

Cross-Clade Reactivity of HIV-1–Specific T-Cell Responses in HIV-1–Infected Individuals From Botswana and Cameroon

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Summary: An effective HIV type 1 (HIV-1) vaccine will likely require elicitation of broadly reactive cell-mediated immune (CMI) responses against divergent HIV-1 clades. We compared anti-HIV-1 T-cell immune responses among 363 unvaccinated adults infected with diverse HIV-1 clades. Response rates to clade B Gag and/or clade B Nef in Botswana (95%) and Cameroon (98%) were similar when compared with those in countries previously studied, including Brazil (92%), Thailand (96%), South Africa (96%), Malawi (100%), and the United States (100%). Substantial cross-clade cell-mediated immune responses in Botswana and Cameroon confirm previous findings in a larger, more genetically diverse collection of HIV-1 samples.

Key Words: HIV, cell-mediated immunity, viral clade

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Worldwide, there are approximately 5 million new HIV type 1 (HIV-1) infections annually, and the incidence

of HIV infection and AIDS-related deaths is still high despite improved access to prevention, treatment, and care.¹ Development of a safe and effective prophylactic vaccine for HIV, as a component of an effective global prevention strategy, is an urgent priority.

The lack of success of first-generation humoral vaccine strategies² has led to continued focus on eliciting antiviral cell-mediated immune (CMI) responses as a component of most HIV-1 vaccine strategies.^{3–6} Vaccines designed to elicit virus-specific CMI responses must overcome the immunologic challenge posed by the considerable diversity of HIV-1 worldwide. It is unclear whether viral diversity limits broadly reactive CMI responses and whether inadequate cross-reactive CMI responses would necessitate construction of several clade-specific vaccines.⁷

To evaluate the breadth of anti-HIV-1 T-cell responses, we used a validated, accurate, and reliable method to systematically quantify CMI responses in unvaccinated HIV-1–infected individuals from diverse geographic areas. We previously presented results on 250 participants from 4 continents, including the countries of Brazil, Malawi, South Africa, Thailand, and the United States,⁸ which were representative of the 3 most predominant HIV-1 viral subtypes (clades A, B, and C) worldwide.⁹ The CMI responses against a series of HIV-1 proteins (Gag, Pol, Nef, Env, Rev, and Tat) were assessed. The 2 viral proteins that yielded the highest CMI responses overall, Nef and Gag, were used to assess cross-reactivity among clades A, B, and C HIV-1 proteins within each country. Based on a ratio of CMI responses to heterologous versus homologous (infecting) clades of HIV-1 (where 1.0 indicates equally strong responses for heterologous vs. homologous clades), cross-clade reactivity of cellular immune responses was substantial for Nef proteins (0.97) and lower for Gag proteins (0.67).

Here we report additional findings on cross-clade reactivity of CMI responses among unvaccinated HIV-1–infected individuals from Botswana and Cameroon to extend our previously reported data in a more genetically diverse collection of HIV-1 samples. The prevalence of HIV-1 in Botswana is one of the highest in the world, with estimates of adult infection often exceeding 30%.¹ Cameroon

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represents a particularly genetically diverse region and is therefore of particular interest in assessing cross-clade reactivity in support of a vaccine development strategy.¹⁰⁻¹²

SUBJECTS AND METHODS

Study Design

Independent ethical review committees at each study site approved the study protocol, and all participants provided written informed consent.⁸ In addition to the 250 HIV-1-positive participants aged 18 to 55 years who have been described and were recruited from clinical sites in Brazil (Rio de Janeiro and São Paulo), Malawi (Thyolo tea estate), South Africa (Soweto), Thailand (Bangkok), and at 10 sites in the United States,⁸ 25 more participants were included in this final analysis based on completion of laboratory assays. This analysis also includes 48 participants who were enrolled in Botswana (Gaborone) and 40 enrolled in Cameroon (Yaounde).

Determination of HIV-1 Clade

For participants from Botswana and Cameroon, we performed sequencing of 3 polymerase chain reaction-amplified gene regions (*gag*, *nef*, V3 loop of *env*). Reconstruction of phylogenetic relationships between generated viral sequences and HIV-1 subtyping was performed by standard phylogenetic packages including software for recombinant analysis. The predominant HIV-1 clade identified in each country was defined as the homologous clade; all other clades were defined as heterologous.

