HIV-uninfected human volunteers. The primary study objectives were to evaluate the safety and tolerability of intramuscular administration of the MEP vaccine + RC529-SE with or without recombinant GM-CSF protein. The secondary objective of the trial was to evaluate the immunogenicity of the product with or without GM-CSF, using IFN- γ ELISPOT assays. Volunteers were 18-50 years of age and were in good general health, were HBV and HCV uninfected, and had normal or near-normal screening values in peripheral blood for standard hematology and chemistry laboratory panels. To enrich the population for those volunteers possessing HLA alleles found in the population at a low frequency who were thought most likely to be associated with cellular responses to the MEP vaccine, HLA screening was performed prior to enrollment. Volunteers demonstrating the presence of one or more of the following MHC alleles were eligible: HLA A3, B7, or B8. This enrollment criteria was instituted in order to ensure that each volunteer had at least one of the MHC alleles capable of presenting vaccine epitopes corresponding to highly prevalent responses found in HIV-infected individuals. Volunteers were excluded if they had previously participated in an HIV vaccine trial, had a history of clinically significant medical illness, or had recently received blood products, immunoglobulin, and other vaccines or investigational agents. 1000 μ g HIV CTL MEP was prepared as an admixture with 50 μ g RC529-SE with or without 250 μ g recombinant GM-CSF, and injected intramuscularly by needle and syringe in the deltoid. Three doses of study product were administered according to the following schedule: day 0, day 28, and day 84. The study schema is provided in Figure 2. Volunteers were followed for 15 months from first study injection.

2.3 Laboratory assessment of immunogenicity

IFN- γ ELISPOT assays were performed at the HVTN Central Laboratory at Duke University on cryopreserved, unfractionated peripheral blood mononuclear cells (PBMC) stimulated overnight with 5 peptide pools spanning the Wyeth HIV CTL multi-epitope peptides (1 pool for each MEP and 1 combined pool). ELISPOT assays were performed on samples obtained at days 0, 42, and 98 (representing pre-vaccination, 2 weeks post 2nd immunization, and 2 weeks post 3rd immunization timepoints). As positive controls, PBMC were also stimulated with phytohemagglutinin (PHA) (1 μ g/ml) as well as a pool of 27 immunodominant 8-10-mer peptides (Anaspec, San Jose, CA) from cytomegalovirus, Epstein-Barr virus and influenza (CEF pool) [27]. Responses to individual or pooled peptides were tested in triplicate. Spot-forming cells (SFCs) were counted using an automated ELISpot reader (Immunospot, Cellular Technology Limited, Cleveland, OH). Responses were considered positive if 1) the mean SFCs in the experimental wells was ≥ 55 detected per 1×10^6 cells and 2) the mean SFCs in the experimental wells was four-fold or greater than the mean of the negative control wells (PBMC in the absence of peptide). Responses were considered reliable if the mean SFC in PHA

stimulated wells was greater than 400, based on validation studies [28]. Among the 278 samples available for testing by ELISPOT assay, samples excluded from the final analysis were those with viability less than 66% after overnight incubation (12 samples excluded), recovery lower than expected (1 sample), and background in unstimulated wells greater than 100 SFC/million PBMC (2 samples). As part of the formal ELISPOT assay validation, the peptides pools used in this study were tested for immunogenicity and background reactivity in blinded PBMC samples from 70 HIV seronegative and 10 HIV seropositive donors. No background reactivity was observed among the seronegative samples, and nine out of the ten seropositive samples responded to one or more of the MEP peptide pools (range 90-2296 SFC/million PBMC).

