Cytotoxic T-Lymphocyte Cross-Reactivity among Different Human Immunodeficiency Virus Type 1 Clades: Implications for Vaccine Development

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Despite recent advances in antiviral therapy for human immunodeficiency virus (HIV) infection, successful global intervention will require an effective vaccine. Expanding evidence suggests that cytotoxic T-lymphocyte (CTL) responses will be an important component of such a vaccine. The varying geographic distribution of HIV type 1 (HIV-1) clades, with the relative absence of clade B HIV-1 outside the developed world, is considered a major obstacle to the development of a single efficacious vaccine. An understanding of cross-reactive CTL responses between different HIV-1 clades is crucial in the design of a vaccine which will be broadly immunogenic. In this study, we examined the ability of HIV-1 Gag-, reverse transcriptase-, and Env-specific CTL clones isolated from individuals infected in the United States to recognize non-B clade viral sequences and found that all were cross-reactive with the majority of non-B clade viral sequences tested. We next studied HIV-1-specific CTL responses in African individuals infected with clade A, C, or G virus and evaluated cross-recognition of clade B virus. Of 14 persons evaluated, all demonstrated cross-reactivity with the U.S. clade B viral constructs. We conclude that significant CTL cross-reactivity exists between clade B and non-B epitopes, suggesting that CTL cross-recognition among HIV-1 clades is more widespread than anticipated and that a vaccine based on a single clade may be broadly applicable.

The majority of human immunodeficiency virus (HIV) infections are in the developing world (46, 63). Whereas recent advances in antiretroviral therapy provide great optimism (11), issues related to cost and availability limit the likelihood that such interventions will have a global impact on the epidemic. The need for an effective vaccine has never been greater.

Although the correlates of immune protection in HIV type 1 (HIV-1) infection are not known, most agree that induction of cytotoxic T-lymphocyte (CTL) responses in addition to neutralizing antibodies will be an important component in an effective HIV-1 vaccine (10, 13, 18, 23, 24, 38). Murine studies provide strong evidence that virus-specific CTLs play an important role as an effective host immune response (8, 48, 49, 52, 64), and emerging data for human cytomegalovirus and Epstein-Barr virus disease suggest a protective role for antiviral CTLs as well (25, 59). In the course of infection with HIV-1, CTLs could play a determinant role in prognosis. HIV-1-specific CTLs are temporally associated with the initial control of viremia during primary infection (3, 4, 35) and decline in persons with disease progression (33). Moreover, strong CTL responses have been detected in individuals who remain asymptomatic with low viral loads (20, 21, 33, 50). HIV-1-specific CTLs have also been detected in heavily exposed uninfected persons (37, 47, 51). These findings have led to an emerging consensus that an effective HIV vaccine will need to induce virus-specific CTLs (10, 13, 18, 23, 24, 38).

The development of a broadly effective AIDS vaccine will

require the induction of immune responses that target specific viral clades in a given geographic region or, preferably, the induction of broadly cross-reactive immune responses. Most vaccines currently in development are based on clade B immunogens. Although this is the predominant clade in the United States and western Europe, clades A, C, D, E, and others predominate in the areas of the world with the greatest burden of infection (7). It has been widely assumed that there will be limited cross-reactivity for CTL responses among different clades in HIV-1 infection, such that different vaccines may be needed for different geographic areas (13, 15, 18). Encouraging recent data demonstrated the ability of a clade B immunogen to induce, from several vaccinees, CTLs capable of cross-reacting with non-B clade viruses (16). Although previous CTL work using clade B targets has been done in Africa (51), analyses of virus-specific CTLs generated in natural infections by diverse HIV-1 clades and the extent of cross-clade recognition are still lacking. Examination of the spectrum of specific cross-reactivity of CTLs between the different clades of HIV-1 will aid the design of an effective HIV vaccine that is applicable to more than one major geographic region.

