Research Article

Clonotypic architecture of a Gag-specific CD8+ T-cell response in chronic human HIV-2 infection

Eirini Moysi1, Samuel Darko*2, Ester Gea-Mallorquí*3, Constantinos Petrovas1, Jorge R. Almeida2, David Wolinsky2, Yanchun Peng4, Assan Jaye5, Guillaume Stewart-Jones6, Daniel C. Douek2, Richard A. Koup7, Tao Dong*4 and Sarah Rowland-Jones*3

1 Tissue Analysis Core, Vaccine Research Centre, Bethesda, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA
2 Human Immunology Section, Vaccine Research Center, NIAID, NIH, Bethesda, MD, USA
3 Viral Immunology Unit, Nuffield Department of Medicine, Oxford, United Kingdom
4 MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom
5 MRC Laboratories, The Gambia, West Africa
6 Structural Biology Section, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
7 Immunology Laboratory, Vaccine Research Center, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA

The dynamics of T-cell receptor (TCR) selection in chronic HIV-1 infection, and its association with clinical outcome, is well documented for an array of MHC-peptide complexes and disease stages. However, the factors that may contribute to the selection and expansion of CD8+ T-cells in chronic HIV-2 infection, especially at the clonal level remain unclear. To address this question, we undertook a detailed molecular characterization of the clonotypic architecture of a HLA-B*3501 restricted Gag-specific CD8+ T-cell response in donors chronically infected with HIV-2 using a combination of flow cytometry, tetramer-specific CD8+ TCR clonotyping, and in vitro assays. We show that the response to the NY9 epitope is hierarchical and narrow in terms of T-cell receptor-alpha (TCRA) and -beta (TCRB) gene usage yet clonotypically diverse. Furthermore, clonotypic dominance in shared origin CTL clones was associated with a greater magnitude of cytokine production and antigen sensitivity at limiting antigen dilution as well as enhanced cross-reactivity for known HIV-2 variants. Hence, our data suggest that effector mobilization and expansion in human chronic HIV-2 infection may be linked to the qualitative features of specific CD8+ T-cell clonotypes, which could have implications for viral control and disease outcome.

Keywords: CTL · Gag · HIV-2 · TCR repertoire

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Correspondence: Prof. Sarah Rowland-Jones
e-mail: sarah.rowland-jones@ndm.ox.ac.uk

*Samuel Darko, Ester Gea-Mallorquí, Tao Dong, and Sarah Rowland-Jones contributed equally to the work.
Introduction

HIV-2 disease differs from HIV-1 in terms of length of viral incubation and clinical outcome. Whereas HIV-1 infection progresses to AIDS within 5–10 years from seroconversion [1], infection with HIV-2 gives rise to a chronic disease characterized by significantly longer disease-free survival times [2–4]. Several explanations have been put forth to account for this disparity including differences in viral dynamics during the asymptomatic phase of infection [5], capsid differences between pathological and non-pathological HIV-2 strains [6], and differences in the magnitude of host responses. More specifically, at high CD4+ T-cell counts (>500 cells/μL), individuals infected with HIV-2 were found to harbor strong HIV-2-specific CD4+ T-cell responses [22], more polyfunctional HIV-specific T-cells [23], and high levels of NK cytotoxicity [9].

