Supporting Information

Chung et al. 10.1073/pnas.1016048108

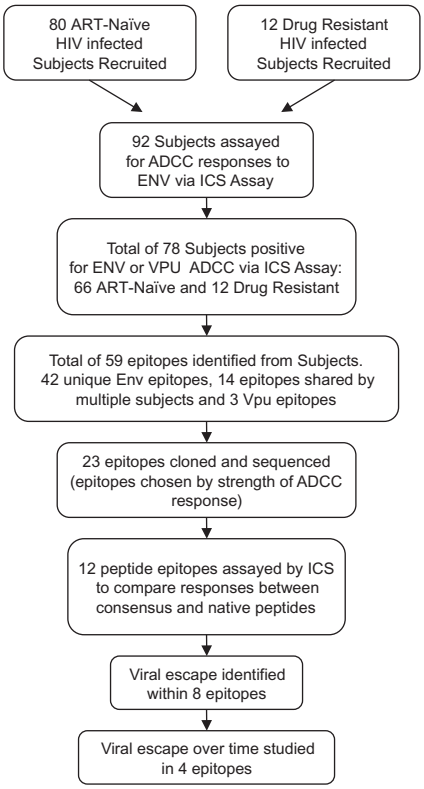
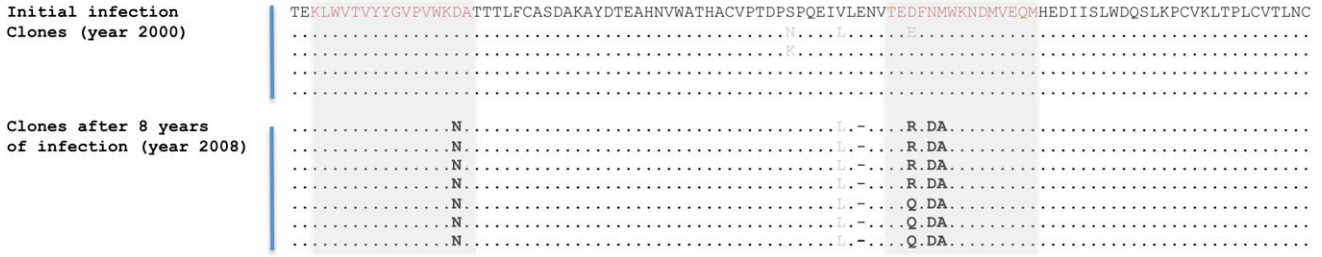


Fig. S1. Flowchart of experimental studies showing the identification of antibody-dependent cellular cytotoxicity (ADCC) epitopes and viral escape. ART, antiretroviral therapy; Env, HIV-1 envelope protein; ICS, intracellular cytokine staining.

A. Clonal analysis of sequence changes during infection



The C1 region of Env was cloned and sequenced from plasma HIV-1 RNA obtained early after initial infection (year 2000) and 8 years later.
Shaded areas represent mapped ADCC epitopes in the subject.
Bolded changes represent variants rarely (≤2%) found in 300 unique Env clones (kindly supplied by Dr B Korber, Los Alamos National Laboratory).

B. Analysis of amino acid changes within and outside ADCC epitopes in Env C1 in multiple subjects:

All changes within C1 ADCC epitopes	5 of 45 aa	
All changes outside epitopes in C1	4 of 151 aa	p = 0.031 Fisher's exact test
Rare† changes within C1 ADCC epitopes	4 of 45 aa	
Rare changes outside epitopes in C1	2 of 151 aa	p = 0.026 Fisher's exact test

† rare changes defined as changes rarely identified (≤2%) in 300 unique subtype B Env clones

Fig. S2. Amino acid changes in and around the ADCC epitope in the conserved domain 1 (C1) envelope. (*A*) Clonal analysis of sequence changes during infection in one subject. Five clones from the year 2000 (within 2 mo of initial infection) and seven clones from the year 2008 are shown aligned to the most common year-2000 variant. Shaded areas represent mapped ADCC epitopes in the subject. Changes shown in bold represent variants rarely (≤2%) found in 300 unique Env clones (kindly supplied by B. Korber, Los Alamos National Laboratory, Los Alamos, NM). (*B*) Analysis of amino acid changes within and outside ADCC epitopes in Env C1 in multiple subjects. All changes and rare changes (defined as changes identified in ≤2% of 300 unique subtype B Env clones) are analyzed separately.

