Journal of Virology

# HLA B*5701-Positive Long-Term Nonprogressors/Elite Controllers Are Not Distinguished from Progressors by the Clonal Composition of HIV-Specific CD8 + T Cells

**Daniel Mendoza, Cassandra Royce, Laura E. Ruff, David R. Ambrozak, Máire F. Quigley, Thurston Dang, Vanessa Venturi, David A. Price, Daniel C. Douek, Stephen A. Migueles and Mark Connors**

***J. Virol.* 2012, 86(7):4014. DOI: 10.1128/JVI.06982-11.**
**Published Ahead of Print 25 January 2012.**

JVI Journals.ASM.org

# HLA B*5701-Positive Long-Term Nonprogressors/Elite Controllers Are Not Distinguished from Progressors by the Clonal Composition of HIV-Specific CD8$^+$ T Cells

Daniel Mendoza,[a] Cassandra Royce,[a] Laura E. Ruff,[b] David R. Ambrozak,[b] Máire F. Quigley,[b,c] Thurston Dang,[d] Vanessa Venturi,[d] David A. Price,[b,c] Daniel C. Douek,[b] Stephen A. Migueles,[a] and Mark Connors[a]

HIV-Specific Immunity Section, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA[a]; Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA[b]; Institute of Infection and Immunity, Cardiff University School of Medicine, Cardiff, Wales, United Kingdom[c]; and Computational Biology Group, Centre for Vascular Research, University of New South Wales, Kensington, Australia[d]

**To better understand the qualitative features of effective human immunodeficiency virus (HIV)-specific immunity, we examined the TCR clonal composition of CD8$^+$ T cells recognizing conserved HIV p24-derived epitopes in HLA-B*5701-positive long-term nonprogressors/elite controllers (LTNP/EC) and HLA-matched progressors. Both groups displayed oligoclonal HLA-B5701-restricted p24-specific CD8$^+$ T-cell responses with similar levels of diversity and few public clonotypes. Thus, HIV-specific CD8$^+$ T-cell responses in LTNP/EC are not differentiated from those of progressors on the basis of clonal diversity or TCR sharing.**

Understanding natural immunologic control of human immunodeficiency virus (HIV) may provide important insights for the development of vaccines and immunotherapies. Rare long-term nonprogressors/elite controllers (LTNP/EC) maintain durable restriction of HIV replication without antiretroviral therapy (25). Evidence suggesting that HIV-specific CD8$^+$ T cells mediate this control (13) includes a dramatic overrepresentation of the HLA-B*5701 allele (8, 12, 19, 21, 29, 33, 37) and a CD8$^+$ T-cell response focused on HLA-B5701-restricted peptides (11, 29). Similarly, LTNP/EC rhesus macaques are enriched for the Mamu B*08 or B*17 alleles (22, 43) and lose control of simian immunodeficiency virus (SIV) replication following antibody-mediated CD8$^+$ T-cell depletion (9). HIV-specific CD8$^+$ T cells in LTNP/EC also show a greater capacity to suppress HIV replication or kill autologous HIV-infected CD4$^+$ T cells than those of progressors (14, 27, 28, 30, 37). Collectively, these data suggest that greater HIV control in LTNP/EC than in progressors is likely mediated by qualitative differences in their CD8$^+$ T-cell responses.

It has been speculated that favorable HLA molecules may select for a diverse repertoire of T-cell receptor (TCR) clonotypes capable of limiting HIV mutational escape (5, 17, 18, 20, 23, 38). Favorable HLA molecules might preferentially select for "public clonotypes" (10, 34, 38–40), which are shared among multiple individuals and may constitute particularly effective CD8$^+$ T-cell responses (1, 2, 15). In the present study, we compared for the first time the clonalities of CD8$^+$ T cells targeting the same immunodominant HIV epitopes in HLA-B*5701$^+$ LTNP/EC and in progressors to better understand qualitative features of an effective HIV-specific CD8$^+$ T-cell response.

Subjects signed institutional review board-approved informed consent forms and were categorized as LTNP/EC or progressors as previously defined (28, 29). Three progressors who had had elevated HIV RNA levels (>10,000 copies/ml) prior to initiating antiretroviral therapy (ART) donated peripheral blood mononuclear cells (PBMC) while receiving ART yet had persistently detectable viremia due to erratic compliance with their regimens (Table 1). PBMC were stained with HLA-B5701 tetramers complexed to the HIV Gag p24 epitopes ISPRTLNAW (IW9; amino acids [aa] 147 to 155), KAFSPEVIPMF (KF11; aa 162 to 172), or QASQEVKNW (QW9; aa 308 to 316) (Beckman Coulter, San Diego, CA) and then surface stained with antibodies to CD3, CD8, CD57, CD25, CD27, CD28, HLA-DR, CD45RO, CD57, and CCR7 (BD Biosciences, San Jose, CA) prior to flow cytometry analysis (16, 28). Tetramer-positive CD8$^+$ T cells were also sorted at >98% purity by flow cytometry, and all expressed TCR$\beta$ gene products were amplified and characterized without bias using a nonnested template-switch-anchored reverse transcription-PCR (RT-PCR) (6, 35). Medians and correlations were analyzed using the Wilcoxon two-sample test and the Spearman rank method, respectively. The Bonferroni method was used to adjust *P* values for multiple testing. Clonotypic diversity was assessed by the standardized number of clonotypes, which accounts for differences in the number of TCR sequences obtained per sample, and Simpson's diversity index, which accounts for the clonal dominance hierarchy and varies between 0 (minimal diversity) and 1 (maximal diversity) (41).