Selection of HIV-1 Antigen Sequences for Study

Using peripheral blood mononuclear cells (PBMCs) isolated from each participant, the T-cell response to each viral antigen was estimated by characterizing the reactivity of

T cells to peptide pools derived from near-consensus HIV-1 Gag, Nef, Pol, Rev, and Tat proteins. Preparation of the peptide pools and complete sequencing methodology were reported previously.⁸

Measurement of T-Cell Responses

In this study, T cells isolated from each research subject were exposed to discrete peptide pools derived from individual homologous and heterologous HIV-1 gene products, and the interferon- γ (IFN- γ) enzyme-linked immunospot (ELISPOT) was used to quantify the T-cell responses to antigenic stimulation. Results were expressed as the number of IFN- γ -expressing cells per million PBMCs. Four participants with background (no antigen) ELISPOT values ≥ 200 IFN- γ -expressing cells/million PBMCs were excluded from the analyses.

Statistical Analyses

Individual ELISPOT responses from HIV-infected individuals depend on the antigen, the infecting clade, and the number of cytotoxic T lymphocytes per million PBMCs. The ratio of ELISPOT responses to a heterologous clade versus ELISPOT responses to the homologous (infecting) clade was calculated for each participant. To calculate the ratios, the response to the predominant clade in each country studied was used as the denominator, and the response to each of the other 2 clades was used as the numerator in separate calculations. The geometric mean of these ratios for all individuals in a sample provides an estimate of the overall ratio of stimulation probabilities for each sample, specific to the gene, the clade of infection, and the stimulating (heterologous) clade. These probability ratios are cross-clade reactivity ratios and were calculated using SAS (Version 8; SAS Institute, Cary, NC); ratios closer to 1 indicate greater cross-clade reactivity, and ratios closer to 0

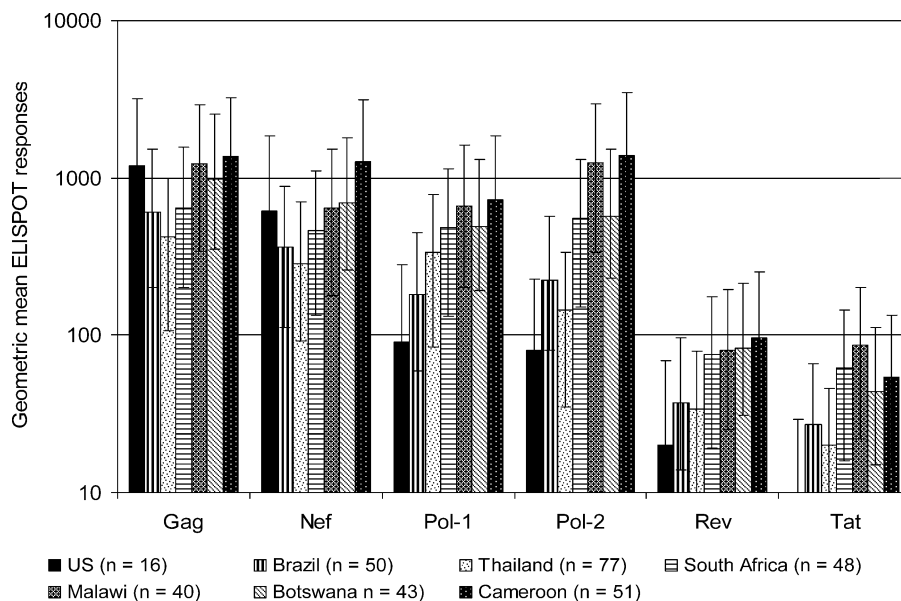


FIGURE 1. HIV-1-specific T-cell responses to clade B antigens. The magnitude of responses is shown as geometric mean \pm SEM of IFN- γ ELISPOT assay responses among 325 HIV-1-infected participants, expressed as the number of IFN- γ -expressing cells per million PBMCs.

indicate less cross-clade reactivity. Additional statistical methodology has been reported.⁸

RESULTS

The median age of the participants from Botswana was 33.5 years; all were black and 33% were female. The Cameroonian participants had a median age of 34.9 years; all were black, and 55% were female. The median CD4⁺ T-cell count (cells per microliter) was 402 in Botswanian participants and 424 in Cameroonian participants. The median plasma viral load was 9165 and 22,700 copies/mL in participants from Botswana and Cameroon, respectively.