Exploratory humoral assays for MEP binding antibodies and for neutralizing antibodies against GM-CSF were performed at Wyeth Research using cryopreserved serum samples from days 0, 14, 42, 98, and 455. MEP binding antibodies were measured by ELISA. Briefly, the four peptides contained in the vaccine were adsorbed onto high binding 96 well microtiter plates. Following antigen binding, plates were blocked with bovine serum albumin. Hyperimmune rabbit serum was used as a positive control, and pre-immune sera as a negative control in setting up the assay. Serial dilutions of reference serum and unknown serum samples were tested in duplicate wells. Serum was pre-diluted 1:5 and then 3-fold dilutions (7 in total) were performed in the microtiter plate. Bound peptide-specific IgG antibodies were detected with a biotinylated mouse monoclonal anti-human IgG secondary antibody, streptavidin conjugated alkaline phosphatase tertiary reagent and para-nitrophenyl phosphate substrate. Color development was terminated with sodium hydroxide and the absorbance read at a wavelengths of 490 and 650 nm. The limit of detection of the assay was determined to be 8 EU (ELISA Units)/ml. The assay was qualified prior to use, and the precision of the assay defined. The within plate variability measurement yielded an average coefficient of variation (CV) of 10%, and the average CV for intermediate precision (operator-to-operator, plate-to-plate variation) of 19%. Identification of a positive response for this assay was the average of pre-bleed values plus 2 standard deviations for subjects in this trial. Data analysis was performed using a four-parameter logistic equation to represent the relation between antibody concentration and optical density of the reference serum and to draw a standard curve.

Detection of GM-CSF specific antibodies was based on their ability to neutralize GM-CSF dependent proliferation of the human erythroleukemia cell line, TF-1. Duplicate two-fold dilutions of sera in 96-well microtiter plates were incubated at room temperature for 1 hr. with GM-CSF (Leukine. 0.25 ng/well). Upon incubation, TF-1 cells (1×10^5) were added to each well and the plates were incubated at 37°C in a 5% CO₂ incubator for 48 hours, at which time the substrate, XTT (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide), was added and incubation continued for an additional 24 hrs. The level of cell proliferation supported by positive and negative controls and test sera was determined by measuring the OD₄₅₀ values of cell supernatants. Data were analyzed by nonlinear regression using 4-Parameter-Logistics transformation (4PL) software. Standards used to develop and validate the GM-CSF assay included commercial purified polyclonal antibodies from rabbit and a pool of human serum that was positive for neutralization antibodies to GM-CSF. A positive response for this assay was considered to be any response above the limit of detection, which was 6 Neutralization Units /ml. The assay was qualified prior to use, and the precision of the assay found as follows: within plate variability yielded an average CV of 6%; the average CV for intermediate precision (operator-to-operator, plate-to-plate variability) was 10%.

2.4 Statistical analysis

Analyses included all participants regardless of number of study injections received. Differences in severity of adverse experiences between the two vaccine groups were tested with Kruskal-Wallis tests. For ELISpot analyses, a positive response to a peptide pool was

determined by the distribution-free resampling criterion to adjust for the multiple peptide-pools [29]. In addition, to be considered positive, the mean background adjusted spot forming cells (SFCs) was required to be ≥ 10 SFC/200,000 PBMC. For the MEP ELISA assay a concentration > 160 EU/ml was considered positive. For the GM-CSF neutralizing antibody assay a value > 6 NU/ml is considered positive. Differences between the vaccine groups in percent positive were tested with Fisher's exact tests. Differences between the distributions of the continuous data values were tested with Wilcoxon rank sum tests. Linear regression models are used to assess the relationship between post-enrollment neutrophil and monocyte counts and GM-CSF neutralizing antibody values, adjusting for baseline blood counts. Statistical tests are two-sided at the alpha equal 0.05 level.

3. Results

3.1 Study Enrollment

A total of 96 individuals were enrolled in this trial at 7 U.S. sites between April 29, 2004 and April 7, 2005. Forty (40) individuals received the CTL MEP vaccine and RC529-SE alone, while 40 received the CTL MEP vaccine and RC529-SE in combination with GM-CSF, and 16 received normal saline as control. Enrollment in this trial was noticeably slow in comparison with other phase I HVTN trials, due primarily to the restrictive HLA enrollment criteria employed in this protocol. The study population was predominantly male (64%) and Caucasian (88%). Seventy-one (89%) of volunteers in the vaccine arms and 81% (13 of 16) control recipients received all three doses and completed study follow-up,

