Proliferative Responses to Human Immunodeficiency Virus Type 1 (HIV-1) gp120 Peptides in HIV-1–Infected Individuals Immunized with HIV-1 rgp120 or rgp160 Compared with Nonimmunized and Uninfected Controls

Karl V. Sitz, Silvia Ratto-Kim, Aimee S. Hodgkins, Merlin L. Robb, and Deborah L. Birx

The proliferative responses to a series of peptides constituting the human immunodeficiency virus type 1 (HIV-1) gp120 sequence were evaluated in 19 HIV-1–infected rgp160 vaccine recipients, 17 HIV-1–infected rgp120 vaccine recipients, 15 HIV-1–infected placebo recipients, and 18 HIV-1–uninfected controls. Many regions of the gp120 molecule were found to contribute proliferative epitopes, although there were clearly regions of relative dominance and silence. Vaccine recipients tended to have broader, more robust, and more frequent peptide recognition than the placebo recipients. Despite the considerable variability in the pattern of peptide recognition among individuals, there was a striking similarity between the rgp160 and rgp120 vaccinee groups as a whole. Low-risk HIV-1–uninfected individuals may react to a few peptides within the gp120 sequence as well, despite a lack of significant response to the whole gp120 protein.

Human immunodeficiency virus type 1 (HIV-1)–specific CD4+ T lymphocytes play an important role in the immunoregulation of HIV-1 infection because of their central role in directing and augmenting both the humoral and cellular arms of the immune response. Unfortunately, CD4+ cells, as well as critical antigen-presenting cells, are primary targets of HIV-1 infection. HIV-1 infection of these cells, in ways that are incompletely understood, leads to associated dysfunction of the immune system. Numerous studies have confirmed the progressive dysfunction in CD4+ proliferative responses to common recall antigens and mitogens associated with progression of HIV-1 infection [1–3]. In that context, recent studies have suggested that CD4+ proliferative responses to HIV-1 proteins may have important prognostic value [4].

The envelope glycoproteins of HIV-1, gp160 and gp120, have been the focus of intense immunologic study since their role in HIV-1 binding and entry into target cells was elucidated (reviewed in [5]). The proliferative response to a whole protein such as gp120 is a function of the complex interaction between individual epitopes of the protein, in the context of major histocompatibility complex class II molecules on antigen-presenting cells and gp120-specific CD4+ T lymphocytes. Investigators have examined the proliferative capacity of peripheral blood mononuclear cells (PBMC) response to whole HIV-1–envelope proteins, as well as their peptide constituents, in HIV-1–infected and uninfected cohorts, in an attempt to better understand the quality and potential deficiency of the anti–HIV-1 envelope T helper repertoire (reviewed in [6]). This study evaluated these responses in the context of specific immunologic augmentation.

Repetitive immunization of HIV-1–infected volunteers with HIV-1 proteins has been attempted in an effort to augment putative protective, HIV-1–specific immune responses (reviewed in [7, 8]). We have undertaken several clinical trials that have evaluated the safety and immunogenicity of recombinant HIV-1 envelope proteins, rgp160 and rgp120, injected into early-stage HIV-1–infected volunteers ([9] and unpublished data). As a consequence, a comparison of the proliferative responses to peptide constituents of the whole gp120 molecule has been possible. The location and quality of the PBMC proliferative responses to overlapping peptides of the gp120 sequence were evaluated in HIV-1–infected rgp160, rgp120, and placebo recipients as well as in low-risk non–HIV-1–infected volunteers.

Materials and Methods

Subjects. Nineteen HIV-1–seropositive volunteers participating in a phase I rgp160 (NL4-3, IIIIB-like; MicroGeneSys, Meriden, CT) vaccine therapy trial and 17 HIV-seropositive volunteers par-
Fifteen HIV-1-seropositive volunteers participating in a phase II rgp160 (MicroGeneSys) vaccine therapy trial, who received placebo injections, served as the unvaccinated controls. Eighteen HIV-seronegative laboratory workers served as the uninfected controls for the peptide proliferation assay.