In this study, we investigated cross-recognition by CTL responses among different HIV-1 clades by two different methods. We first examined the level of cross-reactivity of clade B CTLs with other HIV-1 clades and tested a panel of HIV-specific CTL clones that were isolated from individuals infected in the United States. These CTL clones had been characterized previously with respect to major histocompatibility complex (MHC) class I restriction and epitope specificity for clade B virus. Clones were further selected with specific HLA

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Identity
One amino acid difference
Two amino acid differences

Three amino acid differences
Four amino acid differences
Five or more amino acid differences

FIG. 1. Defined clade B CTL epitopes were examined for cross-clade sequence variability. The fractions of cross-clade conservation and variation for all defined epitopes (6) within a particular clade were calculated. These epitopes have been described in general in the context of B clade HIV-1 infections in the United States and Europe. HIV-1 protein sequence alignments used as a basis for this study were taken from the 1996 molecular immunology database (34), with the exception of the Pol alignment, which was updated in 1997. For example, for the 11 well-defined CTL epitopes in p17, 29 clade A isolate sequences were examined; 34% (pink) had identical sequences, 33% (blue) had one amino acid substitution, and 22% (turquoise), 6% (green), 4% (yellow), and 1% (red) had two, three, four, and five or more amino acid substitutions within the clade A sequences, respectively.

restricting alleles that were representative of HLA allele distributions in sub-Saharan African populations where vaccination efforts are currently being discussed. Corresponding peptides from consensus sequences of clades A, C, D, and E were synthesized, tested for recognition, and compared with the index epitopes. To evaluate the reciprocal hypothesis that HIV-1-specific CTL responses elicited from natural infection with non-B clade viruses could cross-react with clade B viruses, we evaluated CTLs from a group of HIV-1-infected individuals from Senegal, West Africa (41). In this high-risk population of commercial sex workers, heterosexual transmission is considered the route of infection, and the predominant HIV-1 clade is A, with clades C, D, and G less frequent (31).

MATERIALS AND METHODS

Subjects. Subjects infected with HIV-1 clade B were selected on the basis of virus-specific CTL activity and availability of established clonal lines and of known HLA restriction. These clones have previously been characterized, and their optimal epitopes have been defined (5). Individuals infected with non-B clade virus were randomly obtained from the cohort of registered commercial sex workers in Senegal, West Africa (31, 32, 41). Concomitant HIV-2 infection was excluded by immunoblot analysis. Peripheral blood mononuclear cells (PBMC) were separated, cryopreserved on site, and shipped in the vapor phase of liquid nitrogen to the United States for CTL studies.

HLA typing. HLA typing was performed by the Massachusetts General Hospital tissue typing laboratory, using standard serological and molecular typing techniques.

Non-clade B effector cells. Cryopreserved PBMC from HIV-1-infected African subjects were expanded into CD8 cell lines by using heterobifunctional monoclonal antibodies (62). PBMC were enriched for CD8 cells by *in vitro* stimulation with the CD3-CD4 bispecific monoclonal antibody CD3/4b. This antibody has one arm specific for the CD3 cell surface antigen and one arm specific for the CD4 cell surface antigen, with a common Fc portion, and results in the selective expansion of CD8 cells and depletion of CD4 cells (62). Typically cells can be expanded 20- to 40-fold, and the resulting cells are >95% CD8 positive by fluorescence-activated cell sorting (60). CD8 effector cells were then stimulated with autologous B-lymphoblastoid cell lines (B-LCL) infected with recombinant vaccinia viruses expressing HIV-1 clade B Gag-Pol and Env (vAbT 489; Therion Biologics, Cambridge, Mass.) and Nef (26) and inactivated by the UV-psoralen method (40). These cells were incubated with irradiated feeder cells and recombinant interleukin-2 (rIL-2) and assayed for activity after 10 to 14 days in the standard ⁵¹Cr release assay at an effector/target cell (E:T) ratio of 100:1.

Clade B CTL clones. Established HIV-1-specific CTL clones of clade B isolates were expanded in the presence of a CD3-specific monoclonal antibody, rIL-2 (Hoffmann-La Roche, Nutley, N.J.), and irradiated allogeneic PBMC

(feeder cells). The cell lines were maintained in RPMI 1640 (Sigma, St. Louis, Mo.) with 10% heat-inactivated fetal bovine serum (Sigma) supplemented with 50 U of human rIL-2 per ml, with medium exchange twice weekly. HIV-1-specific activity was evaluated after 10 to 14 days, using autologous B-LCL target cells incubated with serial peptide dilutions at an E:T ratio of 5:1.