3.2 Safety and Tolerability

The CTL MEP vaccine and RC529-SE adjuvant with or without GM-CSF exhibited an acceptable safety profile. There were no significant laboratory abnormalities in this trial attributable to the vaccine product. No life-threatening adverse experiences were attributed to the CTL MEP vaccine. Moderate injection site pain and tenderness was the most common reaction, occurring in 65% of participants in the MEP/RC529-SE group, 58% of the MEP/RC529-SE + GM-CSF group, and 6% of controls. The CTL MEP vaccine given with GM-CSF was associated with headaches of greater severity (7 moderate and 1 severe vs. 3 moderate and 0 severe, $p = 0.01$). Other adverse experiences, including local pain and tenderness, were not significantly different between the group receiving GM-CSF and the group receiving the CTL MEP vaccine without GM-CSF. Severe reactions occurred in 7 vaccine recipients: 3 had severe pain and tenderness and 1 had severe malaise in the MEP/RC529-SE group, and 1 each had severe fatigue/malaise, headache/malaise, and allergic reaction in the MEP/RC529-SE + GM-CSF group. Reactogenicity observed in this study is summarized in Table 2. Two additional severe events were assessed as unrelated to vaccine: a control recipient developed hypertension more than 2 weeks post-vaccination, and a participant had elevated CPK and ALT following extremely strenuous exercise.

3.3 Immunogenicity

ELISPOTs were negative at baseline for all participants. Six subjects out of 80 vaccinees demonstrated an HIV-specific immune response (Table 3). All responses were from individuals receiving the CTL MEP vaccine, with no positive ELISpot responses in control recipients. Only one of the positive responders received the CTL MEP vaccine in combination with GM-CSF. Each of the responding subjects had a positive result only at a single timepoint (day 42 or day 98). The number of background-adjusted SFCs was generally low, and reached a maximum mean value of $158/10^6$ cells (volunteer 056-10, day 42, Table 3). The HLA type of the responder subjects is shown in Table 4. Five of six responders were heterozygous for HLA A*0201, and five expressed the A*0301 allele. Because the responses overall were low and infrequent, specific mapping of the epitopes to which these volunteers had responded was not

performed. Also as a result of the low and infrequent responses, attempts to fractionate the PBMCs to define CD4+ and CD8+ responses were not pursued. The low number of ELISPOT responders in this trial prevented meaningful comparisons of the effects of GM-CSF on cellular responses to the CTL MEP vaccine. However, we note that only one of the responders had received GM-CSF.

An exploratory assay assessed binding antibody responses against the four peptides in the CTL MEP vaccine by ELISA. Response rates were low at two weeks following the first immunization, with 7.5% responders in the CTL MEP/RC529-SE group and 7.9% in the CTL MEP/RC529-SE + GM-CSF group. CTL MEP-specific antibody responses at 2 weeks following the 3rd immunization increased to 92% in both groups, and were 100% in both groups at day 98 (14 days post 3rd immunization). Median CTL MEP-specific antibody responses on day 98 were 8569 in the group without GM-CSF and 16324 in the GM-CSF group, representing a statistically-significant difference ($p = 0.04$).

3.4 GM-CSF neutralizing antibodies

The significance of antibodies against GM-CSF is unclear. In this trial, we measured neutralizing antibodies against GM-CSF at the end of the trial as an exploratory assay, and analyzed the data for correlations between anti-GM-CSF antibodies and any hematologic abnormalities such as neutropenia. Neutralizing antibodies against GM-CSF were consistently low in the MEP/RC529-SE group as expected. 25/37 (68%) samples from the GM-CSF group were positive at day 98, and 21/33 (64%) were positive at end of study, day 455. There was no greater incidence of neutropenia or anemia in the GM-CSF group, and no correlation between neutrophil or monocyte counts and the presence of GM-CSF neutralizing antibodies.

