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Three regions of HIV-1 gp160 contain clusters of immunodominant CTL epitopes

Premlata Shankar, Jessica A. Fabry, Donna M. Fong, Judy Lieberman*

The Center for Blood Research, Harvard Medical School, 800 Huntington Avenue, Boston, MA 02115, USA

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Abstract

HIV-1 infection stimulates a strong CTL response that coincides with resolution of viremia in acute infection and declines with development of opportunistic infections. Recognition of HIV gp160 by PBMC-derived T cell lines from 20 HIV-infected subjects is dominated by the response to a small number of peptide epitopes. Overlapping CTL epitopes restricted by multiple MHC Class I elements were identified in 3 relatively conserved regions of gp160 (amino acids 49–68, 591–600 and 844–863). CTL from five of 20 subjects recognized three overlapping immunodominant epitopes in the 49–68 a.a. region restricted by A24, B38, and B55. CTL from four subjects recognized at least three distinct epitopes in a.a. 591–600 in the context of A24, B8, B14, and B27. CTL from seven subjects recognized epitopes within a.a. 844–863 restricted by A30, B7, B8 and B35.

Keywords: Epitope; HIV; gp160; CTL; Restriction; HLA Class I

1. Introduction

HIV-specific CTL are likely to be important in the resolution of the viremia of acute infection and in immune control of viral replication during the asymptomatic stages of HIV infection [1–4]. The study of CTL recognition of HIV-1 epitopes is complicated by the extensive sequence diversity exhibited by the virus. However, highly conserved stretches, essential perhaps for virus integrity and replication, are interspersed amongst the variable regions of the virus. The identification of commonly recognized epitopes and epitope clusters within short protein sequences in conserved regions of the virus should provide important information about the potential immunogenicity of HIV peptide antigens. We have previously shown that the CTL response to HIV-1 in infected individuals is dominated by the recognition of a small number of peptide epitopes [5,6]. In this paper we describe epitope clusters and their restriction elements within three relatively conserved 15–20 amino acid regions of gp160 that are immunodominant in seropositive subjects of diverse MHC background. Such multideterminant peptides recognized in the context of multiple class I alleles are potentially valuable for design of peptide-based vaccines.

2. Materials and methods

2.1. Vaccinia vectors

Vaccinia vectors encoding lacZ (vSC8) and env of the BH8 isolate of HIV-1IIIB/LAI (vPE16) were used to screen cell lines for specific cytotoxicity against control and env-expressing targets. Vaccinia vectors expressing nested truncations of the same env sequence (vPE17, vPE18, vPE8, vPE20, vPE21, vPE22 prepared by P. Earl and B. Moss) were used to localize dominant env CTL epitopes as previously described [7].

* Corresponding author.

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Table 1
The 49–68 a.a. gp160 peptide contains three distinct overlapping CTL epitopes, CTL lines from five subjects were assayed for recognition of 10 mer peptides at an E:T ratio of 5:1

Peptide	% Specific cytotoxicity of CTL line from subject				
	132	203	204	228	237
	A24, A31	A3, A24	A2, A3	A3, A24	A2, A26
	B5, B40	B8, B55	B8, B62	B7, B38	B7, B38
—	3	11	3	13	11
VPVWKEATTT	4	74	10	12	10
VWKEATTTLF	4	10	62	11	7
KEATTTLFCA	4	9	13	14	7
ATTTLFCASD	4	14	10	13	6
TTLFCASDAK	6	17	10	12	8
LFCASDAKAY	18	14	14	54	49

2.2. Cell lines

T cell lines were generated by stimulating PBMC, obtained with informed consent from 20 HIV-seropositive donors at various disease stages, with 2 μg/ml PHA-P (Difco) in media containing 15% fetal calf serum and 600 IU/ml rhu IL-2 (Chiron Corporation, Emeryville, CA) as described earlier. For some experiments, gp160-specific T cell lines were generated using vPE16-infected autologous PBMC as APC as described in [8] and peptide-specific T cell lines were generated as described in [6] using peptide-incubated adherent PBMC as APC. Autologous B lymphoblastoid cell lines (B-LCL) were generated for each subject by standard methods.