Targets cells. Epstein-Barr virus-transformed B-LCL were established from each subject and maintained as described previously (56) in RPMI 1640 medium containing 20% heat-inactivated fetal bovine serum. RPMI 1640 medium used for all cell lines was supplemented with L-glutamine (2 mM), penicillin (50 U/ml), streptomycin (50 µg/ml), and HEPES (10 mM). These B-LCL were infected with either clade B recombinant vaccinia virus expressing Gag (vAbT 141) (36), Env (vAbT 299) (14), reverse transcriptase (RT) (vCF 21) (58), or Nef (26) or non-B clade vectors expressing Env (Quality Biological, Gaithersburg, Md.), Gag, and Nef (Therion Biologics) overnight or were incubated for 1 h with synthetic HIV peptides (30). All cell lines were mycoplasma free.

Generation of recombinant vaccinia viruses containing the gag and nef genes from clades A and E. The gag and nef genes from HIV-1 isolates representing clade A (92UG037) and clade E (90CR402) were cloned by PCR from HIV proviral DNA (17) and were provided by Feng Gao and Beatrice Hahn (University of Alabama). The gag genes contain the entire gag coding sequence, 19 bp of 5' noncoding sequence, and 91 bp of 3' noncoding sequence. The nef genes contain the entire nef coding sequence, 36 bp of 5' noncoding sequence, and 11 bp of 3' noncoding sequence. The HIV genes were inserted individually into a derivative of the New York City Board of Health (NYCBH) strain of vaccinia virus by homologous recombination using a host range selection system (53). The resulting recombinant vaccinia viruses contain the gag or nef gene under the control of the vaccinia virus 40K early/late promoter (19) flanked by DNA sequences from the HindIII M region of the vaccinia virus genome. Recombinant viruses were selected by growth on RK₁₃ cells (ATCC CCL 37). Virus stocks were prepared by clarifying infected RK₁₃ cell lysates followed by centrifugation through a 36% sucrose cushion. Insertion of each HIV gene into the vaccinia virus genome was confirmed by amplification of the recombinant genome by PCR. Expression of each HIV gene was demonstrated by Western blot analysis. The recombinant vaccinia viruses were designated rV-gagA_{UG37} (vT135), rV-nefA_{UG37} (vT139), rV-gagE_{CR402} (vT142), and rV-nefE_{CR402} (vT141).

Cytotoxicity assay. Vaccinia virus-infected targets were prepared by incubating 1×10^6 to 5×10^6 B-LCL in log-phase growth with recombinant vaccinia virus at 1 to 3 PFU/cell for 16 h at 37°C. Cytotoxic assay was performed by the standard ⁵¹Cr release assay (56) using round-bottom microtiter plates containing 10^4 target cells per well. Plates were incubated in a humidified incubator at 37°C for 4 h. All assays were performed in duplicate. Supernatants were then harvested and counted on a Cobra gamma counter (Packard Instrument Company, Downers Grove, Ill.), and percent lysis was determined from the following formula: $100 \times [(experimental\ release - spontaneous\ release) / (maximum\ release - spontaneous\ release)]$. Maximum release was determined by lysis of target cells in detergent (5% Triton X-100). HIV-specific CTL activity was defined as 10% above control. Spontaneous release was <30% of maximal release for all assays. For peptide titration assays, serial 10-fold dilutions from a peptide concentration of 100 µg/ml to 10 pg/ml were performed on 96-well plates. Peptides were added directly to the well and incubated with washed chromium-labeled target cells (final concentration of 10^4 cells/well) for 60 min to a volume of 100 µl. Effector clones (E:T ratio of 5:1) were added to a final volume of 200 µl.

HIV-1 subtyping. DNA was extracted from frozen PBMC by proteinase K-phenol extraction as previously described (39). To amplify the HIV-1 V3 region, a nested PCR was performed with two sets of primers: WT (5'GCT

TABLE 1. Number of isolate sequences used to analyze cross-clade CTL epitope variations with a required minimum of two sequences for each clade^a

Protein	No. of sequences used									Group O
	Clade A	Clade B	Clade C	Clade D	Clade E	Clade F	Clade G	Clade H		
p17	29	32	7	10		5	2	2	2	
p24	27	32		10		5	4	2	2	
gp120	46	107	46	28	20	14	15	2	5	
gp41	38	71	24	11	11	5	3	2	4	
Pol	2	50	7	3					2	
Nef	3	63		3	24				2	

^a The 1996 compendium was used for the alignment except for Pol, the alignment of which was updated in 1997 to include some C-subtype representatives. The compendium alignments are designed to give an overview of variability and contains only one viral sequence per infected individual.