CD8+ T-cells are a critical component of the antiviral response against HIV-1. Their depletion in nonhuman primate models of HIV infection results in rapid and marked increases in viremia [10] and in humans, CTL responses in early infection are temporally associated with virus clearance [11]. In particular, responses directed against conserved structural proteins, such as Gag, strongly correlate with viremia reductions [12–16] and escape from such CD8+ T-cell responses [17,18], functional exhaustion of Gag-specific T cells, and concomitant upregulation of inhibitory receptors, [19,20] predicts disease progression [21]. In some studies, better outcomes have been linked to the selection of CD8 T-cells bearing specific receptors (TCRs) that are shared among multiple individuals [22–24]. Such TCRs, coined “public” have identical T-cell receptor beta chains (TCRB) or T-cell receptor beta variable region (TRBV) amino acid (aa) sequences [25]. However, the exact role of these “public” TCRs remains unclear as their presence is associated both with an increased control of viral replication [22,24] as well as viral escape [26]. Much less is known about the clonotypic characteristics of mobilized CD8 T-cells in HIV-2 infection. To address this knowledge gap, we undertook a detailed characterization of the clonotypic architecture of an HLA-B*35 restricted Gag-specific CD8+ T-cell response (HIV-2 Gag epitope NPVPVGNIY or NY9). We chose to study HLA-B*3501 because it is an allele with good representation in our population of interest (~12% in West Africa; 18.6% in the studied cohort) [27,28]. We dissected the pattern of TCRA and TCRB family mobilization, measured the extent of CDR3 clonotypic diversity and determined the pattern of cytokine production, antigen sensitivity, as well as cross-reactivity of shared origin CTL clones in the NY9-specific CD8+ T-cell pool as a function of their relative frequency. Our data suggest that effector mobilization and expansion in chronic HIV-2 infection is consistent with epitope-based selection and may be linked to the qualitative features of Gag-specific CD8+ T cells, with implications for viral control.

Results

NY9-specific clonotypes exhibit preferential TCR gene segment usage and hierarchical expansion

TCR specificities making up the B*35-restricted NY9-specific response were dissected by TCR clonotyping of antigen-specific T cells isolated directly ex vivo from HIV-2-infected individuals (n = 7). We used an anchored RT-PCR step to amplify all TCRA and TCRB CDR3 chains present in the sorted populations, without the bias and incomplete coverage arising from methodologies employing TRAV-/TRBV-specific PCR primers [29]. Study participants were of the Manjako ethnic group, carriers of the HLA-B*3501 allele, HIV-2 infected, Anti-retroviral treatment naive (ART)-naïve, had detectable IFN-γ ELISPOT responses to the HIV-2 gag epitope NPVPVGNIY (NY9) and viral loads that were either low (<700 copies/μL) or at the limit of detection. At the time of blood sampling, donors had been seropositive for at least 9 years (Fig. 1A). The size of NY9-responses, as revealed by tetramer staining, ranged from 0.1 to 1.32% of the total CD8+ T-cell population (Fig. 1B). All donors were AIDS free at sampling as determined by their CD4% (Fig. 1C). A total of 40 TCRA and 39 TCRB clonotypes were identified (Table 1) and in six out of seven HIV-2 donors (86%) TRAV and TRBV expansions were highly oligoclonal in terms of gene segment usage (Fig. 2A and B). The number of individual families mobilized in response to NY9:B*3501 presentation in these highly oligoclonal samples ranged between 2 and 7 for TRAV and 1 and 5 for TRBV out of 46 and 48 total possible functional families reported in the IMGT database, respectively [30,31]. The most frequently mobilized TRAV families were TRAV12-3 (four of seven donors) followed by TRAV8-6, TRAV12-2, TRAV13-2, and TRAV29 (all detected in three of seven donors) (Fig. 3A). TRBV family mobilization was equally narrow. TRBV7-9 and TRBV29-1 were the most frequently mobilized families among all donors (four of seven donors) followed by TRBV7-2, TRBV5.1, and TRBV19 (both detected in three of seven donors) (Fig. 3B). Furthermore, within each donor the clonotypic structure was found to be highly hierarchical and dominated principally by one to two TCR specificities. This was true for both TCRA as well as TCRB chains (Table 1). Chains with the highest frequency in each sample were defined as dominant. These represented, on average, 46% of total alpha chains detected (SD ± 20.6%) and 53% of total beta chains (SD ± 27.4%). Whenever top representation was shared among two different TCRA or TCRB chains (<10% difference in frequency), these were considered codominant. Thus, expansion of NY9-specific CD8+ T-cells in the donors under study was associated with TCR repertoire narrowing and hierarchical dominance of specific TCR clonotypes.
Table 1. TCR usage in the NY9-specific response is dominated by private TCRs