Table S1. Sequences of identified ADCC epitopes in Env and Vpu

Env peptide no.*	Residues	Amino acid sequence	Region†	Patient no.	NK cells secreting IFN-γ‡ (%)
2	5–19	GIRRNYQHWWGWGTM	Signal peptide	89	0.2
				56	0.7
4	13–17	WWGWGTMLLGLLMIC	Signal peptide	89	0.2
				56	0.9
8–9	29–47	ATEKLWVTVYYGVPVWKEA	C1	91	1.5
				84	1.6
9	33–47	LWVTVYYGVPVWKEA	C1	61	1.8
				89	0.2
				88	0.4
				72	1.2
10–11	37–55	VYYGVPVWKEATTTLFCAS	C1	56	1.3
11	41–55	VPVWKEATTTLFCAS	C1	8	0.3
12	45–59	KEATTTLFCASDAKA	C1	61	1.3
13	49–63	TTLFCASDAKAYDTE	C1	1	0.6
14	53–67	CASDAKAYDTEVHNV	C1	80	0.9
14–15	53–71	CASDAKAYDTEVHNVWATQ	C1	29	0.4
14–17	53–79	CASDAKAYDTEVHNVWATQACVPTDPN	C1	89	0.2
16	61–75	DTEVHNVWATQACVP	C1	56	1.5
19	73–87	CVPTDPNPQEVELVN	C1	56	0.8
19–20	73–91	CVPTDPNPQEVELVNVTEN	C1	80	0.5
23	89–103	TENFNMWKNNMVEQM	C1	91	1.4
				61	7.6
				42	0.4
				88	1.1
				72	0.4
23–24	89–107	TENFNMWKNNMVEQMHEDI	C1	84	5.0
24	93–107	NMWKNNMVEQMHEDI	C1	89	0.3
				19	0.3
30	117–131	PCVKLTPLCVTLNCT	C1	80	0.5
49–50	193–211	STSYRLISCNTSVITQACP	V2-C2	61	1.4
52	205–219	VITQACPKISFEPIP	V2-C2	29	0.8
53	209–223	ACPKISFEPIPIHYC	C2	19	0.5
70–71	277–295	IRSENFTDNAKTIIVHLNE	C2	61	1.5
77–78	305–323	NYNKRKRIHIGPGRAFYTT	V3	31	6.7
				67	3.9
				13	
78	309–323	RKRIHIGPGRAFYTT	V3	91	2.1
				8	0.9
				94	2.0
				86	0.2
78–80	309–331	RKRIHIGPGRAFYTTKNIIGTIR	V3	93	8.3
79–80	313–331	HIGPGRAFYTTKNIIGTIR	V3	56	1.0
80	317–331	GRAFYTTKNIIGTIR	V3	31	0.9
				67	1.2
				13	1.0
87	345–359	TLRQIVSKLKEQFKN	C3	3	1.3
94–96	373–395	EIVMHSFNCGGEFFYCNTSPLFN	C3	8	0.3
106–107	421–439	KQIINMWQEVGKAMYAPPI	C4	61	1.7
137	545–559	LLSGIVQQQNNLLRA	C6	36	0.3
137–138	545–563	LLSGIVQQQNNLLRAIEAQ	C6	61	0.2
143	569–583	LTVWGIKQLQARVLA	C6	8	0.3
154	613–627	ASWSNKSLDDIWNNM	C6	3	2.0
161	641–655	SLIYSLLEKSQTQQE	C6	3	1.2
176	701–715	AVLSIVNRVRQGYSP	C7	92	1.0
176–177	701–719	AVLSIVNRVRQGYSPLSLQ	C7	3	1.0
187	745–759	TSGRLVHGFLAIIWV	C7	29	0.9

Vpu peptide epitope	Residues	Amino acid sequence	Region*	Patient no.	Cells secreting IFN-γ§ (%)
3	9–23	VALVVAIIIAIVVWS	n/a	95	0.3
7	25–39	VIIEYRKILRQRKID	n/a	95	0.5
19	69–81	EMGHHAPWDVDDL	n/a	8	5.4

*Derived from subtype B Env consensus sequence. 15-mer peptides overlapping by 11 amino acids. (supplied by the National Institutes of Health AIDS Research and Reference Reagent Program).

†There are five variable domains (V1–V5) and seven conserved domains (C1– C7).

‡The percentage of NK ($CD3^{-}CD56^{+}$) cells secreting IFN-γ after peptide stimulation of whole blood for 5 h.

§Patients were recruited into two cohorts: Treatment-naïve patients were HIV-infected adults who never had undergone any antiretroviral drug therapy. Treated patients were HIV-infected adults who had undergone extensive antiretroviral drug therapy and who had drug-resistant HIV.