TABLE 2. HLA class I distribution in selected African countries^a

HLA	Distribution (%)						
	Gambia	Mali	Nigeria	Senegal	Zimbabwe	South Africa	U.S. blacks
A2	14.1	15.9	14.7	19.5	15.5	13.0	18.8
A3	4.4	3.7	9.7	6.1	3.0	7.0	8.8
B7	6.7	8.9	7.3	4.5	8.8	10.5	9.7
B14	3.8	3.7	0.0	1.7	4.3	3.3	3.8
B35	16.1	8.2	5.9	13.1	2.1	2.1	8.2

^a Data for Senegal from reference 55a; other data from reference 1.

FIG. 2. HIV-1 clade B CTL clones recognition of non-B clade epitopes. Serial peptide titrations were done with HIV-1 clade B CTL clones, using the index peptides and respective synthetic peptides corresponding to the consensus sequences of non-B clades. The index peptide (clade B) represents the optimal epitope. Peptide concentrations from 100 μ g/ml to 10 pg/ml were used with an E:T ratio of 5:1 in a standard chromium release assay. When sequence data were not available for the consensus clade, representative sequences from known isolates (*; number assigned arbitrarily) that belong to the respective clades were chosen. Control lysis values in assays using irrelevant peptides were <10%.

GGTTTTCGATTCTAAAGTGA [6884 to 6908, positions relative to HXB2 in HIV database]) and WT2 (5' CAATAGAAAAATCCCTCCACAA [7353 to 7377]) for the first round; and published primers KK30 and KK40 (31, 61) for the second round. The PCRs were performed in a 100- μ l volume reaction mixture containing the DNA, 10 μ l of 10 $\times$ PCR Buffer II (Perkin-Elmer PCR Reagents, Roche Molecular Systems, Branchburg, N.J.), 2.5 U of *Taq* polymerase (Perkin-Elmer PCR Reagents), 0.2 mM each dideoxynucleotide, and 2 mM MgCl₂. Each reaction was subjected to 30 cycles of denaturation (45 s at 97°C), annealing (45 s at 57 and 55°C for the first and second rounds, respectively), and extension (45 s at 72°C), followed by a final extension of 3 min at 72°C in an automatic thermal cycler. Negative controls included deionized water and negative peripheral blood lymphocyte DNA in all experiments. The PCR product was purified by agarose gel electrophoresis and purification columns (Qiaquick gel extraction kit; Qiagen Inc., Chatsworth, Calif.) and directly sequenced by using the second-round primers (KK30 and KK40). Sequences were determined by dye terminator cycle sequencing using *Taq* polymerase and an automatic sequencer ABI 373 (both from Perkin-Elmer, Applied Biosystems Division, Foster City, Calif.).

Analysis, alignment, and comparison of the sequences obtained were per-

formed with the GeneWorks software (IntelliGenetics, Mountain View, Calif.) and the Clustal package of multiple alignment programs (Clustal W 1.6), with minor manual adjustment when necessary. Phylogenetic analysis was performed by the neighbor-joining method with 1,000 bootstrap resamplings, using Clustal W 1.6.

Nucleotide sequence accession number. The nucleotide sequences have been submitted to GenBank (accession no. AF02819 to AF02832).

RESULTS

Sequence variation in known CTL epitopes among different HIV-1 clades. CTLs recognize processed viral peptides generally 8 to 11 amino acids in length, which are presented as a molecular complex with MHC class I and β_2 -microglobulin. A single amino acid change within a CTL epitope can lead to lack of recognition, and thus it has been suggested that a vaccine based on a single clade would be unlikely to provide cross-

FIG. 3. Cross-clade recognition of a clade B Env-specific CTL clone that is B7 HLA restricted, using autologous B-LCL infected with recombinant vaccinia virus vectors expressing Env from clade A or B at an E:T ratio of 5:1. Target cells infected with the vaccinia virus NYCBH were used as a control vector.