2.3. Peptides

Overlapping 22 mers spanning amino acids 748–851 (amino acid numbering as in [9]) of the BH8 isolate of HIV-1IIIB with eight amino acid overlaps were synthesized on a Milligen 9050 synthesizer. Overlapping 20 mers with ten amino acid overlaps spanning a.a. 37–851 of the HXB.2 subclone of LAI or of SF2 env were provided by the MRC AIDS Reagent Project. Overlapping 10 mers spanning a.a. 49–68 and 12 mers spanning a.a. 844–863 were commercially synthesized and peptides used to map epitopes in a.a. 591–605 were a kind gift of P. Johnson and B. Walker. Peptide stock solutions (1 mg/ml in 10% DMSO, PBS) were diluted in medium to a concentration of 10–50 μg/ml for generating peptide-specific T cell lines and for cytotoxicity assays.

2.4. Chromium release assay

Cytotoxicity assays were performed against B-LCL target cells that were either untreated, pre-incubated with relevant peptides, or infected with vaccinia constructs encoding complete or truncated HIV-1 proteins as described [5]. For infection, B-LCL in log phase growth were infected with 2–10 pfu/cell of vaccinia virus for approximately 16 h. For peptide experiments, radiolabeled targets were incubated with peptide for 30 min at 37°C before adding effector cells. Each experiment was performed in triplicate and repeated at least once; spontaneous release was less than 20% of the total release in all experiments.

3. Results and discussion

3.1. Identification of three epitopes restricted by multiple MHC alleles within the N terminal peptide spanning a.a. 49–68

We have previously shown that it is possible to characterize immunodominant CTL epitopes reproducibly from mitogen-stimulated cell lines generated from the PBMC of HIV-infected individuals [5]. Autologous target cells infected with recombinant vaccinia vectors expressing nested truncations of the HIV-1 IIIB/LAI envelope gene product are used first to localize epitopes to within approximately 100 a.a. and then a set of overlapping 15–22 mer peptides for the region defined by the vaccinia truncations is used for finer mapping of the epitope. Because the vaccinia recombinants and peptides are derived from the sequences of laboratory strains, relatively conserved epitopes are selectively identified. Using this method, complete epitope analysis has been performed for recognition of HIV