**PBMC isolation.** About 14–20 mL of venous blood from vaccine therapy participants and 30–40 mL of venous blood from HIV-uninfected volunteers were drawn into heparinized tubes and processed within 1–5 h of being collected. PBMC were isolated by ficoll-hypaque density centrifugation as previously described [10]. All proliferation assays were performed from 20 May 1994 to 14 April 1995 using standardized reagents and techniques.

**Synthetic HIV-1 peptides.** The peptides used in this study are described in figure 1. The approximate locations of each peptide in the context of the previously described constant and variable regions are illustrated in the upper panel (figure 1A). The peptides represent a combination of commercially available as well as newly synthesized peptides and are therefore not of uniform length or overlap (figure 1B). They do, however, span the entire gp120 LAI/LAV consensus sequence from the end of the signal sequence until the beginning of the transmembrane sequence of gp41. Disregarding peptide 259, whose sequence is completely contained within peptide 246, the average length of the peptides is ~29 amino acids (range, 23–40) with an average overlap of 10 amino acids (range, 3–13). There was typically excellent sequence homology between the immunogens (rgp120 and rgp160) administered in the two trials and the consensus LAI sequence represented in the peptides. However, peptide sequences 132, 162, and 191, in the V1 and V2 region, differed from the immunogen sequences by 10%–30%, due to a

Figure 1. HIV-1 gp120 peptides used in peripheral blood mononuclear cell proliferation assays. A. Approximate location of peptides in context of described constant and variable regions. B. Amino acid sequences, with corresponding length and overlap. Amino acids overlapping with flanking peptides are underlined.
combination of point heterogeneity as well as deletions (depicted in [11]).

Peptides LAI 74, 99, 132, 162, 246, 306, 348, and 420 were purchased (SynTheCell, Rockville, MD). Purity was ≥90%, carboxy-termini were amidated, and cysteine-containing peptides were mixtures of monomers and dimers. All other peptides were synthesized by standard solid-phase methods using a Waters Excell Synthesizer and Fmoc chemistry (Millipore, Bedford, MA). The carboxy-terminus of each peptide was amidated; the amino-terminus was not derivatized. Cysteines at the amino-terminus were left free; internal cysteines were blocked with acetamidomethyl groups to prevent polymerization.

**Proliferation assays.** The proliferative response was measured by incubating 1 × 10^5 PBMC in wells containing each peptide at 5 μg/mL, 5 μg/mL rgp120(IIIB) (Genentech), and medium only on a 96-well round-bottom plate (Costar, Cambridge, MA). For the rgp120 and rgp160 recipients, the peptide and rgp120 wells were tested in triplicate, whereas the medium wells were tested in 6 replicates, because of a diminished number of available cells. For the placebo recipients and uninfected volunteers, the peptide wells were tested in 6 replicates, the rgp120 wells in 4 replicates, and the medium wells in 12 replicates. After 7 days of incubation, the cells were pulsed with 1.67 μCi/well tritiated thymidine for 18 h, harvested, and counted in an β-counter (Beta Plate, model 1205; Wallac, Uppsala, Sweden). The data were expressed by the lymphocyte stimulation index (LSI), which is the mean counts per minute of antigen-pulsed PBMC divided by the mean counts per minute of unpulsed PBMC, to define antigen specificity. PBMC were arbitrarily defined as positive for the particular antigen if their LSI was ≥5.

**Results**

The frequency of positive PBMC proliferative responses, as defined by LSI ≥5, to each overlapping peptide of the gp120 sequence and the rgp120 molecule is depicted for the combined rgp120 or rgp160 vaccine recipients, the placebo recipients, and the HIV-1–uninfected volunteers (figure 2).