clade protection (13–15). The availability of defined optimal CTL epitopes affords the opportunity to determine the degree of variability within epitopes based on reported sequences. As an initial assessment of interclade epitope variability, defined clade B CTL epitopes (6) were examined (Fig. 1). The fractions of cross-clade conservation and variation for the most well-defined epitopes in specific proteins (gp120, gp41, p17, p24, Nef, and Pol) (6) were computed by using the 1996 HIV molecular immunology database (34) (with the exception of the Pol alignments, which were updated in 1997 to include additional sequences of the C subtype). These alignments consisted of a single representative sequence from infected individuals, and complete protein sequences, compiled from the literature (43). At least two sequences of each clade were required for a protein subtype to be included in the analysis. For example, there are 11 well-defined CTL epitopes in p17. For clade A, 29 isolate sequences were included in the analysis of the p17 epitopes such that a total of 319 (11×29) epitope sequences were examined. Of these clade A epitope se-

quences, 34% were identical to the 11 CTL epitopes described for the p17 region, and similarly 33, 22, 6, 4, and 1% had one, two, three, four, and five or more amino acid substitutions in the clade A sequences, respectively. Sequence analyses were performed with clades A to O where applicable.

The total number of sequences examined for each clade respective to the particular CTL epitope region is shown in Table 1. Subtype E has a mosaic genome and has been defined as subtype E only in the envelope gene; thus, viruses that are subtype E in Env that have been fully sequenced are associated with subtype A in other regions of viral genome (9, 17). Sequences for clade C in p24, clades F to H in Pol, and clades C and F to H in Nef were not represented in the database. Some cross-clade sequence conservation was seen in CTL epitopes from all proteins. Clade B and D sequences, genetically the most similar, show the most potential cross-reactivity, whereas the clade E envelope shows substantially less potential cross-reactivity.

Cross-clade recognition by clade B-induced CTL responses.

The above-described sequence analysis suggests that cross-clade recognition by clade B CTLs could be common, particularly for the most conserved proteins, p24 and RT. However, even in these proteins, one, two, and sometimes more amino acid substitutions occur within epitopes, which might abrogate recognition. To test the effects of sequence variations on CTL recognition experimentally, we chose a panel of HIV-1-specific CTL clones (all generated from individuals infected in the United States) to evaluate cross-clade recognition. Clones restricted by HLA A2, A3, B7, B14, and B35 were chosen on the basis of adequate representation in selected African countries (Table 2) (1, 55a). Previous studies have shown that synthetic peptides that sensitize target cells at limiting peptide concentrations can be used to identify optimal epitopes and that these correspond to naturally processed proteins (54, 55). We performed serial peptide titrations and compared the optimal index clade B peptide with the analogous peptides representing other non-B clade sequences. Clones were tested at an E:T ratio of 5:1 and with peptide concentrations ranging from 100 μ g/ml to 10 pg/ml in a standard chromium release assay. Peptides corresponding to different HIV-1 clades were synthesized by using the consensus sequences obtained through the Los Alamos database (43). At the time these reagents were generated, a consensus sequence for clades C and E had not been defined for Gag and RT. In situations where the consensus sequences were incomplete, we selected representative sequences from known isolates belonging to the respective clades.

Of the nine clones tested for cross-clade activity, eight were cross-reactive against all clades tested at the highest peptide concentrations (Fig. 2). All of the CTL clones tested were cross-reactive with at least one non-B clade strain, in some cases due to sequence conservation and in others due to CTL recognition of the variant peptide. Further evidence of broad cross-reactivity was demonstrated by the B35-restricted, Env-specific CTL response, which was fully cross-reactive over a wide range of peptide concentrations despite up to three amino acid changes within the nine-amino-acid epitope (Fig. 2). CTL activity was also demonstrated in the B7-restricted, Env-specific clone, using autologous B-LCL infected with non-B clade vaccinia virus vectors (Fig. 3); this finding further established that cross-clade recognition persists with the intracellular processing and presentation of antigens. These results indicate that despite the sequence variation within CTL epitopes, cross-clade recognition is often maintained. If Fig. 1 is viewed in terms of one or two amino acid substitutions still

TABLE 3. Summary of CTL activity directed against clade B viral antigens in individuals infected with non-B clade viruses^a

Subject	% specific lysis				
	Control	Gag	RT	Env	Nef
Clade A					
p186	5	7	18	25	24
p225	2	27	7	25	3
p264	10	9	6	25	45
p542	2	19	-1	2	2
p787	3	16	60	32	27
p1108	8	18	19	47	24
p1286	25	42	60	27	21
p1581	0	23	6	4	12
p1913	5	25	5	10	25
p2285	1	8	17	3	-1
Clade G					
p306	8	32	54	15	52
p2187	-1	16	27	14	2
p2675	7	2	16	18	0
Clade C					
p158	3	11	5	13	15

^a PBMC were expanded into CD8⁺ cell-enriched populations and stimulated with autologous B-LCL infected with vaccinia virus constructs rV-env/gag-pol and rV-nef. CTL activity was detected in a 4-h chromium release assay with an effector/target ratio 100:1, using target cells infected with vaccinia viruses expressing Gag, RT, Env, and Nef of HIV-1 clade B (vaccinia virus vector NYCBH was used as control). Boldface values indicate HIV-specific activity (10% or higher than control).

