

Oligoclonal Expansions of CD8⁺ T Cells in Chronic HIV Infection Are Antigen Specific

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Summary

Acute HIV infection is associated with a vigorous immune response characterized by the proliferation of selected T cell receptor V beta (BV)-expressing CD8⁺ T cells. These 'expansions', which are commonly detected in the peripheral blood, can persist during chronic HIV infection and may result in the dominance of particular clones. Such clonal populations are most consistent with antigen-driven expansions of CD8⁺ T cells. However, due to the difficulties in studying antigen-specific T cells *in vivo*, it has been hard to prove that oligoclonal BV expansions are actually HIV specific. The use of tetrameric major histocompatibility complex-peptide complexes has recently enabled direct visualization of antigen-specific T cells *ex vivo* but has not provided information on their clonal composition. We have now made use of these tetrameric complexes in conjunction with anti-BV chain-specific monoclonal antibodies and analysis of cytotoxic T lymphocyte lines/clones to show that chronically clonally expanded CD8⁺ T cells are HIV specific *in vivo*.

Key words: human immunodeficiency virus • T cell receptor • cytotoxic T lymphocytes • tetramer • expansion

CD8⁺ T cells are generally believed to play a major protective role in the immune response to HIV infection during the primary and chronic asymptomatic phase of infection. The appearance of HIV-specific CTLs after primary infection has been shown to be concurrent with a dramatic fall in viral load (1). During the chronic asymptomatic phase of HIV infection, there are high levels of persisting HIV-specific CTLs (2–5), and there is an inverse correlation between HIV-specific CTL frequency and plasma RNA viral load (6). Strong cytotoxic responses to particular HIV epitopes may drive selection of HIV variants with mutations within the epitopes, preventing presentation or recognition of the epitope by the T cells (7, 8). The appearance of virus escape mutants suggests that the T cell response is a major factor influencing survival of HIV within the host.

Primary infection with HIV, SIV (simian immunodeficiency virus), and EBV can stimulate a vigorous CD8⁺ T cell response characterized by the proliferation of (oligo)clonal populations of CD8⁺ T cells. Such clonal expansions can be very large (9–11) and may persist with dominance of particular clones (12, 13). The antigen specificity of persisting clonally expanded CD8⁺ T cells has not been previously analyzed. The pattern of TCR rearrangement is con-

sistent with antigen-driven expansions and could correlate with the observed high degree of HIV-specific CTL activity seen during acute and chronic infection (9, 12).

Tetrameric MHC-peptide complexes, which bind specifically to appropriate MHC-peptide-specific T cells (14) has been used to show that the large CD8⁺ T cell expansions during the acute phase of EBV and lymphocytic choriomeningitis virus are largely antigen specific (15, 16). However, it is not known whether the smaller CD8⁺ T cell expansions seen during acute and chronic viral infections are composed of antigen-specific cells. To determine whether these T cells are antigen specific we have performed a longitudinal analysis of the CD8⁺ TCR repertoire during the chronic asymptomatic stage of HIV infection using the tetrameric MHC-peptide complexes in combination with anti-BV chain-specific mAbs and analysis of CTL lines/clones. Our results identify clonally expanded T cells that are HIV specific *in vivo*.

Materials and Methods

Patients and Controls. Peripheral blood was studied from seven HIV-infected individuals over a period of 2 and 3 yr. HIV-

uninfected individuals, <35-yr-old, were used as controls. Three patients were studied in detail: patient 003 (HLA A2, A3, B7, B51), a long term nonprogressor; patient 868 (HLA A2, A24, B27, B35), a progressor; and patient 065 (HLA A1, A2, B8, B44), a slow progressor.

Isolation and Fractionation of Lymphocyte Preparations. PBMCs were isolated from peripheral blood by Ficoll-Hypaque density gradient centrifugation. CD8⁺ T cells were negatively selected from PBMCs using anti-CD4 conjugated dynabeads (DynaL UK Ltd., Wirral, UK). A2-Gag-specific cells were selected from PBMCs using dynabeads indirectly conjugated to HLA A2-SLYNT-VATL complexes.