All tested *gag* (n = 11), *nef* (n = 9), and *env* (n = 55) sequences from Botswanian participants were clade C. Among Cameroonian participants, on the basis of *env* sequencing (n = 44), clade types were 57% clade A, 14% clade D, 5% recombinant A/G, 5% recombinant A/G/F/J, and 2% each of clade G, recombinants A/F, F/J, F/K, A/G/J, A/D/H, and group

O. Based on *gag* sequencing (n = 17), clade types were 59% clade A; 12% each of clade D, F, and A/J recombinant; and 6% unclassified/clade A. Based on *nef* sequencing (n = 27), HIV-1 clade distributions included 25% of A/E recombinant, 21% of clade G, 14% of A/G recombinant, 11% of G/F recombinant, 7% of clade F, and 4% each of clade A and D, or D/B, G/B, A/E/G, and G/E recombinants.

Results from Botswanian and Cameroonian participants augment and are consistent with the previously reported comparison of CMI responses to HIV-1 proteins. Overall, ELISPOT results were available for 325 participants for all 5 HIV-1 antigens. Quantitative ELISPOT responses (Fig. 1) to clade B Gag, Nef, Pol-1, and Pol-2 were higher than responses to Rev and Tat for the overall study population ($P < 0.0001$) and within each country ($P < 0.007$). The proportion of participants with positive ELISPOT responses to clade B Gag (93%), Nef (84%), Pol-1 (82%), and Pol-2 (79%) were higher than those with positive ELISPOT responses to Rev (30%) or Tat (19%). The differences in

TABLE 1. Ratios of Cellular Immune Response Against HIV-1 Gag and Nef Proteins, for Heterologous Clades Versus Homologous Clades, Among 363 HIV-1-infected Participants

Country, Clade Comparison	Immune Response Ratio, Geometric Mean (95% CI)	
	Gag	Nef
New countries		
Botswana* (n = 48)		
A vs. C	0.51 (0.37–0.71)	1.04 (0.85–1.26)
B vs. C	0.81 (0.71–0.93)	1.08 (0.83–1.40)
Cameroon† (n = 40)		
B vs. A	0.84 (0.66–1.06)	1.18 (1.05–1.31)
C vs. A	0.92 (0.72–1.19)	0.90 (0.74–1.11)
Previously reported countries with updated data‡		
Brazil§ (n = 57)		
A vs. B	0.76 (0.64–0.90)	0.90 (0.75–1.09)
C vs. B	0.82 (0.68–0.97)	0.95 (0.79–1.14)
Malawi* (n = 54)		
A vs. C	0.52 (0.42–0.63)	0.74 (0.59–0.92)
B vs. C	0.69 (0.58–0.82)	1.00 (0.83–1.21)
South Africa* (n = 50)		
A vs. C	0.49 (0.41–0.58)	0.84 (0.64–1.09)
B vs. C	0.53 (0.42–0.66)	0.98 (0.84–1.15)
Thailand¶ (n = 77)		
B vs. A	0.65 (0.51–0.82)	0.73 (0.59–0.92)
C vs. A	0.54 (0.44–0.66)	0.92 (0.72–1.17)
United States (n = 37)		
A vs. B	0.63 (0.48–0.81)	0.89 (0.70–1.12)
C vs. B	0.65 (0.54–0.77)	0.63 (0.42–0.95)

Values in the table represent the geometric means of ELISPOT response ratios against Gag or Nef for each study population. For each participant, the ratio of ELISPOT responses to one clade versus another was calculated. The geometric mean of the ratio for the study population was then calculated. For each country, the ratios of heterologous clades relative to the homologous clade that is predominant in that country are shown. Ratios closer to 1 indicate greater cross-clade reactivity.

*Clade C is the predominant HIV-1 clade.

†For Gag, clade A is the predominant HIV-1 clade. However, for Nef, the clade distribution was heterogeneous, representing principally clade G and G recombinants followed by clade A recombinants. Therefore, no homologous clade could be defined, and the immune response ratios for Nef cannot be considered to be comparisons with the prevailing *nef* clade in Cameroon.

‡These data are shown for comparison purposes only.

§HIV-1 clade frequencies are as follows: clade B, 78%; clade F, 15%; clade C, 5%.

¶HIV-1 clade frequencies are as follows: CRF01_AE, 90%; clade B, 10%.

||Clade B is the predominant HIV-1 clade.

HIV-1 = human immunodeficiency virus type 1.

the proportion of positive ELISPOT responders were statistically significant for the overall study ($P < 0.0001$) and within each country ($P < 0.0001$), except in the 15 participants from the United States ($P < 0.06$) (data not shown). Overall, the ELISPOT response rate to clade B Gag or Nef proteins was 96%. The proportion of positive ELISPOT responses were 88% to all 3 clade (A, B, and C) Gag proteins, 74% to all 3 clade Nef proteins, and 93% to either all 3 clade Gag or all 3 clade Nef proteins. Response rates to clade B Gag and/or clade B Nef in Botswana (95%) and Cameroon (98%) were similar when compared with those in countries previously studied, including Brazil (92%), Thailand (96%), South Africa (96%), Malawi (100%), and the United States (100%).