For the combined vaccine recipients there was broad and frequent recognition of the gp120 peptides and whole gp120 molecule (figure 2). The overall reactivity to the whole rgp120 molecule was ~75%. Although almost all of the gp120 sequence was recognized by at least 1 volunteer, there was significant variability in the rate of response between peptides. For example, peptide 152 was recognized by 53% of the vaccine recipients while peptide 283 was never recognized. In vaccine recipients, 7 peptides (112, 152, 191, 269, 319, 337, and 443) were recognized by ≥20%, while 11 other peptides were recognized by <10%. The remaining 8 peptides were recognized by 10%–20% of vaccine recipients.

In contrast to the HIV-1–infected vaccine recipients, a similar cohort of HIV-1–infected individuals who received placebo injections had a relative paucity of proliferative responses (figure

![Figure 2](image-url)

**Figure 2.** Frequency of individual peptide and whole rgp120 protein reactivity among combined HIV-1–infected vaccine recipients (rgp120 or rgp160), HIV-1–infected placebo recipients, and low-risk HIV-uninfected laboratory workers. “Positive reactivity” was defined as lymphocyte stimulation index ≥5.
2). Only 2 (13%) of 15 placebo recipients had proliferative responses to the whole gp120 protein. The whole gp120 protein responders had concomitant reactivity to 3 and 5 peptides, respectively (data not shown). All 18 of the total peptide responses from the placebo recipients came from 5 individuals with ≥2 responses each (range, 2–6 peptides/individual). The average LSI was 8.5 for all positive peptide reactivities and 5.9 for the 2 gp120 reactivities (data not shown).

The frequency of positive proliferative responses is also depicted for 18 low-risk HIV-uninfected laboratory workers in figure 2. There were 8 peptides recognized by 7 of the uninfected volunteers, for a total of 15 positive reactivities. Peptides 132, 152, 269, and 337 were recognized by ≥2 individuals, while peptides 162, 191, 319, and 391 were recognized by 1 person each. However, 2 individuals accounted for 10 of the 15 reactivities. Both of these individuals recognized peptides 132 and 152 (which share a 10 amino-acid sequence overlap) as well as peptides 269 and 337. Interestingly, despite sometimes multiple peptide reactivities, there was no significant reactivity to the whole gp120 molecule.

The frequency of positive proliferative responses to each overlapping peptide of the gp120 sequence and the whole rgp120 molecule is depicted separately for the 19 rgp160- and 17 rgp120-immunized volunteers in figure 3. Taking into account individual variability, the overall findings of peptide reactivity were quite similar between the 2 groups of volunteers. Six of the 7 peptides that were most commonly recognized as a group were recognized by ≥20% of both subgroups of vaccinees, with <10% variability between them. Peptide 443 activity did occur in ≥20% of both subgroups but was recognized by 18% more rgp160 recipients than rgp120 recipients. Conversely, peptide 74 was recognized by 19% more rgp120 than rgp160 recipients. There were other regions of ≥10% discrepancy, primarily in peptides that were infrequently reactive for the entire group (peptides 99, 246, 409, and 480); in fact, peptides 99 and 409 were uniquely recognized by the rgp120 recipients. The overall frequency of whole rgp120 protein reactivity was essentially identical between the groups (76% vs. 74%).

In addition to the similarities in the frequency of peptide recognition between the rgp120 and rgp160 vaccinee groups, there were no statistical differences in the magnitude of the positive responses (LSI >5; data not shown). The mean LSI of all positive responses for the combined rgp120 and rgp160 vaccine groups ranged from 5 to 45. For 21 of the 25 positive peptide reactivities, the mean LSI was ≥10, indicating a relatively robust proliferative response. The correlation between the frequency and magnitude of peptide reactivity was relatively poor (data not shown).

There was tremendous heterogeneity in the breadth and magnitude of individual patterns of peptide recognition, even when controlling for the magnitude of the whole rgp120 protein re-
response. For example, 3 individuals with similarly moderate LSI to whole rgp120 (LSI ~25–30), demonstrated significant heterogeneity in the patterns of peptide reactivity (figure 4). Volunteer 33613 demonstrated reactivity to ≥10 peptides, while volunteer 32657 had a single peptide reactivity (peptide 191). Both of these individuals had relatively modest peptide LSI (range, 5–13) compared with volunteer 32873, who had 2 peptide reactivities (peptides 152 and 269) that were equivalent to the whole rgp120 response (LSI ~25), as well as a modest reactivity to peptide 443.