Between 0.8 and 6.2% of CD8$^+$ T cells from 7 HLA-B*5701-positive LTNP/EC (10 specificities) and 8 progressors (10 specificities) (Table 1) stained positively with at least one HLA-B5701/p24 tetramer (Fig. 1A and B and data not shown). No significant differences were observed between LTNP/EC and progressors within the tetramer-positive populations regarding activation status, costimulation, antigen exposure, or homing (CD25, HLA-DR, CD27, CD28, CD45RO, CD57, or CCR7 expression) ($P > 0.5$ for all comparisons; data not shown). The predominant phenotype of HLA-B5701/p24 tetramer-positive CD8$^+$ T cells was

**Received** 2 December 2011 **Accepted** 9 January 2012
**Published ahead of print** 25 January 2012
Address correspondence to Mark Connors, mconnors@nih.gov.

doi:10.1128/JVI.06982-11

**TABLE 1** Patient characteristics

| Patient type and no. | HLA class I allele(s) | | | Yr of diagnosis | No. of T cells (cells/$\mu$l) | | Viral load (copies/ml) | Tetramer(s)[a] |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | | $CD4^+$ | $CD8^+$ | | |
| LTNP/EC | | | | | | | | |
| 4 | 1,31 | 8,57 | 6,7 | 1985 | 943 | 1,012 | <50 | IW9, QW9 |
| 7 | 1,2 | 57 | 6 | 1985 | 325 | 342 | <50 | IW9, KF11 |
| 33 | 2,30 | 13,57 | 6 | 1995 | 1,334 | 1,557 | <50 | IW9 |
| 34 | 1,2 | 8,57 | 6,7 | 1989 | 1,523 | 1,111 | <50 | KF11, QW9 |
| 30 | 31,74 | 51,57 | 7,16 | 1990 | 306 | 747 | <50–58 | KF11 |
| 6 | 11,30 | 52,57 | 7,12 | 1986 | 737 | 998 | <50–152 | QW9 |
| 8 | 11,23 | 44,57 | 4,6 | 1985 | 490 | 1,033 | <50–567 | IW9, KF11 |
| Progressors (untreated) | | | | | | | | |
| 35 | 2,3 | 57 | 6,7 | 2000 | 1,144 | 1,220 | 1,800 | KF11 |
| 20 | 1 | 52,57 | 6,12 | 1985 | 1,068 | 1,091 | 25,017 | KF11 |
| 106 | 3,30 | 18,57 | 5,18 | 1986 | 420 | 1,382 | 24,564 | IW9, KF11 |
| 131 | 2,11 | 35,57 | 4,6 | 1989 | 421 | 1,082 | 76,038 | IW9, KF11 |
| 107 | 3 | 40,57 | 3,7 | 1987 | 405 | 1,418 | 90,130 | IW9, KF11 |
| Progressors (treated) | | | | | | | | |
| 105 | 2,80 | 8,57 | 2,7 | 1990 | 616 | 1,026 | 2,473 | KF11 |
| 101 | 1,31 | 51,57 | 6,15 | 1986 | 419 | 992 | 3,421 | KF11 |
| 103 | 2,11 | 55,57 | 3,6 | 1991 | 653 | 1,130 | 3,968 | KF11 |

[a] HLA-B5701/HIV Gag p24 tetramers. IW9, ISPRTLNAW (positions 147 to 155); KF11, KAFSPEVIPMF (162 to 172); QW9, QASQEVKNW (308 to 316).

$CD25^-$ $CD27^+$ $CD45RO^+$ $CCR7^-$ $CD57^{+/-}$ in both groups (data not shown), consistent with previous reports (4, 7, 16, 32).

In analyses of the clonotypic composition of HLA-B5701/p24 tetramer-positive cells, a mean of 65 clones were examined for each sorted specificity, representing >1,000 TCR sequences (6, 36). Overall, the immunodominant B5701-restricted HIV p24 epitope-specific $CD8^+$ T-cell populations in both groups were oligoclonal, comprising a median of 6.5 clonotypes (range, 1 to 12) per specificity (Table 2 and Fig. 1A and B). Only the QW9-specific cells of LTNP/EC 4 were monoclonal (Table 2). These data are consistent with previous observations in chronic human viral infections (6, 23).