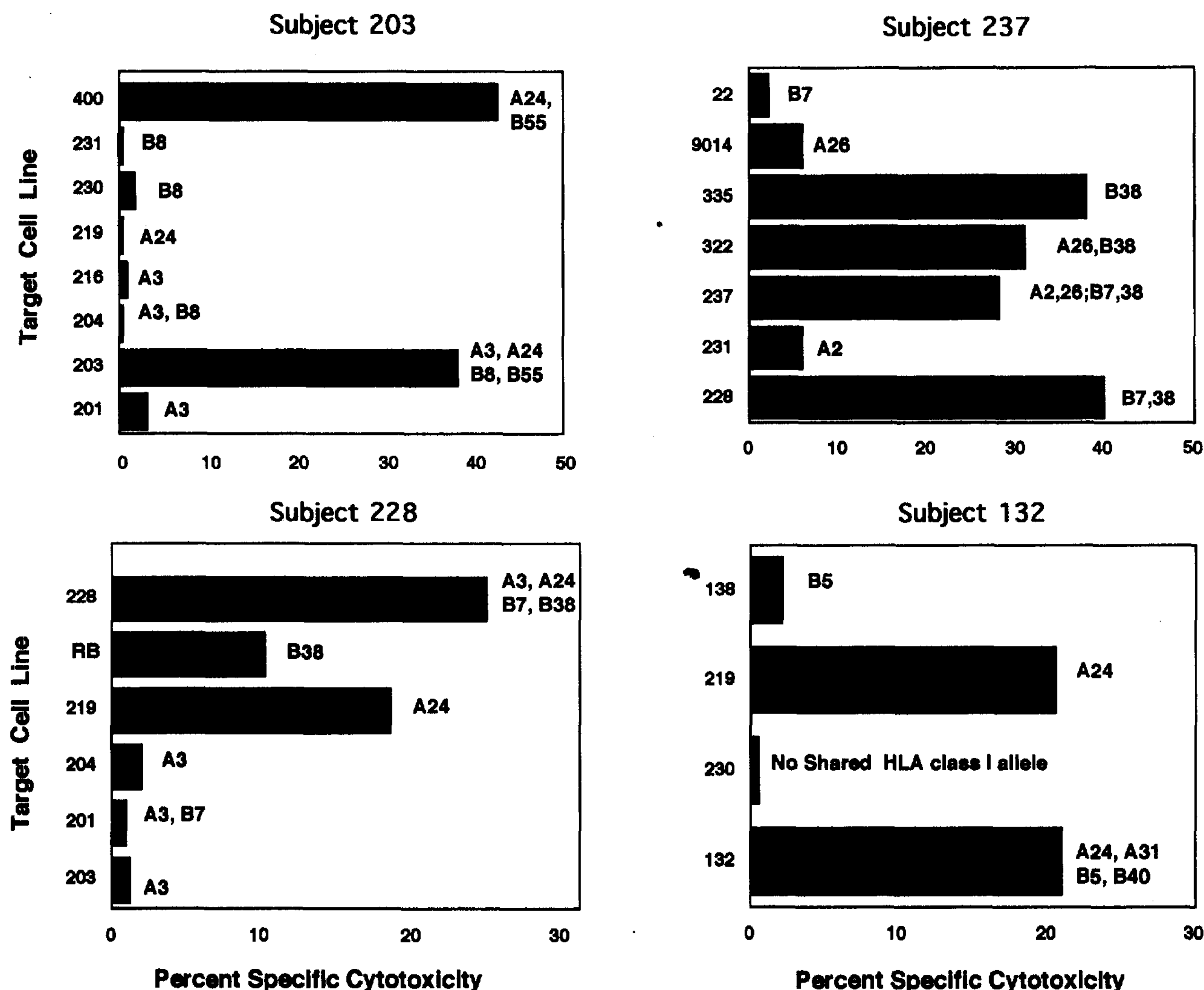

Fig. 1. The N terminal gp160 peptide VPVWKEATTTLFCASDAKAY (a.a. 49–68) is recognized in the context of B55 (subject 203), B38 (subjects 228 and 237) and A24 (subjects 228 and 132). Peptide-specific T cell lines were used as effectors against autologous and heterologous peptide-pulsed B-LCL targets matched at one or more class I alleles in a 4 h ^{51}Cr release assay at an E:T ratio of 5:1. Peptide-specific lysis, obtained by subtracting the lysis of targets in the absence of peptide, is shown.

gp160 by 20 subjects, of whom 10 have been previously described in part [5,6].

The 20 mer peptide VPVWKEATTTLFCASDAKAY (a.a. 49–68) in the N terminus was recognized as immunodominant by CTL from five of 20 subjects of diverse MHC background. To refine the epitope further, autologous B-LCL targets were pulsed with a set of overlapping 10 mers (Table 1). Of the five subjects who recognized the peptide, T cell lines from three subjects (132, 228 and 237) recognized an identical 10 mer epitope LFCASDAKAY (a.a. 59–68). The epitope was recognized in the context of HLA-A24 in subjects 132 and 228 (Fig. 1). There was some degeneracy in the restriction by CTL from subject 228 with probable presentation by B38. A third subject (237) recognized the same 10 mer in the context of B38. Subject 203 recognized the adjacent 10 mer peptide VPVWKEATTT (a.a. 49–58) in the context of B55. Although this subject carries the A24 allele, his CTL did not recognize the A24 restricted epitope. The fifth subject (204) recognized another 10 mer peptide VWKEATTTLF (a.a. 51–60) in this region. The restriction element for this epitope could not be identified despite the use of multiple HLA matched targets. The restricting element could not be identified even for two CD8 CTL clones from this individual that recognized this peptide epitope (data not shown). It is possible that the restriction element is either a minor subtype of one of the A or B alleles or an HLA C allele for which typing was not carried out. The three new epitopes that we have identified in this study, are adjacent to the HLA-A2 epitope LMVTVYYGV (a.a. 41–49) described in seropositive subjects vaccinated with recombinant gp160 [10] and to the HLA-A3 epitope

TVYYGVPVWK (a.a. 44–53) and a B18 epitope contained in a.a. 38–62 recognized by CTL from two seronegative subjects vaccinated with recombinant vaccinia expressing gp160 and then boosted with recombinant gp160 [11]. Thus there are at least six CTL epitopes within a span of 30 amino acids in the N terminus of gp160.