Discussion

Elucidation of CD4+ T cell epitopes is critically important to the understanding of the immunoregulation of HIV-1 infection. Humoral as well as cellular immune mechanisms are regulated by specific CD4+ recognition of HIV-1 epitopes in the context of HLA class II presentation. Once CD4+ epitopes are fully delineated in the context of natural infection, immunotherapy, and viral escape via mutation, potential correlates of protective immunity may be identified and augmented by specific vaccination. Although there are several studies that have examined the proliferative responses to regions of the HIV-1 envelope in natural history cohorts [12–16] and envelope-immunized volunteers [17], we present the first comparative description of HIV-1 envelope proliferative responses in HIV-1–infected volunteers who have undergone repetitive immunization with either rgp120 or rgp160 in phase I clinical trials. Although these phase I trials, using multiple dose and schedule combinations, were not specifically designed for direct comparison, the similarities in the volunteer cohorts and multiple immunizations over relatively long periods invited qualitative comparisons. We then compared the responses of these envelope vaccine recipients to those of HIV-1–infected individuals who received placebo injections and low-risk HIV-1–uninfected laboratory workers.

There was a striking breadth of regions of the gp120 molecule that contributed T helper epitopes in HIV-1–infected volunteers who underwent repeated rgp160 or rgp120 immunization. Only a single peptide (peptide 283), in the early V3 region, was not recognized by any of the vaccinee cohort. The 7 most commonly recognized peptides, with >30% recognition, were clustered in three broad regions of the sequence. The first 3 frequently recognized peptides were clustered from the carboxyl end of C1, through V1 and V2, and into early C2. Other commonly recognized regions were in the carboxyl end of C2, the carboxyl end of V3 into early C3, and the carboxyl end of C4. Because the peptides tested had high homology with the immunizing proteins, rgp120 and rgp160, and not necessarily with an individual’s infecting viral sequence, epitopes found in highly

Figure 4. Variability in recognition of gp120 peptides among 3 individuals whose peripheral blood mononuclear cells had similar reactivity to whole rgp120 protein.
variable regions may have limited effects on HIV-1 immunoregulation.

Although direct comparison of the peptide reactivity in this study to that in other published studies is somewhat difficult due to differences in peptide sequences, patient populations, and proliferation assay methodologies, some general comparisons can be made (table 1) [6, 13–16]. Six of the 7 most commonly recognized peptides among vaccinees have been previously seen in similar regions in other studies [6, 13–16]. Epitopes in the peptide 443 region, which is just carboxyl to the previously described T1 epitope [17–19], have apparently not been reported previously in humans [6, 13–16].

Other regions that commonly contained proliferative epitopes in other studies are also listed in table 1. These regions were far less commonly recognized in our study. Other commonly described T helper epitopes, such as T2 (peptide 99), P18 (peptide 306), and T1 (peptide 420) [17–19], were infrequently recognized in this study, which is compatible with the findings of Geretti et al. [13]. Because of the experimentally observed breadth of the epitopes within gp120 following repetitive immunization and the highly diverse HLA expression within our population, we suspect that computer algorithms predicting CD4⁺ epitopes may not be as sensitive as previously reported [20–22]. We are currently evaluating a novel in vivo technique for rapidly determining an individual’s T helper epitope repertoire [10].

It is notable that our comparable cohort of placebo recipients generally recognized many fewer peptides than both vaccine recipients and HIV-1–infected volunteers in the other published reports mentioned above (except for [13], which was quite similar). This is likely a result of the much more stringent criterion of positivity (LSI ≥5) compared with the other studies and of the acquisition of new and boosted proliferative epitopes during the course of envelope vaccination [23–28]. The high LSI values for the whole rgp120 protein and the constituent peptides among the rgp120 and rgp160 vaccinees, in comparison with the placebo, uninfected, and other, previously described HIV-1–infected cohorts, would tend to confirm the idea of augmented cellular immune responses. Acquisition of broad CD4⁺ epitope recognition, particularly in conserved regions, is a primary goal of HIV-1 preventative and therapeutic vaccination.