| ID  | TRAV       | CDR3               | TRAJ | Freq. | ID  | TRBV   | CDR3               | TRBJ | Freq. |
|-----|------------|--------------------|------|-------|-----|--------|--------------------|------|-------|
| 1   | 12-2       | CAVQLFSDGQKLFF    | 16-1 | 42    | 1   | 5-1    | CASSSLGGSEAFF      | 1-1  | 54    |
|     | 8-6        | CAVSDA GNTGKLFIF  | 37-1 | 18    | 29-1| CSGVGYNTEAFF      | 1-1  | 24    |
|     | 6          | CALDMGMLTF        | 44-1 | 11    | 7-2 | CASSPVGGADGYTF    | 1-2  | 1     |
|     | 1-2        | CAVDTGTAKLTF      | 44-1 | 3     | 19  | CASSQGNNQPQHFF    | 1-5  | 1     |
|     | 12-2       | CAAQLFSDGQKLFF    | 16-1 | 1     | 29-1| CSVEDAPGRADTQHF   | 2-3  | 3     |
|     | **Seqs:**  | **75**            |      |       |     |        |                    |      |       |
| 2   | 12-3       | CAMDTGTAKLTF      | 44-1 | 23    | 2   | 7-2    | CASSLSPGWSEAFF     | 1-1  | 82    |
|     | 4          | CLVGEGLSGNTPLVF   | 29-1 | 20    | 1   | 6-1    | CASSEDVPGNEQFF     | 2-1  | 4     |
|     | 12-2       | CAVYNARLMTF       | 31-1 | 19    | 7-2 | CASSLDGKSYEQYF    | 2-7  | 1     |
|     | 26-1       | CIIVRESGGSNYKLTF  | 53-1 | 16    | 7-9 | CASSSYQGAGTEAFF   | 1-1  | 1     |
|     | 29          | CAASSQGGYEKLIF    | 57-1 | 2     |     |        |                    |      |       |
|     | 4          | CLVGEGRSGNTPLVF   | 29-1 | 1     |     |        |                    |      |       |
|     | 13-2       | CAERNNANLMTF      | 39-1 | 1     |     |        |                    |      |       |
|     | **Seqs:**  | **82**            |      |       |     |        |                    |      |       |
| 3   | 29          | CAAASEQGGSSEKFVLF| 57-1 | 58    | 3   | 5-1    | CASTFEAGGYPNEQFF   | 2-1  | 47    |
|     | 12-1       | CVVNFETGFGKTIF    | 9-01 | 13    | 7-9 | CASSLYQGAATEGF    | 1-1  | 37    |
|     | 12-3       | CAVSMTYNTDKLFIF   | 34-1 | 11    | 19  | CASSRQEFAGNEQF    | 2-1  | 2     |
|     | 8          | CAVANAGAFQKLFIF   | 8-1  | 4     | 27  | CASSLTYDAGNTIYF   | 1-3  | 2     |
|     | 12-1       | CVANFETGFGKTIF    | 9-01 | 1     |     |        |                    |      |       |
|     | **Seqs:**  | **87**            |      |       |     |        |                    |      |       |
| 4   | 12-3       | CAMDTGTAKLTF      | 44-1 | 74    | 4   | 7-2    | CASSLSPGWNEQFF     | 2-1  | 41    |
|     | 8-6        | CAVSDRSITGLYLF    | 18-1 | 2     | 29-1| CSVAWGQIMTEAFF    | 1-1  | 34    |
|     | 8-6        | CAVSDRSNTQKLFIF   | 37-1 | 3     | 29-1| CSGVGYNTEAFF      | 1-1  | 10    |
|     | 13-2       | CAENGGATNKLIF     | 32-1 | 2     | 10-2| CASSWSTSYNEQFF    | 2-1  | 2     |