3.2. Identification of two multi-determinant overlapping epitope regions in gp41

Two regions of gp41 were also recognized as immunodominant in CTL lines from multiple patients. A 15 mer peptide a.a. 591–605 (ERYLRDQQLLGIWGC) was recognized by four subjects (201, 203, 230, 231). This region has previously been shown to contain minimal epitopes ERYLKDQQL (a.a. 591–599) restricted by B14 and YLKDQQLL (a.a. 593–600) restricted by B8 and A24 [12,13]. Using overlapping peptides provided by P. Johnson and B. Walker, we identified the B14-restricted epitope ERYLKDQQL in two B14-expressing patients (201, 231) (Table 2 and Fig. 2). CTL from subject 203 (A3,24;B8,55) recognized the YLKDQQLL peptide in the context of both B8 and A24. Because we used polyclonal T cell lines as effectors, we cannot ascertain without cloning whether the same TcR sees this peptide in the context of more than one MHC allele. In a third individual, a new minimal peptide RYLKDQQL (a.a. 592–599) was recognized in the context of B27. Of note, B8-expressing subjects 230 and 231 did not recognize the B8-restricted epitope contained in the 15 mer despite recognition of overlapping epitopes restricted by other class I alleles. In other model systems, the dominance of one MHC molecule over another in presenting overlapping epitopes has been attributed to determinant capture by the higher affinity interaction of the dominant peptide with its restricting MHC molecule [14,15]. However, differences in peptide processing or an individual's TcR repertoire could also play a role.

Table 2
The 591–605 a.a. gp41 peptide contains three distinct overlapping CTL epitopes, CTL lines from four subjects were assayed for recognition of truncated peptides at an E:T ratio of 5:1

	% Specific cytotoxicity of CTL line from subject			
	201	203	230[a]	231
	A3, A32	A3, A24	A1, A11	A1, A2
Peptide	B7, B14	B8, B55	B8, B27	B8, B14
–	3	5	47	4
AV-ERYLKDQQLLG	25	51	61	14
AV-ERYLKDQQLL	27	67	70	17
AV-ERYLKDQQL	26	7	64	21
AV-ERYLKDQQ	4	2	44	10
VERYLKDQQLLGIW	22	57	64	14
ERYLKDQQLLGIW	18	58	59	12
RYLKDQQLLGIW	6	54	58	7
YLKDQQLLGIW	4	44	47	8
ERYLKDQQL	44	47	62	35
YLKDQQLL	5	51	41	8

[a]T cell line 230 acquired a high level of background cytotoxicity after peptide stimulation with B-LCL APC. No stored samples were available to generate a second line using PBMC as APCs to reduce the background.

The other region of gp41 which was found to contain multiple MHC restricted epitopes is a 20 mer region in the extreme C terminus (a.a. 844–863). For the further mapping of this region, recognized as immunodominant by CTL from seven of 20 subjects, we synthesized three overlapping 12 mers: YRAIRHIPRRIR, RHIPRRIRQGLE, RRIRQGLERILL. In an earlier paper we documented a B8 restricted 12 mer epitope a.a. 852–863 RRIRQGLERILL and an A30 restricted epitope within this 20 mer [5]. In this study, T cell lines from three additional individuals recognized the 20 mer. Subject 204 did so in the context of B8 (data not shown). Taken together with our earlier data, CTL from four of eight B8-expressing subjects studied recognized the C terminal epitope as immunodominant, suggesting that this peptide is readily processed and binds with high affinity to the B8 molecule. Subject 237 recognized the 20 mer C terminal peptide in the context of HLA B7 (Fig. 3). The restriction element for recognition of the C terminal peptide by subject 219 CTL was B35. The B35-restricted epitope was mapped to the C terminal 12 mer (data not shown). Therefore the C terminal 20 mer contains epitopes restricted by at least 4 alleles—B7, B8, A30, and B35.

Allele-specific motifs have been described for the sequences of CTL epitopes based on known MHC-restricted epitopes and on the sequences of peptides eluted from MHC molecules from infected and uninfected cells for many of the common class I alleles, including A24, B7, B8 and B27 [16–20]. All the epitopes described in this report restricted by these class I alleles contain sequences that loosely fit the known binding motifs. The HLA-B55 restricted epitope VPVWKEATTT is the first report of a B55-restricted epitope. Two groups have suggested that HLA-B55