4. Discussion

Peptide-based vaccines could conceptually provide a valuable means of eliciting HIV-specific cell-mediated immune responses. Synthetic peptide vaccines have not been in the forefront of approaches to stimulate CD8+ CTL responses in humans, however, because they have not proven as effective in raising CTLs as have live vector-based vaccines [3,30]. The ideal synthetic peptide vaccine would generate potent pathogen-specific CD4+ and CD8+ immune responses, and would stimulate responses against epitopes that are subdominant in the context of natural infection or of a live vector-based vaccine. This potential has not yet been reached in human clinical trials. In this phase I trial, the potential of a multiepitope peptide vaccine to elicit HIV-specific T cell responses was evaluated. The vaccine consisted of a novel combination of four T helper/CTL epitope fusion peptides given with a new chemically-synthesized analog of MPL. GM-CSF was included in this evaluation for its potential to enhance antigen presentation, a potential that in preclinical studies seemed well justified [14, 21,22,24]. The CTL MEP vaccine was not initially designed as a stand-alone HIV vaccine, but rather as a means of priming for cellular responses that could then be boosted by a live vector or by adjuvanted DNA vaccines. However, as the CTL MEP vaccine tested here proved to be only minimally immunogenic in humans, plans for its use as a priming agent in a prime-boost regimen were subsequently abandoned.

A previous trial performed by the AIDS Vaccine Evaluation Group (AVEG), the clinical trials network that preceded the HVTN, evaluated a C4-V3 HIV envelope multiepitope peptide given to humans in incomplete Freund's adjuvant. This trial (AVEG 020) generated unacceptable local injection site reactions, including the formation of painful, draining, sterile abscesses and flu-like illness in several volunteers. The extreme local reactogenicity of this product led to the premature termination of the trial by the DSMB, the only time in the history of the AVEG/HVTN that this has occurred [31]. Although incomplete Freund's adjuvant was likely the major cause of the severe local reactions in AVEG 020, we were careful to monitor for similar

reactions in the present multiepitope peptide trial. Local pain and tenderness remained significant for the volunteers in this trial, although it did not approach the severity of that observed in AVEG 020. Local reactions were moderate, but were transient and did not prevent any volunteers from completing the vaccination schedule. We also noted that there was no detectable contribution of GM-CSF to the occurrence or severity of local injection site reactions.

A potentially valuable aspect of the conduct and evaluation of this trial was the measurement of anti-GM-CSF antibodies. The trial employed a licensed recombinant GM-CSF product (Leukine) that is commonly administered to neutropenic cancer patients. The safety record of GM-CSF made it seem unlikely that we would generate serious adverse effects from its use in this trial. However, we administered GM-CSF intramuscularly in combination with RC529-SE adjuvant, whereas for clinical indications it is given subcutaneously and without adjuvant. We hypothesized that this could lead to more potent neutralizing antibodies than in its most common use, and thus we measured neutralizing activity and monitored for adverse effects on leukocyte counts. The pathologic consequences of induction of anti-GM-CSF antibody is not clear, as it has been observed that plasma from 0.3% of normal healthy plasma donors possess neutralizing antibodies to GM-CSF, and 94% to 100% of commercially available human immunoglobulin preparations contain anti-GM-CSF neutralizing antibody activity [32,33]. Nevertheless, it is possible that anti-GM-CSF antibodies could play a pathogenic role, as suggested by the correlation between the severity of pulmonary alveolar proteinosis and titer of anti-GM-CSF antibodies in this patient population [34]. The potential of GM-CSF used as an adjuvant to generate antibodies that can functionally neutralize the cytokine has been studied in mice [35]. In this study, GM-CSF antibodies were readily elicited, and inhibition of GM-CSF activity was detected without any apparent adverse consequences. The present study was the first human vaccine clinical trial to measure antibodies raised following use of recombinant GM-CSF protein + adjuvant in a vaccine preparation. More than half of GM-CSF recipients developed measurable GM-CSF neutralizing antibodies based on a functional assay. It was reassuring that despite apparently high levels of neutralizing antibody, no adverse events were associated with the presence of anti-GM-CSF antibodies in this study. We note that one volunteer had a very high titer of such antibodies (2686 NU/ml) at day 98, yet had normal hematologic studies. Thus, in this small study, we found no evidence for adverse effects associated with anti-GM-CSF antibodies.