|     |            |                    |      |       | 7-2 | CAGSLPGWNEQFF     | 2-1  | 1     |
|     | **Seqs:**  | **81**            |      |       |     |        |                    |      |       |
| 5   | 13-2       | CAVDCGDTGGFTIF    | 9-1  | 58    | 5   | 27     | CASSFTIDSYEQYF     | 2-7  | 41    |
|     | 12-3       | CAVSMCPYNNNDMRF   | 43-1 | 11    | 11-2| CASSFSHNTAETF     | 1-1  | 32    |
|     | 10          | CVVSAMSSSYNQLIW   | 33-1 | 16    | 29-1| CAAAYGNTAETF      | 1-1  | 10    |
|     |            |                    |      |       | 10-3| CAINHAGDTQYF      | 2-3  | 1     |
|     |            |                    |      |       | 7-9 | CASSLYQGAACEQYF   | 2-7  | 2     |
|     | **Seqs:**  | **85**            |      |       |     |        |                    |      |       |
| 6   | 26-2       | CILREGYGGATNKLIF  | 32-1 | 16    | 6   | 6-5    | CASSYMSYEQYF       | 2-7  | 12    |
|     | 29          | CAAALLNNANMNLMFLF| 39-1 | 10    | 5   | 5-1    | CASSLEMGFQNYEQFF   | 2-1  | 11    |
|     | 8-1        | CAFYSAGASYQKTF    | 28-1 | 8     | 4-1 | CASSQGTDTGKKLF    | 1-4  | 9     |
|     | 30          | CGTVGSSGYSIPTF    | 6-1  | 8     | 4-1 | CASSQAEQGWWGEQYF  | 2-7  | 8     |
|     | 38-2       | CAYRSDSWGKLFQ     | 24-2 | 5     | 19  | CASSMGGQYNQFF     | 2-1  | 7     |
|     | 38-2       | CAYRRDGTSKLFIF    | 44-1 | 5     | 16  | CASSPAAGMRNEQF    | 2-7  | 6     |
|     | 8-6        | CALNTGGFKTFI      | 9-1  | 5     | 7-9 | CASSPRGHNNEQF     | 2-1  | 6     |
|     | 21          | CAVRNYTNGAKSTF    | 27-1 | 4     | 6-5 | CASKSRRGNTEAFF    | 1-1  | 5     |
|     | 13-1       | CAAHSWGKLFQ       | 24-2 | 4     | 20-1| CASMGPWGGSMGRTYNEQF| 2-1  | 4     |
|     | 12-2       | CAVNALLSDQGKLF    | 16-1 | 4     | 6-5 | CASSQGVDFEQYF     | 2-7  | 3     |
|     | 14          | CAMREGQAGNKLFIF   | 17-1 | 2     | 20-1| CAS'REGLYSNQPQHF  | 1-5  | 2     |
|     | 39          | CAVDRMDSYYKLIF    | 12-1 | 2     | 29-1| CSVGYRGTSFEAFF    | 1-1  | 2     |
|     | 25          | CAGIKAAANGKLFIF   | 17-1 | 1     | 11-2| CASSGLDRDNEKLF    | 1-4  | 1     |
|     | 12-1       | CVVNSGRSDDYKLSF   | 20-1 | 1     | 4-2 | CASSPOAGGSNTGELF  | 2-2  | 1     |
|     |            |                    |      |       | 7-6 | CASSISGPWDEHQEF    | 2-1  | 1     |
|     | **Seqs:**  | **74**            |      |       |     |        |                    |      |       |
| 7   | 13-1       | CAANRHDKVIF       | 50-1 | 53    | 7   | 19     | CASSPQHGDQYF       | 2-3  | 94    |
|     | 10          | CVVSAANLGTYKYIF   | 40-1 | 36    |     |        |                    |      |       |
|     | **Seqs:**  | **89**            |      |       |     |        |                    |      |       |