Table S2. Comparison of consensus and subjects' native viral epitopes

Subject no.	Peptide*	Sequence†	Frequency‡	Amino acid changes
31	Env 77–78	NYNKRKRIHIGPGRAFYTT	Consensus	
		YN.T.RS.....FA.	2/3 (66%)	7
		YN.T.RS.....FAN	1/3 (33%)	8
31	Env 80	GRAFYTTKNIIGTIR	Consensus	
		....FA.GE...D..	2/3 (66%)	5
		....FANGEV..D..	1/3 (33%)	7
8	Env 78	RKRIHI--GPGRAFYTT	Consensus	
		..S.R.QR.....V.I	13/13 (100%)	6
56	Env 2	GIRRNYQHWWGWGTM	Consensus	
		...K.C.RL.R....	8/9 (89%)	5
		...K.C.RL.R.S..	1/9 (11%)	6
3	Env 87	TLRQIVSKLKEQFKN	Consensus	
		..K..A...R...G.	2/3 (66%)	4
		..K..A...R..YG.	1/3 (33%)	5
67	Env 80	GRAFYTTKNIIGTIR	Consensus	
		.....A.GE...D..	4/7 (57%)	4
		.....A.GD...D..	1/7 (14%)	4
		.....A.GDV..D..	2/7 (29%)	5
67	Env 77–78	NYNKRKRIHIGPGRAFYTT	Consensus	
		.N.T..S.P.....A.	7/7 (100%)	5
56	Env 4	WWGWGTMLLGLLMIC	Consensus	
		L.R.....M....	8/9 (89%)	3
		L.R.S.....M....	1/9 (11%)	4
8	Env 96	CGGEFFYCNTSPLFN	Consensus	
		.....STQ...	13/13 (100%)	3
72	Env 23	TENFNMWKNNMVEQM	Consensus	
		..Q.DA.....	3/9 (33%)	3
		..R.DA.....	6/9 (66%)	3
80	Env 19–20	CVPTDPNPQEVELVNVTEN	Consensus	
		.....V.D....H	5/7 (72%)	3
		.....V.D....Y	1/7 (14%)	3
		.....I.D....H	1/7 (14%)	3
93	Env 78–80	RKRIHIGPGRAFYTTKNIIGTIR	Consensus	
		..G.....GE...D..	2/2 (100%)	4
84	ENV 8–9	ATEKLWVTVYYGVPVWKEA	Consensus	
		.A.P.....	3/4 (75%)	2
		.I.Q.....E.....	1/4 (25%)	3
56	Env 19	CVPTDPNPQEVELVN	Consensus	
		.....V.G.	9/9 (100%)	2
80	Env 13–14	TTLFCASDAKAYGTEVHNV	Consensus	
		.....K.....	3/7 (43%)	1
		.....K..A...	4/7 (57%)	2
56	Env 16	DTEVHNVWATQACVP	Consensus	
		.....H....	8/9 (89%)	1
		.....	1/9 (11%)	2
84	Env 23–24	TENFNMWKNNMVEQMHEDI	Consensus	
		.....	3/4 (75%)	0
		..H.....A.....	1/4 (25%)	2
8	Env 94	EIVMHSFNCGGEFFY	Consensus	
		...T.....	13/13 (100%)	1
8	Env 143	LTVWGIKQLQARVLA	Consensus	
		.....I..	Bulk Seq	1
8	Vpu 19	EMGHHAPWDVDDL	Consensus	
		.....I...	10/10 (100%)	1
72	Env 9	LWVTVYYGVPVWKEA	Consensus	
		.....N.	9/9 (100%)	1
42	Env 23	TENFNMWKNNMVEQM	Consensus	
		..E.....	3/3 (100%)	1
8	Env 11	VPVWKEATTTLFCAS	Consensus	
		.....	6/6 (100%)	0
56	Env 10–11	VYYGVPVWKEATTTLFCAS	Consensus	
		.....	9/9 (100%)	0

*Peptide ADCC epitopes identified by ADCC intracellular cytokine staining (ICS) assay. Peptides derived from subtype B Env consensus sequence; 15-mer peptides overlapping by 11 amino acids (supplied by the National Institutes of Health AIDS Research and Reference Reagent Program).

†Sequence of consensus peptide epitope (top) and sequence of patients' native viral epitope obtained by extracting viral RNA from patients' plasma and bulk sequencing or cloning and sequencing across identified epitopes.

‡Frequency of clones from patients' virus with specific sequence (*X*/*Y*, where *X* = number of clones with each specific sequence and *Y*= total number of clones). Consensus identifies the sequence as HIV-1 Clade B consensus sequence across the epitope.