Peptide-based approaches to HIV vaccine development remain suboptimal, due primarily to weak immunogenicity. Methods of enhancing the local innate immune response and the magnitude of subsequent adaptive responses must be pursued in order to advance this line of investigation. It remains possible that modifications such as the addition of lipids to peptides can lead to sufficient enhancement of immune responses to make this approach viable [36]. The results reported here suggest that neither the MPL-like adjuvant employed (RC529-SE) nor the use of a cytokine co-adjuvant (GM-CSF) are sufficient to make this very weak immunogen into a potent one. We note that the lack of efficacy of RC529-SE in stimulating responses to the peptide component of this vaccine formulation may not be a reflection of its ability to act as an adjuvant for other antigens, as evidenced by the adjuvant properties of RC-529 in combination with a commercially available Hepatitis B vaccine [25]. The immunogenicity results overall were disappointing, and point out a central problem with the administration of synthetic peptides to humans for purposes of vaccination. We note that much higher magnitude CTL responses have been generated in humans by live vector approaches such as recombinant adenovirus vector vaccines [37,38]. In light of these results, peptide-based HIV vaccines for the stimulation of CTL responses will require significant enhancements before they can be counted among the most promising vaccine approaches.

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Fig. 1. Design and sequence of CTL multi-epitope peptides

Four multi-epitope peptides were used in combination in this study. Each peptide consists of a T helper epitope from *env* or *gag* fused to one of four CTL “hotspots” derived from *gag* or *nef*.

				Vaccination Schedule in Months (Days)		
Study Group	N	Peptide/RC529-SE Dose (µg)	GM-CSF Dose (µg)	0 (0)	1 (28)	3 (84)
1	40	1000/50	-	Vaccine	Vaccine	Vaccine
	8	-	-	Control	Control	Control
2	40	1000/50	250	Vaccine/GM-CSF	Vaccine/GM-CSF	Vaccine/GM-CSF
	8	-	-	Control	Control	Control

Total = 96

Fig. 2. Study Schema

Study participants received CTL MEP with RC529-SE with or without GM-CSF according to the schedule as shown.

Table 1
Amino acid sequence and allelic restriction^a of the multi-epitope T_H-CTL
Peptides A, B, C, and J

PEPTIDE “A”	
Allele	Sequence^b: gp160 (421-436) - p24 (30-40)
---	KQIINMWQEVGKAMYA-KAFSPEVIPMF
A2	NMWQEVGKAM
B57, B*5701, B*5703, B58, B*5801, B63	KAFSPEVIPMF
B57, B*5703	KAFSPEVI
B57, B58, B63	FSPEVIPMF
B35	SPEVIPMF
PEPTIDE “B”	
Allele	Sequence: p24 (130-146) - p24 (121-150)
---	YKRWIILGLNKIVRMYS-NPPIPVGEIYKRWIILGLNKIVRMYSPTSI
A*0201	ILGLNKIV
B8, B*0801	GEIYKRWII
B*0801	GEIYKRWIIL
B8, B*0801	EIYKRWII
A2, A24, A*2402	EIYKRWIIL
A*2402	IYKRWIIL
A24, A*2402, B27	IYKRWIILGL
B27, B*2703, B*2705	KRWIILGLNK
A2, A3	ILGLNKIV
B27	KRWIILGLNKIV
B27	RWIILGLNK
B27	RWIILGLNKIVRMY
A3, A11, A33	IILGLNKIVR
A3, A11	ILGLNKIVR
B7 supertype	ILGLNKIVRMY
B62	LGLNKIVRMY
B62	LGLNKIVRMYS
B15, B*1501, B62	GLNKIVRMY
Cw18, Cw*1801	VRMYSPTSI
B52, B*5201	RMYSPTSI
PEPTIDE “C”	
Allele	Sequence^b: gp41 (827-841) - nef (66-81)
---	DRVIEVVQGAYRAIR-VGFPVRPQVPLRPMTYK
A2, A*0201	RVIEVVQGA
A*3301	EVVQGACR
A*0201	VGFPVTPQV