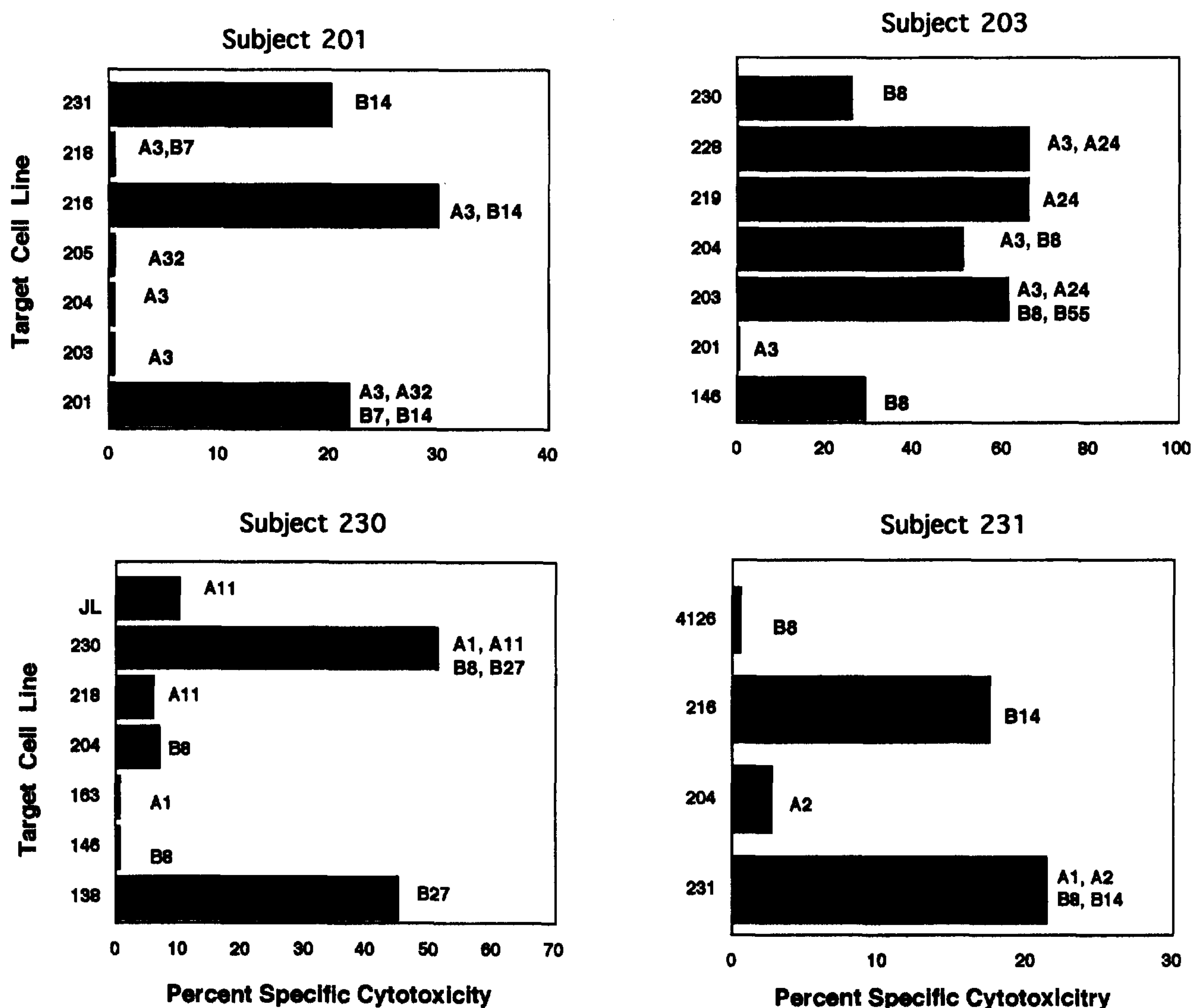

Fig. 2. The gp41 15mer peptide ERYLKDQQLLGWGIC (a.a. 591–605) is restricted by B14 (subjects 201 and 231), A24 (subject 203) and B27 (subject 230). Peptide-specific T cell lines from subjects 201, 203, 230 and 231 were used as effectors against autologous and heterologous peptide-pulsed B-LCL targets matched at one or more class I alleles in a 4 h ^{51}Cr release assay at an E:T ratio of 5:1. Peptide-specific lysis, obtained by subtracting the lysis of targets in the absence of peptide, is shown.

may belong to the recently identified HLA-B7 supertype family, which bind peptides with a critical proline at position 2 and hydrophilic residues at the C terminal anchor [21,22]. The sequence of this epitope lends support to this B55 relationship. The epitopes described in this paper have also been verified in some cases by T cell cloning (data not shown) and by our consistent ability to enhance epitope-specific cytotoxicity by incubating CTL lines with peptide-incubated APC.

Two of the three multi-determinant regions we have identified occur very close to the termini of gp160. The a.a. 49–68 region is within 12 a.a. of the signal peptide cleavage site and the a.a. 844–863 region is at the extreme C terminus. It is possible that the occurrence of regions of common CTL recognition at the ends of gp160 is not accidental. It may be that these regions of the protein are more accessible to proteolysis for peptide epitope processing.