FIG. 4. Epitope mapping for CD8 cell line of p306 (clade G) was accomplished by using clade B truncated vaccinia virus vectors and synthetic peptides. The E:T ratio was 50:1; vaccinia virus NYCBH was used as the control vector.

allowing recognition, then one would expect extensive cross-clade reactivity.

Clade B recognition by CTLs generated from non-B clade infection. To evaluate the CTL cross-recognition in individuals infected with non-B clade HIV-1, we obtained cryopreserved PBMC from HIV-1-infected individuals from Senegal. The PBMC were selectively expanded into CD8⁺ cells and tested for HIV-specific CTL activity by using autologous B-LCL infected with HIV-1 clade B vaccinia virus constructs. Fourteen consecutive individuals were evaluated. Ten were infected with clade A virus, three were infected with clade G virus, and one was infected with clade C virus. All subjects demonstrated recognition of at least one of the clade B vaccinia virus constructs, using vectors expressing Gag, Env, RT, and Nef proteins (Table 3). For each individual, cross-clade CTL recognition was confirmed by a second method of *in vitro* stimulation, using irradiated autologous CD4⁺ expanded cells that had been superinfected with HIV-1 IIIB; concomitantly, seven seronegative Senegalese individuals were evaluated, and no HIV-1-specific CTL activity was detected in assays using the same protocols (data not shown). The levels and range of lysis values are similar to what has been observed for individuals infected with clade B virus (22, 29, 56–58). Subjects p158 (clade C) and p2675 (clade G) demonstrated lower but reproducible levels of lysis of target cells. Both subjects had more advanced HIV disease compared to the others (both subjects were classified as CDC stage A3). It is unclear whether the lower CTL activity reflects the advanced HIV infection, poor recognition of clade B targets, or both. Confirmatory assays were performed in 11 of these 14 individuals, with persistence of HIV-1

clade B-specific CTL activity at E:T ratios ranging from 20 to 50 (data not shown).

Cross-clade recognition was further defined for five of the Senegalese subjects by using expanded CD8 cell lines and synthetic clade B HIV-1 peptides. HLA restriction analysis was also performed in these subjects, and in each case, recognition was restricted by class I antigens. An example is shown in Fig. 4. The analysis was performed in a subset of subjects expressing the class I allele A3, for which optimal epitopes have been defined in clade B infection. For these five individuals, cross-clade conservation was seen in the epitopes identified (9 to 12 amino acids) for at least one other clade, using consensus sequence data from the Los Alamos database (Table 4). We also verified, in four individuals, cross-clade CTL activities, using the recombinant vaccinia vectors expressing non-B clade Env, Gag, and Nef, and found comparable lysis of targets expressing these different clade constructs (Fig. 5), indicating that cross-clade recognition was maintained when epitope presentation was achieved by intracellular processing of viral proteins. These data suggest extensive cross-clade CTL recognition and define epitope conservation as one explanation for the observed recognition.

DISCUSSION

Vaccines appear to be the most cost-effective means of control and prevention of HIV-1 infection, which currently afflicts over 20 million people in the world (63). The emerging consensus regarding development of an HIV-1 vaccine is that efficacy will require the induction of CTL responses (10, 13, 18,

TABLE 4. Non-B clade CTL epitopes^a

Subject	Epitope	HLA	Conservation
Clade A			
p186	PRRIRQGLER (Env)	B27	Clades A, B, and E
	QVPLRPMTYK (Nef)	A3	Clades A, B, and D
p225	KIRLRPGGKK (p17)	A3	Clades A, B, C, and D
p542	KIRLRPGGKK (p17)	A3	Clades A, B, C, and D
p1913	QVPLRPMTYK (Nef)	A3	Clades A, B, and D
Clade G			
p306	KIRLRPGGKK (p17)	A3	Clades A, B, C, and D
	QVPLRPMTYK (Nef)	A3	Clades A, B, and D

^a Epitope mapping and HLA restriction of CTLs generated from non-B clade HIV-1 infection. Epitope mapping was done with clade B truncated recombinant vaccinia virus vectors and peptides. Cross-clade conservation of epitope sequences was determined by using consensus sequence data from the Los Alamos database.