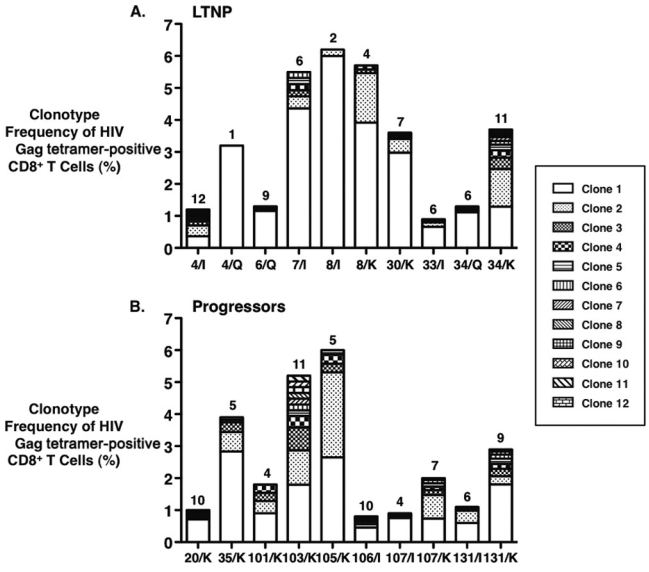


**FIG 1** Clonal composition of HLA-B5701/HIV Gag p24 tetramer-positive $CD8^+$ T-cell populations in LTNP/EC and progressors. (A and B) Summary data showing the frequencies of each TCR clonotype (Table 2) relative to the frequencies of the corresponding HLA B5701/HIV Gag p24 tetramer-positive $CD8^+$ T cells in 7 HLA-B*5701-positive LTNP/EC (A) and 8 HLA-B*5701-positive progressors (B). Three HLA-B5701/HIV Gag p24 tetramers containing B57 bound to one of three immunodominant Gag p24-derived epitopes (I, IW9; K, KF11; and Q, QW9) were used to stain and sort antigen-specific $CD8^+$ T cells for clonotypic analysis. Results for two different specificities are shown for patients 4, 8, 34, 107, and 131. The numbers above each stacked bar denote the total number of clonotypes for the depicted epitope-specific $CD8^+$ T-cell population.

To analyze repertoire diversity, subject 107's IW9-specific TCR repertoire (18 TCRs) and subject 101's KF11-specific repertoire (14 TCRs) were excluded due to low numbers of sequences. The remaining data were standardized for sampling differences (41). There were no significant differences between LTNP/EC and progressors in either the standardized numbers of epitope-specific clonotypes per repertoire ($P > 0.5$) (Fig. 2A) or Simpson's diversity index ($P = 0.31$) (Fig. 2C) for the IW9- and KF11-specific repertoires. QW9-specific repertoires were omitted from the comparisons since they were only detected in the LTNP/EC group ($n = 3$) (Fig. 2B and D). Additionally, there were no significant correlations between HIV RNA levels and either the number or diversity of $CD8^+$ T-cell clonotypes per repertoire ($P > 0.5$ for all correlations; data not shown).

We next examined whether there were public TCR clonotypes shared between these patients or with prior reports (10, 38, 44). Only 2 public clonotypes were identified in LTNP/EC that were not shared with patients in this study but that had previously been reported in two progressors (Table 2) (44). In progressors, only two sequences were shared between two individuals and one was previously reported (Table 2) (44). Thus, public clonotypes were not a dominant feature of the repertoires of either LTNP/EC or progressors.

Collectively, these data suggest that the large differences in immunologic control between HLA-B*5701-positive LTNP/EC and progressors are unlikely to be mediated by differences in the TCR