3.3. Restriction by the most prevalent HLA class I alleles is uncommon

None of the dominant epitopes we have identified in gp160 were restricted by the most common HLA class I alleles (A1, A2 and A3) despite their fair representation in the study (Table 3). Since peptides that bind to A2 with high affinity have been identified and A2- and A3-restricted epitopes have been recognized after vaccination [10,11], we performed several experiments to validate our method and verify that it did not systematically miss A2 or A3 restricted epitopes. For four subjects (201, 202, 203, 231), of which two express A3

and one expresses A1 and A2, we stimulated PBMC with recombinant vaccinia expressing gp160 (vPE16) as described in [8]. This enhanced recognition of the epitope identified in the mitogen-stimulated T cell line, but did not identify any new epitopes. Fig. 4 shows the results for the A3+ subject 201. For five subjects expressing either A2 or A3, we stimulated PBMC with either an immunodominant peptide epitope we had identified from the mitogen-stimulated T cell line or with highly conserved A2 or A3 binding gp160 peptides reported in vaccinated subjects. The cell lines exposed to our epitope had enhanced peptide specific lysis but the cell lines generated by exposure to the A2 or A3 binding peptides did not recognize those epitopes above background (Fig. 5). We have also generated over 20 gp160-specific clones by direct cloning of PBMC in the presence of anti-CD3 and IL-2 from subjects 113, 146, 201, 203 and 204, who express A1, A2 or A3. All of these clones recognized the epitopes identified in the mitogen-stimulated lines and none recognized any new A1, A2 or A3 restricted epitopes (data not shown). We also attempted to block HIV-specific lysis of gp160-vaccinia infected targets by T cell lines from 4 A2.1-expressing subjects (136, 163, 204, 214) with the A2.1-specific antibody PA2.1. There was no diminished lysis suggesting that CTL recognition of gp160 in these subjects did not involve presentation by A2.1 (data not shown).

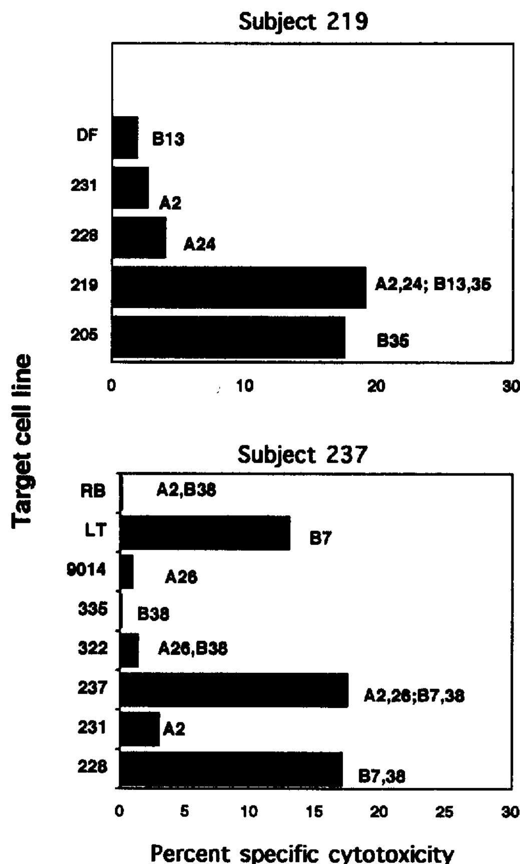

Fig. 3. Recognition of gp41 C terminal peptide YRAIRHRRIRQGLERILL (a.a. 844–863) by peptide-specific T cell line 219 is restricted by B35 and by T cell line 237 is restricted by B7. Cytotoxicity was assayed at an E:T ratio of 5:1 and background lysis in the absence of peptide has been subtracted.

Our study suggests that targeting single epitopes that are recognized in the context of a particular common MHC molecule may not be the most useful strategy. None of the restricting elements we identified were amongst the most prevalent class I alleles. The expression of an MHC allele also did not insure that it restricted CTL recognition even when other nearby epitopes were recognized. This finding agrees with the study of gag epitope recognition by Buseyne et al. [23]. In this paper we have shown this for the A24-restricted epitope which was not recognized by CTL from subject 203 and for two separate B8-restricted epitopes not recognized by subjects 230 and 231.