B*0702	FPVTPQVPL
B*0702	TPQVPLRPM
A*1101	PLRPMTYK
A11	VPLRPMTYK
A1, B8	VGFPVTPQVPLRPMT
B7, B7 supertype, B35, B*3501, Cw*0401	FPVTPQVPL
B7, B*0702	FPVTPQVPLR
B7, B7 supertype, B35, B*4201, B*4202, B81, B*8101, CW*0401	TPQVPLRPM
B7, B7 supertype, B35, B*3501, B51	TPQVPLRPMTY
B35, B51	PQVPLRPMTY
A2, A3, A*0301, A3 supertype, A11, A*1101, B8, B27, B35	QVPLRPMTYK
A3, B35, B*3501	VPLRPMTY

PEPTIDE “J”

Allele	Sequence: gp160 (421-436) - p24 (8-27)
---	KQIIINMWQVVGKAMYA-GQMVHQAI SPRTLNAWVKVV
A3 supertype	MVHQAI SPR
B*1510	HQAI SPRTL
B*5701	IS PRTLNAW
B*0702	SPRTLNAWV
B57	GQMVHQAI SP
Cw3	GQMVHQAI SPRTL
A3 supertype	QMVHQAI SPR
B*1510	VHQAI SPRTL
Cw3	QAI SPRTL
A25, A*2501	QAI SPRTLNAW
B57, B63	AIS PRTLNAW
B57, B58, B*5801, B63, Cw*0602	IS PRTLNAW
B7, B*8101	SPRTLNAWV
A2	RTLNAWVKV
A*0201	TLNAWVKV
A2, A*0201, A*0202	TLNAWVKVV

^aBased on sequence and epitope data maintain within the Los Alamos National Laboratory HIV immunology Database (<http://www.hiv.lanl.gov/content/immunology>)

^bEach peptide epitope is presented in its appropriate location relative to the sequence of HXB2.

Table 2
Reactogenicity Maximum severity of local and systemic reactogenicity symptoms for each participant, all injections combined

Symptom	Control N = 16		MEP/RC529-SE N = 40		MEP/RC529-SE + GM-CSF; N = 40		Total N = 96	
	N	%	N	%	N	%	N	%
Injection Site Pain and/or Tenderness								
None	4	25.0	0	0	1	2.5	5	5.2
Mild	11	68.8	11	27.5	16	40.0	38	39.6
Moderate	1	6.3	26	65.0	23	57.7	50	52.1
Severe	0	0	3	7.5	0	0	3	3.1
Erythema/Swelling/Induration								
None	15	93.8	27	67.5	30	75.0	72	75.0
>0 to 10 cm ²	1	6.3	4	10.0	6	15.0	11	11.5
>10 to 25 cm ²	0	0	3	7.5	2	5.0	5	5.2
>25 cm ²	0	0	6	15.0	2	5.0	8	8.3
Malaise/Fatigue								
None	12	75.0	12	30	15	37.5	39	40.6
Mild	4	25.0	18	45.0	9	22.5	31	32.3
Moderate	0	0	9	22.5	14	35.0	23	24.0
Severe	0	0	1	2.5	2	5.0	3	3.1
Headache								
None	13	81.3	24	60.0	13	32.5	50	52.1
Mild	3	18.8	13	32.5	19	47.5	35	36.5
Moderate	0	0	3	7.5	7	17.5	10	10.4
Severe	0	0	0	0	1	2.5	1	1.0

Table 3
ELISPOT results, mean background-adjusted spot-forming cells (SFCs)/million PBMC

Volunteer	Peptide Pool	Visit Day	GM-CSF	Mean background - adjusted SFCs
056-10	ABCJ	42	No	75
	B	42	No	158
056-37	B	42	No	70
056-42	ABCJ	42	No	58
056-64	ABCJ	98	No	94
	B	98	No	112.5
056-67	ABCJ	42	Yes	82.5
056-71	A	42	No	79

Table 4**HLA Type of Responders**

056-10	A*0201	A*0301	B*40	B*44
056-37	A*0201	A*0301	B*0702	B*0702
056-42	A*0301	A*0301	B*0702	B*0801 or B*0822
056-64	A*0201	A*0205	B*0801/22	B*4901
056-67	A*0201	A*0301	B*1501	B*1801 or B*1817N
056-71	A*0201	A*0301	B*3503	B*3503