**TABLE 2** HLA-B5701-restricted HIV Gag p24-specific CD8$^+$ TCR$\beta$ repertoires[a]

| Patient | TRBV | CDR3 Sequence | TRBJ | Frequency (%) |
|---|---|---|---|---|
| 4-I | 7-8 | CASSLRGGGASYNEQF | 2-1 | 30.6 |
| | 7-8 | CASSPWTYGTGTLNEKLF | 1-4 | 27.8 |
| | 11-2 | CASSLKLADEQF | 2-1 | 11.1 |
| | 4-1 | CASSQDGLLNSYEQY | 2-7 | 5.6 |
| | 12-5 | CASGLGRGWNTEAF | 1-1 | 5.6 |
| | 2 | CASSRQLGTGELF | 2-2 | 2.8 |
| | 4-1 | CASSQDPTANSEAF | 1-1 | 2.8 |
| | 4-1 | CASSQDSTSYGYT | 1-2 | 2.8 |
| | 4-1 | CASSQEGHSNQPQH | 1-5 | 2.8 |
| | 7-2 | CASSLVLLGNSPLH | 1-6 | 2.8 |
| | 7-9 | CASSLLQGAASPLH | 1-6 | 2.8 |
| | 19 | CASSIWLSGPNYGYT | 1-2 | 2.8 |
| | | Sequences: 36 | | |
| 4-Q | 27 | CASGTKYYGYT | 1-2 | 100.0 |
| | | Sequences: 50 | | |
| 6-Q | 27 | CASSSQGYGRAQH | 1-5 | 88.9 |
| | 3-1 | CASSQYLLAGARGTYEQY | 2-7 | 2.2 |
| | 12-4 | CASSQGGVQPQH | 1-5 | 2.2 |
| | 7-9 | CASSTPSIPWSTDDFFEQY | 2-7 | 1.1 |
| | 12-3 | CASSWRVMNTEAF | 1-1 | 1.1 |
| | 12-4 | CASRPDRAGSNQPQH | 1-5 | 1.1 |
| | 27 | CASGSQGYGRAQH | 1-5 | 1.1 |
| | 27 | CASSSQGHGRAQH | 1-5 | 1.1 |
| | 27 | CASSSQGYGGAQH | 1-5 | 1.1 |
| | | Sequences: 90 | | |
| 7-I | 7-9 | CASSHGQGMKTQY | 2-5 | 79.3 |
| | 20-1 | CSALQLGGAGANEQF | 2-1 | 6.9 |
| | 7-9 | CANSHGQGMKTQY | 2-5 | 3.5 |
| | 7-9 | CASSLGRGMKTQY | 2-5 | 3.5 |
| | 7-9 | CASSYGQGMKTQY | 2-5 | 3.5 |
| | 7-9 | CATSPGQGMKTQY | 2-5 | 3.5 |
| | | Sequences:29 | | |
| 8-I | 5-1 | CASTYDRGFTGELF | 2-2 | 96.8 |
| | 5-1 | CASTYDRGFTGGLF | 2-2 | 3.2 |
| | | Sequences: 62 | | |
| 8-K | 19 | CASSMTYGYT | 1-2 | 68.8 |
| | 19 | CASSGSYGYT | 1-2 | 27.1 |
| | 19 | CASRMTYGYT | 1-2 | 2.1 |
| | 19 | CASSTTYGYT | 1-2 | 2.1 |
| | | Sequences: 48 | | |
| 30-K | 7-6 | CASSLMSGTDTQY | 2-3 | 82.8 |
| | 18 | CASSDRSQYGYT | 1-2 | 11.8 |
| | 7-6 | CASNLMSGTDTQY | 2-3 | 1.1 |
| | 7-6 | CASSLMPGTDTQY | 2-3 | 1.1 |
| | 7-6 | CASTLMSGTDTQY | 2-3 | 1.1 |
| | 15 | CATGPGAREQY | 2-7 | 1.1 |
| | 29-1 | CSVEDQTDPGYT | 1-2 | 1.1 |
| | | Sequences: 93 | | |
| 33-I | 27 | CASRPGQGGYEQY | 2-7 | 73.5 |
| | 4-2 | CASSQDPSGNPMETQY | 2-5 | 14.7 |
| | 2 | CASSSGTASTDTQY | 2-3 | 2.9 |
| | 4-2 | CASSPDPSGNPMETQY | 2-5 | 2.9 |
| | 5-1 | CASRYPLDLRNTGELF | 2-2 | 2.9 |
| | 10-3 | CAISGASHEQF | 2-1 | 2.9 |
| | | Sequences: 34 | | |
| 34-Q | 6-6 | CASTQGRGGSGYT | 1-2 | 86.1 |
| | 3-1 | CASSQAGGDAEAF | 1-1 | 2.8 |
| | 5-1 | CASSPGTANSGNTIY | 1-3 | 2.8 |
| | 9 | CASSVDAGFTDTQY | 2-3 | 2.8 |
| | 12-4 | CASSLRGDSNQPQH | 1-5 | 2.8 |
| | 28 | CASSPPSGIYNEQF | 2-1 | 2.8 |
| | | Sequences: 36 | | |
| 34-K | 6-1 | CASSGTSYGYT | 1-2 | 34.9 |
| | 7-6 | CASSLRRDSGPPSNQPQH | 1-5 | 31.8 |
| | 19 | CATTGQDYGYT | 1-2 | 9.5 |
| | 27 | CASSLLGTVGNQPQH | 1-5 | 6.4 |
| | 6-1 | CASSGTAYGYT | 1-2 | 4.8 |
| | 19 | CASTGGSYGYT | 1-2 | 3.2 |
| | 20-1 | CSASSGQGSGNTIY | 1-3 | 3.2 |
| | 6-1 | CASSGANYGYT | 1-2 | 1.6 |
| | 6-1 | CASSGDSYGYT | 1-2 | 1.6 |
| | 19 | CASSGQDYGYT | 1-2 | 1.6 |
| | 30 | CAWSGQVYGYT | 1-2 | 1.6 |
| | | Sequences: 63 | | |