In comparing the rgp120 and rgp160 vaccinees, there was remarkable similarity in the frequency and magnitude of the peptide and whole rgp120 responses. Each of the 7 most commonly recognized peptides was recognized by >20% of both vaccinee subgroups. Reactivity to the whole rgp120 protein was essentially identical, with a frequency of ~75%, consistent with a much larger cohort of rgp160 recipients in a phase II trial ([23] and unpublished data). The similarities are notable, because the rgp120 immunogen is expressed in a mammalian system, with mammalian glycosylation and preserved CD4⁺ binding, while the rgp160 immunogen is expressed in an insect system, is unable to bind CD4⁺, and has insect glycosylation.

Because the context of epitope presentation has been experimentally demonstrated to be important [29–31], we postulate that the presentation of epitopes is generally very similar between the rgp120 immunogen and the rgp160 immunogen. However, minor differences, such as the finding that peptides 99 and 409 were recognized by rgp120 recipients but not rgp160 recipients, could be due to intrinsic differences in antigen presentation or could have occurred by chance alone.

Despite the similarities between the peptides recognized by the subgroups of vaccinees as a whole, there was significant variability in patterns of peptide recognition among individuals. This should not be surprising, in light of the diversity of the HLA types, combined with presumed T cell receptor diversity, in this population. However, the significant variability in the breadth and magnitude of peptide reactivity, despite similar whole rgp120 recognition, among individuals may have profound implications for HIV-1 immunoregulation. Even robust recognition of a limited number of variable sequences may predispose an individual to viral escape mutation when compared with an individual with broad recognition of conserved envelope sequences. One goal of HIV-1 vaccine design should be the acquisition of cellular immune responses directed toward a broad array of conserved sequences that permit continued recognition of mutant whole proteins [29, 32, 33].

The lack of peptide reactivity, despite the significant whole rgp120 reactivity seen in a few individuals, could be a result of sequence heterogeneity between the immunogen and 1 (or a few) of the peptides or potential differential antigen processing between the peptides and the whole molecule [29]. There was typically good sequence homology between the immunogens and the consensus LAI sequence used to synthesize the peptides. However, peptide sequences 132, 162, and 191 differed from the immunogen sequences by >10%. This sequence heterogeneity in regions that were the site of reactivity for many individuals could explain the occasional finding of individuals with significant whole rgp120 reactivity but no peptide reactivity.

Low-risk HIV-uninfected individuals’ PBMC may proliferate to peptides within the gp120 sequence. The most frequently recognized regions among the uninfected controls in this study

<p>| Table 1. Comparative frequencies (%) of selected peptide recognition. |</p>
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(peptides 132–191, 269, and 337) correspond very well to regions reported to be recognized by uninfected individuals in a previous study [14]. The lower frequency of peptide recognition by uninfected controls in the present study is likely to be a result of a more stringent criterion for positivity (LSI ≥ 5). Interestingly, and consistent with previous reports [29, 34], proliferative responses to constituent peptides in low-risk HIV-1–uninfected individuals did not confer proliferative responses to the whole rgp120 protein. Without inferring prior noninfectious exposure to HIV-1, these proliferative responses may represent cross-reactivity with homologous sequences from other infectious or environmental sources, as previously postulated [14, 35].

Our study identified, in 10–20 amino acid segments, the frequency and magnitude of proliferative responses to HIV-1 gp120 in a large group of HIV-1–infected rgp120 or rgp160 vaccine recipients, HIV-1–infected placebo recipients, and low-risk HIV-1–uninfected controls. This information should be beneficial in future vaccine design and evaluation.

Acknowledgments

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References