23, 24, 38). Vaccines in current development are based on clade B immunogens; this clade is present in Europe and the United States but is found rarely in developing countries (7). Reformulating these potentially effective vaccines for each of the different clades could delay the delivery of promising interventions to the neediest populations.

In this study, we examined immunologic cross-recognition by using CTLs derived from both U.S. and African subjects. Our results indicate striking CTL cross-reactivity among persons infected with clade B and non-B viruses. This is apparent not only when CTL responses from infected Africans are tested for recognition of the prototypic U.S. viral strain but also when one examines the ability of selected CTL clones derived from persons in the United States to recognize corresponding non-B viral sequences. Among CTL clones which were generated in response to infection with clade B virus, all demonstrated

cross-recognition of at least one other clade, and several were cross-reactive with all strains tested. Among CTL responses from Africans infected with non-clade B viruses, all 14 subjects tested demonstrated cross-recognition of clade B virus, using recombinant vaccinia virus constructs. Several of these individuals demonstrated evidence of epitope conservation in at least one other HIV-1 clade. Mapping studies of these epitopes in a subset of HLA A3-positive persons revealed frequent recognition of A3-restricted epitopes well described in individuals infected with clade B virus. These data thus demonstrated cross-clade CTL recognition and define epitope conservation as one explanation for this recognition.

Given the heterogeneity among the HIV-1 clades and the sensitivity of CTLs to epitope sequence variation, we did not anticipate the degree of detectable CTL cross-reactivity among different clades. Single amino acid changes can abrogate CTL recognition, either by interfering with binding to the class I MHC molecule or due to alterations in T-cell receptor recognition (27, 28, 42, 45). However, in our study, single or even double amino acid substitutions frequently did not abrogate recognition. Furthermore, comprehensive analysis of sequence variability within well-defined CTL epitopes supports the experimental results, suggesting that one would encounter extensive interclade CTL cross-reactivity. Another factor which may abrogate CTL recognition is sequence change either within or adjacent to CTL epitopes can alter protein processing, such that immunogenic epitopes are no longer presented for recognition (2, 12, 44). However, all of the CTL responses tested demonstrated some degree of cross-recognition, including cross-recognition of processed viral proteins, and all infected African subjects recognized clade B virus. Although one previous study has demonstrated CTL cross-recognition among the HIV-1 viral clades by vaccine-induced CTLs (16), cross-clade recognition by CTL induced in natural HIV-1 infections has not been defined. This study demonstrates extensive cross-

FIG. 5. Cross-clade recognition from African CTLs, using autologous B-LCL infected with recombinant vaccinia vectors that express Env, Gag, or Nef from various clades. An E:T ratio of 50:1 was used for all (except p306, which was used at an E:T ratio of 10:1); NYCBH was used as the control.

reactivity with clade B and non-B viruses. Although all African subjects tested demonstrated cross-reactive responses with U.S. strains, with several subjects maintaining recognition of more than one clade, it is possible that responses to autologous strains are of higher or broader magnitude and that cross-recognition among other nontested clades differs. Our analyses (Fig. 1) also indicate that the proteins most likely to stimulate cross-clade immune responses are Gag p24 and RT, which is logical as they are the most conserved proteins overall. Further cross-clade analyses that include additional epitope mapping and determination of HLA restriction are currently under way in our laboratory. The understanding of cross-clade CTL responses will be further enhanced as vaccinia virus constructs from additional clades become available.

The relative contributions of CTL to protective immunity are not clear, but most vaccine strategies include the induction of CTL responses as an important goal. A number of vaccines capable of inducing CTL responses, including both recombinant poxvirus and polynucleotide vaccines, are in phase I trials that are ongoing or soon to begin. At least one study has shown that clade B immunogens can induce cross-clade CTL responses (16). Our data, in a larger cohort of persons infected with clade B and non-B clade viruses, demonstrate significant cross-reactivity for CTL responses, with evidence of either epitope conservation or cross-clade recognition, and provide support for initiating trials with clade B immunogens in Africa. Further studies will need to be performed with additional non-B clade viruses to determine whether this cross-recognition applies for clades other than those included in this analysis.

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