In conclusion, our study suggests that seropositive subjects of diverse MHC background commonly recognize epitopes within three 15–20 a.a. long multideterminant regions of gp160 (Table 3). Of 20 subjects whose CTL recognized gp160-expressing targets, CTL from 5 recognized a dominant epitope in a.a. 49–68, from four recognized a.a. 591–605 and from seven recognized a.a. 844–863. For 13 of the 20 subjects (65%), at least one of these regions contained an immunodominant CTL epitope. Such short peptides, all contained in relatively well conserved regions of gp160, are good candidates for peptide based vaccines because they can overcome the MHC barrier. Their recognition in naturally infected subjects also implies that these epitopes are well presented on infected cells. Similar overlapping epitope clusters have been described for HIV nef [24,25].

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Table 3
Summary of immunodominant env CTL epitopes recognized by T cell lines from 20 HIV-seropositive subjects, the minimal epitope and restricting element were not identified in all cases

Subject	CD4 count	CDC	MHC			Peptide epitope sequence	MHC
	(/mm³)	Stage	A	B	AA #		restriction
101	410	A2	23,w33	17,18	788–809	IVELLGRRGWEALKYWWNLLQY	ND
113	318	A2	2,30	27,w60	844–863	YRAIRHIPRRIRQGLERILL	A30
120	300	A2	2,–	15,18	219–238	PIPIHYCAPAGFAILKCNNK	ND
132	300	A2	24,31	5,40	802–823	YWWNLLQYWSQELKNSAVNLLN	ND
					59–68	LFCASDAKAY	A24
138	780	A1	2,–	5,27	788–809	IVELLGRRGWEALKYWWNLLQY	B27
142	87	C3	1,31/32	8,w60	852–863	RRIRQGLERILL	B8
146	280	A2	2,32	7,8	852–863	RRIRQGLERILL	B8
					259–278	RPVVSTQLLLNGSLAEEEVV	B7
147	280	C2	ND	ND	199–218	SLTSCNTSVITQACPKVVSFE	ND
153	600	B1	ND	ND	109–128	EQMHEDIISLWDQSLKPCVK	ND
					209–228	TQACPKVSFEPIPIHYCAPA	ND
201	470	A2	3,32	7,14	591–599	ERYLKDQQL	B14
202	400	A2	ND	ND	209–228	TQACPKVSFEPIPIHYCAPA	ND
203	360	B2	3,24	8,55	593–600	YLKDQQLL	A24,B8
					49–58	VPVWKEATTT	B55
204	440	A2	2,3	8,62 [15]	852–863	RRIRQGLERILL	B8
					51–60	VWKEATTTLF	ND
214	360	B2	2,–	21,–	69–78	DTEVHNVWAT	ND
					119–139	WDQSLKPCVKLTPLCVSLK	ND
					199–218	SLTSCNTSVITQACPKVVSFE	ND
					249–268	VSTVQCTHGIRPVVSTQLLL	ND
219	240	A2	2,24	13,35	844–863	YRAIRHIPRRIRQGLERILL	B35
228	250	A2	3,24	7,38	59–68	LFCASDAKAY	A24,B38
230	250	A2	1,11	8,27	592–599	RYLKDQQL	B27
231	350	B2	1,2	8,14	591–599	ERYLKDQQL	B14
237	260	B2	2,26	7,38	852–863	RRIRQGLERILL	B7
					59–68	LFCASDAKAY	B38
4126	630	A1	28,–	8,–	852–863	RRIRQGLERILL	B8

ND = not determined.