Cytofluorimetric Analysis. Two- and three-color staining of PBMCs and CTL lines/clones were carried out as previously described (17) using a combination of the following: a panel of anti-human TCR BV chain-specific mAbs, a second layer rabbit anti-mouse directly conjugated to FITC (DAKO Ltd., High Wycombe, Buckinghamshire, UK), PE-conjugated (DAKO Ltd.) HLA A2 or B27 MHC-peptide tetrameric complexes, an anti-CD8 mAb directly conjugated to PE, and an anti-CD8 mAb directly conjugated to Tricolor (Caltag Labs., San Francisco, CA). The panel of anti-human TCR BV chain-specific mAbs formed part of the TCR antibody workshop (18) and included the following: E2.2E7.2 (BV2); 8F10 (BV3); IMM157 (BV5S1); MH3-2 (BV5S2); OT145 (BV6S5); 3G5D5 (BV7.1); JR2 (BV8); AMKB1-2 (BV9); S511 (BV12); H131 (BV13S1); C1 (BV17); ELL1.4 (BV20); IG125 (BV21S3); IMM546 (BV22); and HUT787 (BV23). The nomenclature used for the TCR BV elements is as described by Arden et al. (19). Samples were analyzed on a FACS[®] using the CELLQUEST software (Becton Dickinson, San Jose, CA).

Production of Tetrameric MHC-Peptide Complexes. Soluble peptide-MHC tetramers were produced as previously described (15) for HLA A2 and HLA B27. The HLA A2 peptide ligands were SLYNTVATL (p17Gag 77-85) and ILKEPVHGV (Pol 476-484) (20, 21). The B27 peptide ligand was KRWIIMGLNK (p24Gag 263-272) (22).

Molecular Cloning and Sequencing of TCR BV Chains. mRNA was extracted and first strand cDNA synthesis was carried out as previously described (16). The cDNA was used as a template in PCR reactions using a 3' CB primer (CGTTTGTCGTCGAC-CTCCT TCCCATTCCACC) and a 5' BV primer using the same PCR conditions previously described (17). The 5' BV primers were as follows: BV3, GTCTCTAGAGAGAAGAAGAGCGC; BV5S1, CTCGGCCCTTTATCTTTG; BV5S2, ATAAGAATG-CGGCCGCCAGGCCCTGGGTCAGGGGCC; BV8, ATA-AGAATGCGGCCGCCGCTACTTTAACAACAACGTTCCG; B21S3, ATAAGAATGCGGCCGCCGCGCAGATCCTGGGAG-AGGGCC. The PCR products were purified and cloned, and plasmid DNA was extracted as previously described (13). Double-stranded sequencing was performed using T7 DNA polymerase (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK).

Results and Discussion

The Chronic Asymptomatic Stage of HIV Infection Is Associated with Expansions of Specific BV Families of the CD8⁺ TCR Repertoire. To determine whether the chronic asymptomatic stage of HIV infection is associated with expansions of CD8⁺ BV families, freshly isolated PBLs from HIV-infected individuals were analyzed using 14 human TCR BV-specific mAbs. Seven individuals were studied, all of

whom had seroconverted at least 5 yr previously. In all seven patients, expansions of particular TCR BV chains were found in the CD8⁺ TCR repertoire. Results for three individuals are shown in Table 1. Some of the expansions were large: in patient 065, 11.21% of CD8⁺ T cells expressed BV22, compared with 3.1% (\pm 1.68 SD) in the controls. It was common to see more than one BV expansion in these patients. For example, patient 868 had significant expansions in BV13S1 and BV21S3 (Table 1). In contrast, the percentage of use of each BV chain by CD8⁺ T cells from 10 healthy controls analyzed fell within a narrow range (data not shown).

The CD8⁺ BV Expansions Are (Oligo)clonal and May Persist for At Least 2 yr. To determine whether the restricted CD8⁺ BV T cell expansions persisted, the CD8⁺ TCR repertoires of three patients were reanalyzed two more times over a period of 2 or 3 yr. We found that all the BV expansions seen in patients 868 and 065 had persisted for 2 yr, whereas the expansion seen in patient 003 had persisted for >3 yr (data not shown). In some cases, the expression levels of the expanded CD8⁺ BV cells had increased or showed a transient increase. For example, the BV8 expansions of 7.65% seen in patient 003 transiently increased to 17.5% only to fall again to 7.5% over a period of 3 yr. In addition, new CD8⁺ BV expansions were found in all three patients: patient 868 exhibited a new expansion in BV8, patient 003 had a new expansion in BV13S1, and patient 065 had a new expansion in BV8 and BV20 (data not shown).