| Patient | TRBV | CDR3 Sequence | TRBJ | Frequency (%) |
|---|---|---|---|---|
| 20-K | 7-9 | CASSGTDYGYT | 1-2 | 71.2 |
| | 7-9 | CAQGGSNYGYT | 1-2 | 5.3 |
| | 7-9 | CASSSERPSNEQY | 2-7 | 5.3 |
| | 2 | CASRAQGPIYEQY | 2-7 | 2.6 |
| | 19 | CASSGTNYGYT | 1-2 | 2.6 |
| | 19 | CASTGGDYGYT | 1-2 | 2.6 |
| | 19 | CASTGPYGYT | 1-2 | 2.6 |
| | 23-1 | CASSQFDRALHQTQY | 2-5 | 2.6 |
| | 28 | CASSLSGSSYNEQF | 2-1 | 2.6 |
| | 30 | CALTRSYGYT | 1-2 | 2.6 |
| | | Sequences: 38 | | |
| 35-K | 7-9 | CASSPGQGFKTQY | 2-5 | 72.6 |
| | 27 | CASSPGQGGYEQY | 2-7 | 15.7 |
| | 7-6 | CASSYGQAGEQY | 2-7 | 7.8 |
| | 7-9 | CASSLGRGQETQY | 2-5 | 2.0 |
| | 27 | CASSPGQGGYGQY | 2-7 | 2.0 |
| | | Sequences: 51 | | |
| 101-K | 6-1 | CASTGQGYGYT | 1-2 | 50.0 |
| | 7-9 | CAEGGRDYGYT | 1-2 | 21.4 |
| | 5-1 | CASSGRNEQF | 2-1 | 14.3 |
| | 7-9 | CASSLGDRAFRNIQY | 2-4 | 14.3 |
| | | Sequences: 14 | | |
| 103-K | 6-1 | CASTGSYGYT | 1-2 | 34.5 |
| | 4-3 | CASSQETPRGPPLAKNIQY | 2-4 | 20.7 |
| | 6-1 | CASTGQGYGYT | 1-2 | 13.8 |
| | 11-2 | CASSISYEQY | 2-7 | 6.9 |
| | 4-3 | CASSQETPRGPPLARNIQY | 2-4 | 3.5 |
| | 6-1 | CASSGGEYGYT | 1-2 | 3.5 |
| | 7-9 | CASSPRTGPNYGYT | 1-2 | 3.5 |
| | 19 | CASNGQNYGYT | 1-2 | 3.5 |
| | 19 | CASNGRNYGYT | 1-2 | 3.5 |
| | 19 | CATTGGNYGYT | 1-2 | 3.5 |
| | 28 | CASKLAGGYYEQY | 2-7 | 3.5 |
| | | Sequences: 29 | | |
| 105-K | 7-7 | CASSPWGDGTDTQY | 2-3 | 44.2 |
| | 7-9 | CASSAPDSQETQY | 2-5 | 44.2 |
| | 2 | CASPREFNTEAF | 1-1 | 4.7 |
| | 5-4 | CASTTLGQDQETQY | 2-5 | 4.7 |
| | 2 | CASSDSTGVRQPQH | 1-5 | 2.3 |
| | | Sequences: 43 | | |
| 106-I | 7-8 | CASSFGTGGVGELF | 2-2 | 57.5 |
| | 7-8 | CASSSATGITGELF | 2-2 | 12.5 |
| | 7-8 | CASSARQGSRTDTQY | 2-3 | 10.0 |
| | 7-8 | CASSQATGNTGELF | 2-2 | 5.0 |
| | 3-1 | CASRMGANTEAF | 1-1 | 2.5 |
| | 7-7 | CASSNPDGTGDNEQF | 2-1 | 2.5 |
| | 7-8 | CASSPLTGATYNEQF | 2-1 | 2.5 |
| | 7-8 | CASSDLTGLTGELF | 2-2 | 2.5 |
| | 7-8 | CASNFGTGGVGELF | 2-2 | 2.5 |
| | 27 | CASSLSQGTILYEQY | 2-7 | 2.5 |
| | | Sequences: 40 | | |
| 107-I | 7-9 | CASSAGQGWKEQY | 2-7 | 83.3 |
| | 7-8 | CASSGTQVQPQH | 1-5 | 5.6 |
| | 7-9 | CASNAGQGWKEQY | 2-7 | 5.6 |
| | 7-9 | CASSAGQGWKGQY | 2-7 | 5.6 |
| | | Sequences: 18 | | |
| 107-K | 7-3 | CASSLIWDRGNYGYT | 1-2 | 36.8 |
| | 25-1 | CASSGSGTGYGYT | 1-2 | 36.8 |
| | 7-6 | CASSLMQGTDTQY | 2-3 | 7.9 |
| | 19 | CASSGQGYGYT | 1-2 | 5.3 |
| | 19 | CASTGTYGYT | 1-2 | 5.3 |
| | 24-1 | CATSDPNRDTDTQY | 2-3 | 5.3 |
| | 19 | CATAESYGYT | 1-2 | 2.6 |
| | | Sequences: 38 | | |
| 131-I | 28 | CASSRYEQY | 2-7 | 54.2 |
| | 7-9 | CASFLNTEAF | 1-1 | 35.4 |
| | 28 | CASSLYEQY | 2-7 | 4.2 |
| | 5-5 | CASSPADSQETQY | 2-5 | 2.1 |
| | 7-9 | CASFPNTEAF | 1-1 | 2.1 |
| | 28 | CASSGIGVF | 1-4 | 2.1 |
| | | Sequences: 48 | | |
| 131-K | 19 | CASTGSYGYT | 1-2 | 62.2 |
| | 6-1 | CASSGGSYGYT | 1-2 | 8.9 |
| | 19 | CASSGTYGYT | 1-2 | 7.8 |
| | 19 | CATTGGNYGYT | 1-2 | 5.6 |
| | 25-1 | CASSDPGQYGYT | 1-2 | 5.6 |
| | 19 | CATDTGSYGYT | 1-2 | 4.4 |
| | 19 | CASSNTYGYT | 1-2 | 3.3 |
| | 6-7 | CASSGGWYGYT | 1-2 | 1.1 |
| | 19 | CACSRSYGYT | 1-2 | 1.1 |
| | | Sequences: 90 | | |