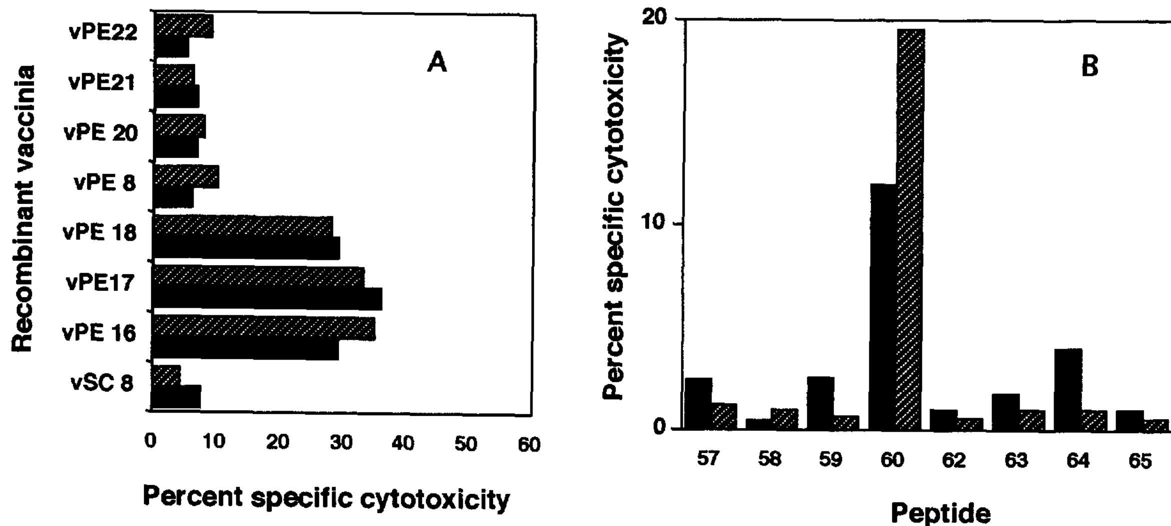

Fig. 4. After exposure of PBMC from subject 201 to autologous cells infected with vPE16, vaccinia-gp160 recombinant virus, there was enhanced lysis of gp160-expressing targets but no epitopes were recognized aside from a.a. 591–599, identified in the mitogen-stimulated T cell line. (A) Lysis of targets expressing truncations of gp160 after infection with vaccinia viruses [7] revealed that for both the mitogen stimulated cell line (solid bars) and the gp160-stimulated cell line (hatched bars), gp160 recognition was focused in the N terminal region of gp41 contained in vPE18. Overlapping peptides (B) spanning the region encoded by vPE18, but excluded from vPE8, which encodes gp120, showed that both cell lines recognize the 15 mer peptide 60 (a.a. 591–605). The E:T ratio for the mitogen-stimulated T cell line was 25:1, for the gp160-stimulated line, 5:1.

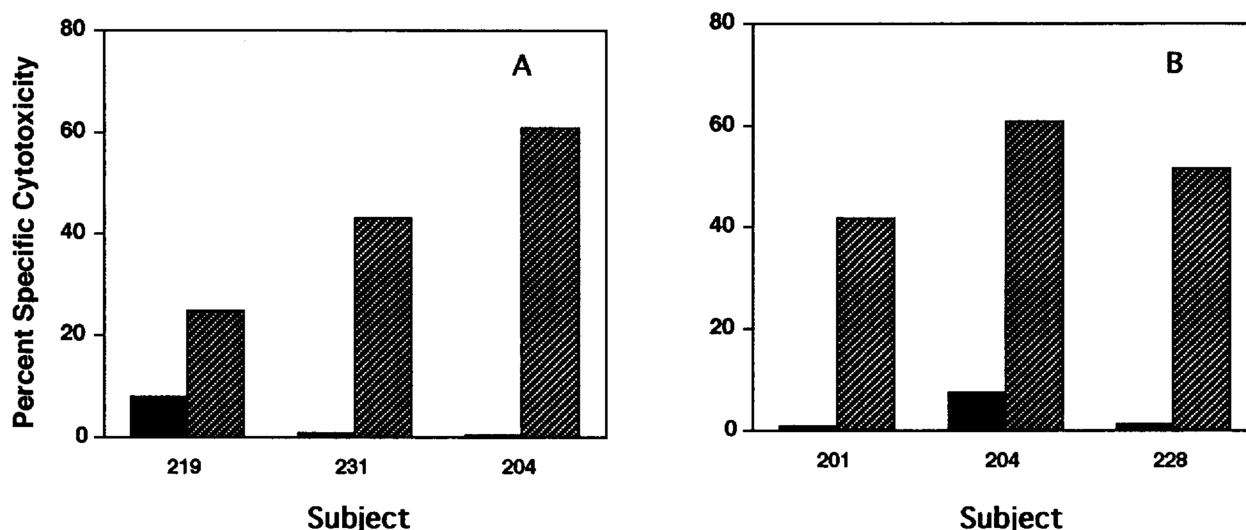

Fig. 5. Stimulation of PBMC with (A) the A2-restricted peptide LMVTVYYGV (a.a. 41–49), or (B) the A3-restricted peptide TVYYGVPVWK (a.a. 44–53) did not elicit peptide-specific lysis above background (solid bars) in A2- or A3-expressing subjects, respectively. However, recognition of targets incubated with the immunodominant peptide characterized for the mitogen-stimulated cell lines (hatched bars) was enhanced after exposure to a dominant peptide for that subject. E:T ratio = 5:1.

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