Table 1. The CD8⁺ TCR Repertoire of Three HIV-infected Individuals during the Chronic Asymptomatic Stage of the Disease

TCR BV	868*	065*	003†	Controls (s.d.)§
2	5.03	4.15	6.56	5.52 (1.67)
3	2.99	3.31	2.26	4.33 (2.18)
5S2	3.62	2.00	5.67	3.25 (0.83)
6S5	2.02	2.02	3.16	1.77 (1.34)
7S1	1.93	1.00	2.24	1.59 (0.79)
8	3.59	6.86	7.65	4.48 (0.94)
9	4.04	0.71	2.16	2.16 (0.63)
12	1.71	1.54	1.75	2.24 (1.13)
13S1	5.79	12.69	4.79	1.92 (1.04)
17	3.51	6.8	4.77	6.91 (2.05)
20	2.82	4.0	2.99	1.62 (0.84)
21S3	7.44	7.02	3.76	2.44 (0.43)
22	4.96	11.21	4.27	3.1 (1.68)
23	1.59	1.59	3.27	2.79 (1.35)

*Percentage of CD8⁺ T cells in 1996.

†Percentage of CD8⁺ T cells in 1995.

^{||}Percentage of CD8⁺ T cells stained with mAbs specific for human TCR BV chains shown. Expansions \geq mean + 3 SD are shown in bold.

[§]Data from 10 HIV-uninfected controls.

Table 2. The Amino Acid Sequences of the CDR3 Regions of TCR BV Chains from CD8⁺ PBL Expansions over a Period of 2 yr

Patient	TCR BV	CDR3	BJ	1996	1998
868	21S3 CAS	SLSLAVQNNEQF	FG 2S1	7/8	0/10
	21S3 CAS	SLSLGSSDNELF	FG 2S1	0/8	5/10
003	8 CAS	SSDRSDQPQH	FG 1S5	11/11	4/15

To determine the clonality of these persisting expansions we derived the sequences of the CDR3 regions of some of the expanded TCRs. The BV21S3 expansion of 7.44% ($2.44 \pm 0.43\%$ SD in controls) in patient 868 was clonal with seven out of the eight transcripts being identical (Table 2). In patient 003, the BV8 expansion of 17.5% ($4.48 \pm 0.94\%$ SD in controls) was also clonal, representing 11 out of 11 transcripts sequenced (Table 2). We derived the sequences of these TCR β chains 2 yr later. In patient 868, the original BV21S3 clonotype had been replaced by a new clonotype that had similarity to the old. The CDR3 length of this new clonotype is identical to the old clonotype. Furthermore, many of the amino acids in the CDR3 region are identical, and the BJ chain used is the same (Table 2). In patient 003, the BV8 clonotype was found to persist but at a lower frequency than first found (Table 2). Thus, (oligo)clonal CD8⁺ T cell expansions can persist during the chronic asymptomatic stage of HIV infection for at least 2 yr.

The persistence of these oligoclonal CD8⁺ T cell expansions is most consistent with them being antigen driven, although a restricted TCR repertoire is not necessarily related to HIV pathogenesis, since oligoclonal expansions have been reported in normal individuals (17, 23). Also, BV-expanded T cells could be a result of cell division by cross-reactive antigens or through proliferation due to cytokine-mediated bystander activation (24–26). However, oligoclonal expansions are much more common in HIV-infected individuals than in young, healthy controls, and

recent studies using MHC–peptide tetrameric complexes have shown that the majority of expanded CD8⁺ T cells are antigen specific during acute EBV and lymphocytic choriomeningitis virus infection (15, 16).

The Same TCRs of Cultured CTL Lines/Clones Are Expanded In Vivo. To determine whether these expanded BV chains seen in the CD8⁺ TCR repertoire were HIV specific, the BV usage of CTL lines and clones derived from these patients were analyzed.

In some cases, the dominant BV chain used by a CTL line was also expanded in the PBL. For example, in patient 003 the dominant receptor used by an A2-Gag-specific CTL line was found to be BV8, which at its highest expression level represented 17.5% of the CD8⁺ T cells in this patient. In patient 868, a B27-Gag-specific CTL line primarily used BV13S1 and again this was expanded in the PBL of this patient. However, in some cases the dominant BV chain used by a CTL line/clone was not expanded in the PBLs of these patients as assessed by anti-BV-specific mAbs. An A2-Gag-specific CTL clone derived from patient 868 was found to express the BV5S2 chain that represented between 3.62 and 4.18% of CD8⁺ T cells over the course of 2 yr in this patient and as such is not greater than three standard deviations above the mean staining of CD8⁺ BV5S2 T cells in control populations ($3.25 \pm 0.83\%$ SD). BV5S2 was also found to be the dominant receptor of another B27-Gag-specific CTL line taken from this patient. In patient 065, the dominant BV receptor used by an A2-Pol-specific CTL line was BV3, representing between 2.24 and 3.97% of CD8⁺ T cells, and again this was not expanded in the PBL of this patient using the above criteria ($4.33 \pm 2.18\%$ SD in controls). An A2-Gag clone derived from patient 003 was found to express the BV5S1 chain, which made up 2.63% of CD8⁺ T cells in this patient is also not classified as an expansion ($2.98 \pm 0.92\%$ SD in controls).