[a] I, K, and Q represent HLA-B5701-restricted CD8$^+$ T-cell repertoires specific for Gag epitopes IW9, KF11, and QW9, respectively, for a given patient. Public clonotypes were defined as identical TCR$\beta$ amino acid sequences observed in more than one individual with reference to an extensive database, including both previously reported and unpublished sequences. Sequences in gray are public clonotypes that are also found in prior reports. Clonotypes shared between patients in this report are shown in color. TRBV, TCR$\beta$ variable allele; CDR3, complementarity-determining region 3; TRBJ, TCR$\beta$ joining allele.

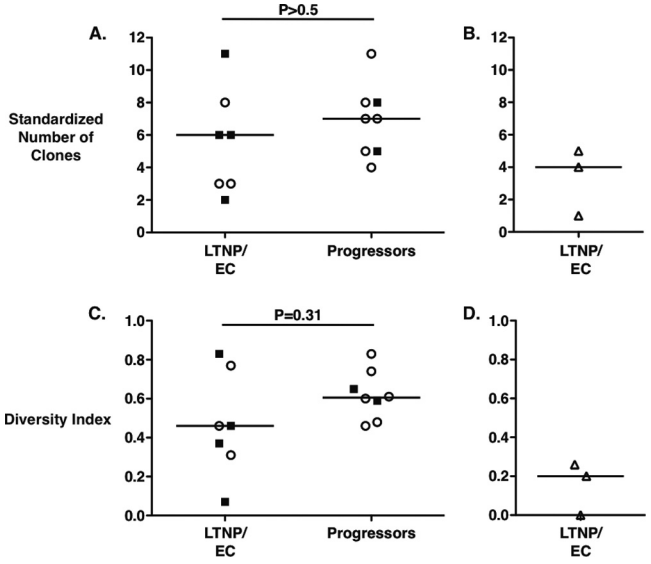


**FIG 2** Comparison of HLA-B5701/HIV Gag p24 tetramer-positive CD8$^+$ T-cell repertoire diversities between HLA-B5701-positive LTNP/EC and progressors (A to D). Two diversity measures, the standardized number of clonotypes (A and B) and Simpson's diversity index (C and D), were estimated for a standard sample size of 29 TCR$\beta$ sequences per repertoire. Simpson's diversity index accounts for both the variety of amino acid sequence clonotypes and their clone sizes, ranging in value from 0 (minimal diversity) to 1 (maximal diversity). Horizontal lines indicate the median values. Squares, circles, and triangles represent the HLA-B5701-restricted HIV Gag p24-derived epitopes IW9, KF11, and QW9, respectively.

repertoire of immunodominant HIV-specific CD8$^+$ T-cell populations. We observed similar clonotype numbers and diversity in these cohorts regardless of viral load or specificity. The trend toward greater TCR polyclonality in progressors than in LTNP/EC might be explained by the recruitment of a more polyclonal response in the context of higher viral loads in the former group.

In prior work, it has been suggested that greater clonal diversity within the HIV-specific memory CD8$^+$ T-cell repertoire may mediate superior control of HIV (17, 18, 24, 38). It has been further proposed that protective HLA class I alleles might mediate their effect through thymic deletion of fewer self-reactive clones, providing a more diverse naïve repertoire (20). In either case, it is hypothesized that greater clonal diversity might mediate superior control through improved recognition of peptide variants. However, many prior studies did not include true LTNP/EC and did not compare responses within a given HLA and specificity (17, 18, 24, 38). In addition, differences in TCR contact residues within targeted epitopes, suggesting differential abilities to contain nonanchor position variation, have not been observed between B*5701-positive LTNP/EC and progressors (3, 26, 31). Therefore, if TCR clonal diversity were an important parameter differentiating LTNP/EC from progressors, some detectable difference in clonality between these groups would be expected, considering the vast differences in their capacity to control HIV replication.