Evaluation of Cross-clade CMI Responses

The quantitative assessment of the ratios of ELISPOT responses to the 3 major HIV-1 clades for Gag and Nef proteins that was previously completed was updated with the results from the Botswanian and Cameroonian participants. The geometric mean ratios of cellular immune responses in the overall study population were 0.91 [95% confidence interval (CI), 0.82–1.00] against clade A Gag versus clade B Gag, 1.07 (95% CI, 0.99–1.16) against clade C Gag versus clade B Gag, 0.95 (95% CI, 0.87–1.03) against clade A Nef versus clade B Nef, and 0.96 (95% CI, 0.87–1.05) against clade C Nef versus clade B Nef. The geometric means of cross-clade ratios were also calculated independently for each country and are presented in Table 1.

The lower cross-clade reactivity for anti-Gag responses among Botswanian participants confirms our previous finding of lower anti-Gag responses from countries with clade C predominant viruses (eg, Malawi and South Africa). However, cross-clade attenuation was not evident in the anti-Nef CMI responses among participants from Botswana, and the difference in cross-clade reactivity to Nef and Gag proteins was significant ($P < 0.001$). The immune response ratios among Cameroonian participants had the highest observed values for both anti-Gag (0.92 for clade C vs. clade A) and anti-Nef (1.18 for clade B vs. clade A) proteins.

DISCUSSION

Cytotoxic T-cell lymphocyte responses are considered an important immunologic component in the development of an effective HIV-1 vaccine. Development of a vaccine that has potential benefit to combat AIDS on a global level requires inclusion of antigens that elicit a CMI response against genetically diverse HIV-1 variants. Otherwise, a vaccine predicated upon induction of a CMI response would, in principle, require an ongoing effort to regularly revise the vaccine formulation to parallel the continuing evolution of this highly genetically agile pathogen.

Our data from HIV-1-infected participants in Botswana and Cameroon demonstrate that CMI responses to Nef, Gag, and Pol proteins were quantitatively higher than responses to Rev and Tat proteins and are consistent with the results obtained in the 5 previously studied countries. Overall, 93%

of participants responded to either all 3 clade Gag or all 3 clade Nef proteins, with anti-Nef cross-clade reactivity exceeding that of anti-Gag in Botswana. Despite the viral diversity of HIV-1 in Cameroon, which has a non-clade B-predominant virus and is an extremely active area for HIV-1 recombination because of the cocirculation of all major groups and subtypes of HIV-1, the response rate to clade B Gag and/or Nef proteins was 98%.

A few additional points should be noted when interpreting our findings. First, 57% and 59% of our samples in Cameroon were subtyped as clade A based on sequencing of the *env* V3 loop and *gag* regions, respectively. However, on the basis of Nef sequences, samples were primarily clade G and G recombinants, followed by clade A recombinants. Therefore, no homologous clade could be defined for Cameroon, and all 3 clades must be considered heterologous. This could help explain our finding that the geometric mean ratio of CMI responses against clade B versus clade A Nef proteins was >1.0 (1.18; 95% CI, 1.05–1.31). Of note, regardless of the fact that all 3 clades were heterologous, immune response ratios were substantial despite the predominance of circulating recombinant and unclassified forms of HIV-1 in Cameroon, and the geometric mean response to *gag* and *nef* clade A and C antigens were comparable with those shown in Figure 1 to clade B antigens (data not shown). Second, it is possible that the ELISPOT assay is insufficient to predict CMI response to antigen; however, the IFN- γ ELISPOT assay correlates well with other methods of quantifying T-cell responses, has been widely used to reliably quantify both HIV-1-specific and general CMI responses, and has a low ($<1.0\%$) false-positive rate.¹³ Finally, as others have suggested, we acknowledge that immune responses to an actual HIV vaccine administered to uninfected individuals may differ from immune responses in HIV-infected participants.¹⁴

Based on the 3 clades responsible for most HIV-1 infections worldwide, we have shown substantial breadth of CMI responses regardless of infecting virus genotype. Our results from Botswana and Cameroon further support the hypothesis that the development of a CMI-based HIV vaccine composed of antigens based on a single clade could be broadly effective and should be investigated further.

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