The sequences of the dominant TCR β chains used by these CTL lines/clones were found to be monoclonal (Table 3). The same TCR β chains were also sequenced from the PBLs of these patients and in all cases the clonotype of

Table 3. The Amino Acid Sequences of the CDR3 Regions from CTL Lines or Clones Derived from Chronically Infected HIV Patients

Patient	Specificity	TCR BV chain of CTL line/clone					TCR In PBL			
		BV	CDR3	BJ	1996	1998				
868	A2-Gag*	5S2	CAS	SDTVSYEQY	FG 2S7	7/15 [‡]	3.62 [§]	4/9 [‡]	4.18 [§]	
	B27-Gag	5S2	CAS	TGSQPQSYEQY	FG 2S7	0/15	3.62	1/9	4.18	
003	A2-Gag*	5S1	CAS	SFDSGNSPLH	FG 1S6	N/D	N/D	7/14	2.63	
	A2-Gag	8	CAS	SSDRSDQPQH	FG 1S5	12/12	17.5	4/15	7.5	
065	A2-Pol	3	CAS	SFVVPDTQY	FG 1S1	N/D	3.31	3/9	3.97	

*Designates a CTL clone.

[‡]The transcript frequency of the frequency of the CTL clonotype present in the PBL of each patient in 1996 and 1998.

[§]Percentage of CD8⁺ T cells stained with mAbs specific for the human TCR BV chains is shown.

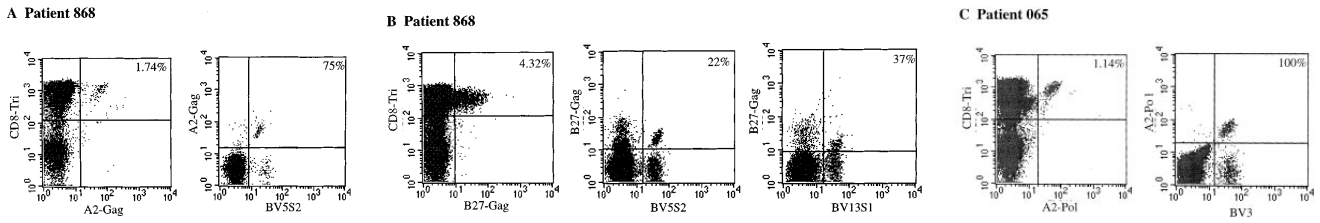


Figure 1. The TCR usage of HIV-specific CD8⁺ T cells in vivo. PBL taken from patients 868 and 003 were stained with HLA A2–SLYNTVATL tetrameric complexes. The PBL of patient 868 was also stained with a HLA B27–KRWIIMGLNK tetrameric complex, whereas the PBL taken from patient 065 was stained with the HLA A2–ILKEPVHGV tetramer. The tetramer was used to stain PBL in conjunction with CD8 and a panel of BV chain–specific mAbs. In patient 868, the A2–Gag tetramer stained 1.74% of the circulating CD8⁺ T cells, of which BV5S2 made up 75% (A). The B27–Gag tetramer also stained 4.32% of the circulating CD8⁺ T cells from this patient, of which BV13S1 and BV5S2 made up 37 and 22%, respectively (B). In patient 065, the A2–Pol tetramer stained 1.14% of the circulating CD8⁺ T cells, of which BV3 made up 100% (C).

the CTL line/clone was detected in the PBL. In patient 868, the BV5S2 A2–Gag clonotype was found to be present in the PBL in 7 out of 15 BV5S2–primed transcripts sequenced (Table 3). The BV5S2 B27–Gag clonotype was not found in the PBL at this time point. However, 2 yr later the B27–Gag clonotype was present in one out of nine transcripts sequenced from the PBLs (Table 3). In addition, the A2–Gag clonotype was found to persist being present in four out of nine transcripts sequenced (Table 3). In patient 003, the BV8 clonotype found in the PBL was identical to the BV8 clone used by the A2–Gag line derived from this patient. This BV8 clonotype also persisted >1 yr later at a lower frequency (4 out of 15 BV8 transcripts sequenced) (Table 3). The BV5S1 clonotype of the A2–Gag–restricted CTL clone derived from this patient was also found in the PBLs at a frequency of 7 out of 14 BV5S1–primed transcripts sequenced (Table 3). Finally, in patient 065, the BV3 clonotype used by the A2–Pol line was found in three out of the nine transcripts sequenced from the PBL (Table 3).