It has also been suggested that LTNP/EC may possess public clonotypes that preferentially contain HIV (10, 34, 44). Public-clonotype HIV-specific CD8$^+$ T cells with high functional avidity that could be particularly efficacious might be selected in multiple individuals (15). However, we did not observe significant numbers of public clonotypes in either LTNP/EC or progressors, in contrast to prior results in which a single clonotype was found in the KF11-specific responses of 3 HLA B*5701-positive donors (10, 44). Furthermore, we previously detected no differences between LTNP/EC and progressors in the avidities of gamma interferon (IFN-$\gamma$)-secreting HIV-specific CD8$^+$ T cells responding to cognate peptides (26, 27). Selection of Gag-specific public clonotypes in the rhesus macaque-SIV infection model has been observed to correlate inversely with subsequent viral load (34). However, these studies were conducted during acute SIV infection. It is likely that the forces governing public clonotype selection in acute lentiviral infection or vaccination may differ from those involved in chronic HIV infection. It remains possible that additional study of HIV-specific CD8$^+$ T-cell clonotypes in the setting of acute infection or vaccination (42) may further our understanding of immunologic control of HIV and other chronic viral infections in humans.

## ACKNOWLEDGMENT

This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Disease, NIH.

## REFERENCES

1. **Almeida JR, et al.** 2007. Superior control of HIV-1 replication by CD8$^+$ T cells is reflected by their avidity, polyfunctionality, and clonal turnover. J. Exp. Med. **204**:2473–2485.
2. **Almeida JR, et al.** 2009. Antigen sensitivity is a major determinant of CD8$^+$ T-cell polyfunctionality and HIV-suppressive activity. Blood **113**: 6351–6360.
3. **Bailey JR, Williams TM, Siliciano RF, Blankson JN.** 2006. Maintenance of viral suppression in HIV-1-infected HLA-B*57$^+$ elite suppressors despite CTL escape mutations. J. Exp. Med. **203**:1357–1369.
4. **Champagne P, et al.** 2001. Skewed maturation of memory HIV-specific CD8$^+$ T lymphocytes. Nature **410**:106–111.
5. **Davenport MP, Price DA, McMichael AJ.** 2007. The T cell repertoire in infection and vaccination: implications for control of persistent viruses. Curr. Opin. Immunol. **19**:294–300.
6. **Douek DC, et al.** 2002. A novel approach to the analysis of specificity, clonality, and frequency of HIV-specific T cell responses reveals a potential mechanism for control of viral escape. J. Immunol. **168**:3099–3104.
7. **Ellefsen K, et al.** 2002. Distribution and functional analysis of memory antiviral CD8$^+$ T cell responses in HIV-1 and cytomegalovirus infections. Eur. J. Immunol. **32**:3756–3764.
8. **Flores-Villanueva PO, et al.** 2001. Control of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. Proc. Natl. Acad. Sci. U. S. A. **98**:5140–5145.
9. **Friedrich TC, et al.** 2007. Subdominant CD8$^+$ T-cell responses are involved in durable control of AIDS virus replication. J. Virol. **81**:3465–3476.
10. **Gillespie GM, et al.** 2006. Strong TCR conservation and altered T cell cross-reactivity characterize a B*57-restricted immune response in HIV-1 infection. J. Immunol. **177**:3893–3902.
11. **Goulder PJ, et al.** 1996. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. AIDS Res. Hum. Retroviruses **12**:1691–1698.
12. **Han Y, et al.** 2008. The role of protective HCP5 and HLA-C associated polymorphisms in the control of HIV-1 replication in a subset of elite suppressors. AIDS **22**:541–544.
13. **Hersperger AR, Migueles SA, Betts MR, Connors M.** 2011. Qualitative features of the HIV-specific CD8$^+$ T-cell response associated with immunologic control. Curr. Opin. HIV AIDS **6**:169–173.
14. **Hersperger AR, et al.** 2010. Perforin expression directly *ex vivo* by HIV-specific CD8$^+$ T-cells is a correlate of HIV elite control. PLoS Pathog. **6**:e1000917.
15. **Iglesias MC, et al.** 2011. Escape from highly effective public CD8$^+$ T-cell clonotypes by HIV. Blood **118**:2138–2149.
16. **Jagannathan P, et al.** 2009. Comparisons of CD8$^+$ T cells specific for human immunodeficiency virus, hepatitis C virus, and cytomegalovirus reveal differences in frequency, immunodominance, phenotype, and interleukin-2 responsiveness. J. Virol. **83**:2728–2742.