Thus, in most cases the expanded BV chains seen in the PBLs appear to be HIV specific as shown by the presence of the same clonotype in the PBL and CTL line/clone. Furthermore, some of the smaller expansions, detectable only by sequence analysis, show that analysis of TCR repertoire by the use of BV TCR–specific mAbs can be insufficient to identify clonally expanded T cell populations. This result also suggests that the CD8⁺ TCR repertoire is even more restricted than previously thought. This is supported by recent studies that have used CDR3 size spectratyping to analyze the TCR repertoire in HIV-infected individuals (27, 28).

The BV Usage of HIV-specific CD8⁺ T Cells In Vivo Directly Correlates with the BV Usage of CTL Line/Clones. To determine directly the BV usage of circulating HIV-specific CD8⁺ T cells in these patients, MHC–peptide tetrameric complexes were used to stain the PBL with anti-CD8 mAb and a panel of anti-BV-specific mAbs.

In patient 868, the A2–Gag tetramer stained 1.74% of circulating CD8⁺ T cells, of which BV5S2 made up 75% (Fig. 1 A). These A2–Gag–specific cells were found to persist for at least 2 yr (data not shown). The B27–Gag tetramer also stained 4.32% of circulating CD8⁺ T cells from this patient. BV13S1 made up 37% of these HIV-specific

cells, and BV5S2 made up another 22% (Fig. 1 B). Thus, in this patient, the same BV receptors that dominated the CTL lines/clones are used by the circulating HIV-specific CD8⁺ T cells seen in vivo. In patient 065, the A2–Pol tetramer stained 1.14% of circulating CD8⁺ T cells. These HIV-specific cells all used BV3 as their TCR (Fig. 1 C). Again, the same BV chain dominated the A2–Pol line derived from this patient. The A2–Gag tetramer stained 3.2% of circulating CD8⁺ T cells in patient 003. Several different BV chains stained with tetramer-positive cells, including BV8 and BV5S1, which stained ~7 and 27%, respectively (data not shown). This is consistent with the TCR BV chain expressed by the A2–Gag–restricted CTL line and clone derived from this patient.

In all the patients studied, the TCR usage of the circulating HIV-specific cells were made up of the BV chains used by the CTL lines/clones. However, some of the expanded BV chains seen in the CD8⁺ TCR repertoire were not used by the CTL lines/clones we analyzed, and they did not stain with the tetrameric MHC–peptide complexes we used. For example, patient 868 exhibited expansions in BV5S2, BV8, BV13S1, and BV21S3, of which only BV5S2 and BV13S1 are shown to be HIV specific. One explanation for this is that these expanded T cells are cytotoxic to as yet undefined HIV epitopes. In addition, we only analyzed the A2- and B27-restricted CTL response in these patients. The other expanded T cells could be restricted by other MHC class I alleles expressed by the patients. The fact that such expanded CD8⁺ T cells are oligoclonal in nature is consistent with antigen-specific expansion.

Table 4. The BV5S2 Amino Acid Sequences of A2–Gag–specific Cells Sorted from 868 PBL Using an A2–Gag Tetrameric Complex

BV	CDR3	BJ	Frequency
5S2	CAS SDTVSYEQY	FG 2S7	10/18*
5S2	CAS SLVGQGVNEQY	FG 2S5	8/18

*This sequence was also used by the A2–Gag–specific CTL clone from this patient (see Table 3).

The Clonotype of HIV-specific Cells In Vivo Is Identical to that Used by CTL In Vitro. Although the HIV-specific cells in vivo use the same TCR BV chain as the CTL lines/clones, the clonotype of the TCR may be different. To determine whether the BV5S2 clonotype of the A2-Gag CTL clone present in the PBL is used by HIV-specific T cells in vivo, A2-Gag-specific cells were sorted from the PBL of patient 868. BV5S2 sequence analysis of the A2-Gag-specific cells revealed the presence of the same BV5S2 clonotype seen in the CTL clone in 10 out of 18 transcripts sequenced. One

other clonotype was also present. (Table 4). Thus, the dominant receptors used by CTL lines/clones in vitro are expanded in the PBLs and are HIV specific.

In conclusion, we have shown that clonally expanded populations of CD8⁺ T cells detected by the use of anti-human BV chain-specific mAbs and sequence analysis are HIV specific in vivo during the chronic asymptomatic stage of HIV infection. Thus, the majority of expanded CD8⁺ T cells are driven to proliferate by antigen and can persist for more than two years.

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