17. **Kalams SA, et al.** 1996. T cell receptor usage and fine specificity of human immunodeficiency virus 1-specific cytotoxic T lymphocyte clones: analysis of quasispecies recognition reveals a dominant response directed against a minor in vivo variant. J. Exp. Med. **183**:1669–1679.
18. **Kalams SA, et al.** 1994. Longitudinal analysis of T cell receptor (TCR) gene usage by human immunodeficiency virus 1 envelope-specific cytotoxic T lymphocyte clones reveals a limited TCR repertoire. J. Exp. Med. **179**:1261–1271.
19. **Kloosterboer N, et al.** 2005. Natural controlled HIV infection: preserved HIV-specific immunity despite undetectable replication competent virus. Virology **339**:70–80.
20. **Kosmrlj A, et al.** 2010. Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. Nature **465**:350–354.
21. **Lambotte O, et al.** 2005. HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication. Clin. Infect. Dis. **41**:1053–1056.
22. **Loffredo JT, et al.** 2007. $CD8^+$ T cells from SIV elite controller macaques recognize Mamu-B*08-bound epitopes and select for widespread viral variation. PLoS One **2**:e1152.
23. **Lopes AR, et al.** 2003. Greater $CD8^+$ TCR heterogeneity and functional flexibility in HIV-2 compared to HIV-1 infection. J. Immunol. **171**:307–316.
24. **Meyer-Olson D, et al.** 2006. Fluctuations of functionally distinct $CD8^+$ T-cell clonotypes demonstrate flexibility of the HIV-specific TCR repertoire. Blood **107**:2373–2383.
25. **Migueles SA, Connors M.** 2010. Long-term nonprogressive disease among untreated HIV-infected individuals: clinical implications of understanding immune control of HIV. JAMA **304**:194–201.
26. **Migueles SA, et al.** 2003. The differential ability of HLA $B*5701^+$ long-term nonprogressors and progressors to restrict human immunodeficiency virus replication is not caused by loss of recognition of autologous viral gag sequences. J. Virol. **77**:6889–6898.
27. **Migueles SA, et al.** 2002. HIV-specific $CD8^+$ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nat. Immunol. **3**:1061–1068.
28. **Migueles SA, et al.** 2008. Lytic granule loading of $CD8^+$ T cells is required for HIV-infected cell elimination associated with immune control. Immunity **29**:1009–1021.
29. **Migueles SA, et al.** 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc. Natl. Acad. Sci. U. S. A. **97**:2709–2714.
30. **Migueles SA, et al.** 2009. Defective human immunodeficiency virus-specific $CD8^+$ T-cell polyfunctionality, proliferation, and cytotoxicity are not restored by antiretroviral therapy. J. Virol. **83**:11876–11889.
31. **Miura T, et al.** 2009. HLA-associated viral mutations are common in human immunodeficiency virus type 1 elite controllers. J. Virol. **83**:3407–3412.
32. **Papagno L, et al.** 2002. Comparison between HIV- and CMV-specific T cell responses in long-term HIV infected donors. Clin. Exp. Immunol. **130**:509–517.
33. **Pereyra F, et al.** 2008. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. J. Infect. Dis. **197**:563–571.
34. **Price DA, et al.** 2009. Public clonotype usage identifies protective Gag-specific $CD8^+$ T cell responses in SIV infection. J. Exp. Med. **206**:923–936.
35. **Price DA, et al.** 2005. Avidity for antigen shapes clonal dominance in $CD8^+$ T cell populations specific for persistent DNA viruses. J. Exp. Med. **202**:1349–1361.
36. **Quigley MF, Almeida JR, Price DA, Douek DC.** 2011. Unbiased molecular analysis of T cell receptor expression using template-switch anchored RT-PCR, p 10.33.1–10.33.16. *In* Coligan JE, et al (ed), Current protocols in immunology. John Wiley and Sons, New York, NY.
37. **Saez-Cirion A, et al.** 2007. HIV controllers exhibit potent $CD8^+$ T cell capacity to suppress HIV infection *ex vivo* and peculiar cytotoxic T lymphocyte activation phenotype. Proc. Natl. Acad. Sci. U. S. A. **104**:6776–6781.
38. **Simons BC, et al.** 2008. Despite biased TRBV gene usage against a dominant HLA B57-restricted epitope, TCR diversity can provide recognition of circulating epitope variants. J. Immunol. **181**:5137–5146.
39. **Turner SJ, Doherty PC, McCluskey J, Rossjohn J.** 2006. Structural determinants of T-cell receptor bias in immunity. Nat. Rev. Immunol. **6**:883–894.
40. **van Bockel DJ, et al.** 2011. Persistent survival of prevalent clonotypes within an immunodominant HIV gag-specific $CD8^+$ T cell response. J. Immunol. **186**:359–371.
41. **Venturi V, Kedzierska K, Turner SJ, Doherty PC, Davenport MP.** 2007. Methods for comparing the diversity of samples of the T cell receptor repertoire. J. Immunol. Methods **321**:182–195.
42. **Venturi V, et al.** 2011. A mechanism for TCR sharing between T cell subsets and individuals revealed by pyrosequencing. J. Immunol. **186**:4285–4294.
43. **Yant LJ, et al.** 2006. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with control of simian immunodeficiency virus SIVmac239 replication. J. Virol. **80**:5074–5077.
44. **Yu XG, et al.** 2007. Mutually exclusive T-cell receptor induction and differential susceptibility to human immunodeficiency virus type 1 mutational escape associated with a two-amino-acid difference between HLA class I subtypes. J. Virol. **81**:1619–1631.