Seqs, number of sequences screened per sample; Freq, frequency of TCR clonotype in the sample under study. Public TCRA and TCRB are shown in red.
NY9-specific TCRs are mostly private, diverse, and display common TRAV and TRBV CDR3 motifs

Public T-cell responses, namely responses in which unrelated individuals share TCRs with identical V and J gene usage, have been previously shown to arise in the context of HIV-1 infection [22, 24, 32]. Structural explanations focusing on the shape of the peptide-MHC complex as well as sequence-based explanations, such as near-germline recombination of TCR gene segments and differences in the production frequency of TCRs (biased recombination), have been examined as likely explanations for this phenomenon [25]. Given the limited TCRA and TCRB family mobilization in our samples, we then asked if the observed oligoclonality could arise as a consequence of public TCR bias. To address this question, we examined the Vα-Jα and Vβ-Dβ-Jβ gene usage of detected TCRA and TCRB chains at the nucleotide as well as at the protein level. Our analysis revealed limited evidence for public TCR sharing restricted to one high-frequency TCRA (CAMDTG-TASKLTF; TRAV12-3/TRAJ44-1) and one TCRB chain (CSVGYGN-TEAFF; TRBV29-1/TRBJ1-1) (Fig. 2A and B and Table 1). These public chains were shared among three different individuals, with the sharing occurring pairwise between donors 2 and 4 for TCRA and donors 1 and 4 for TCRB. Next, we analyzed the nucleotide sequence lengths in private TCRs and found variable nucleotide (nt) additions (Fig. 3C and D). This ranged from 0 to 12 nt for TRA VC D R3 s and 0 to 27 nt for TRB V s C D R3 s (Fig. 3C and D). In line with the near-germline TCR hypothesis, the number of CDR3 nucleotide additions in the public TCRA chains was minimal (1 nt addition in a total 39 bp of CDR3 length). The number of nt additions for the shared TCRB chains, however, was greater (7 nt additions in a total 30 bp of CDR3 length) (individual data not shown). Hence, public TCRs did not appear to be the driving force for the observed oligoclonality in our samples. We also estimated the degree of TCR diversity in each sample using the Shannon Evenness Index which assumes values between 0 (monoclonality) and 1 (maximum diversity). We observed that despite being oligoclonal, NY9-specific TRAV and TRBV CDR3 segments were relatively diverse (Fig. 2A and B). When TCR diversity was
Figure 2. TCR usage of NY9-specific CD8+ T cells in HIV-2 infection, which is characterized by oligoclonal TCRA and TCRB gene distributions. Pie charts showing the distribution of (A) TCRA chains and (B) TCRB chains in the donors under study (n = 7) as defined by TCR clonotyping of associated CDR3 aa sequences from the cardinal Vα segment C (Cysteine) residue to the conserved Jα-segment F (Phenylalanine) residue for TRAV and from the cardinal Vβ segment C (Cysteine) residue to the conserved Jβ-segment F (Phenylalanine) residue for TRBV. Public sequences are shown in red text. Numbers displayed centrally (blue circles) denote the number of distinct TRAV or TRBV families mobilized in each donor whereas numbers on the right side of each chart denote the total number of sequences analyzed. ESI = Shannon entropy index. Colors are assigned randomly to the various TRAV or TRBV families based on the relative frequency of representation.

studied as a correlate of the magnitude of the NY9 response, a statistically significant inverse trend was observed for TCRA, with a lower tetramer magnitude correlating with higher variability (Spearman’s $r = -0.75$, $p = 0.0331$; Supporting information Fig. S1A). A similar trend was observed for TCRB, but the association did not reach statistical significance. Hence, despite an oligoclonal TRAV and TRBV gene segment usage mobilized NY9-specific TCR CDR3 sequences are relatively diverse.

Since TCR specificity for a particular pMHC complex can be bestowed by specific “anchor” residues in the alpha and beta...
Figure 3. NY9-specific CD8+ T cells show oligoclonal TRAC and TRBC expansions and variable numbers of CDR3 nucleotide additions in HIV-2 infection. Cumulative distribution of (A) TRAV and (B) TRBV gene usage in the seven donors under study. Each donor is represented by a different color. Most frequently used families are denoted by a red asterisk. (C) Bar graphs summarizing the CDR3 nucleotide (nt) addition lengths of all detected alpha and (D) beta chains obtained from bulk TCR sequencing and their corresponding frequencies in the donors under study.
CDR3 regions [33], we also examined if selection of particular clonotypes into the NY9 pool could be attributed to similarities in their aa motifs. Cluster analysis of CDR3 aa sequences revealed the presence of four CDR3 motif clusters among TCRA sequences (Fig. 4A and C) and two CDR3 clusters for TCRB (Fig. 4B and D). Hence, the presentation of the NY9-epitope in chronic HIV-2 infection in the context of the B*3501 allele favors the recruitment and expansion of TCR clonotypes with CDR3 TCR aa motifs consistent with MHC-restricted, epitope-based selection.

Mobilization and expansion of NY9 clonotypes is linked to their functional sensitivity

Highly avid, polyfunctional CD8+ T-cell clonotypes have been shown to preferentially expand in controlled HIV infection [34,35]. To determine if the dominance of particular NY9-specific CD8+ T-cell clonotypes in HIV-2 infection could stem, at least in part, from an advantageous functional profile tetramer-specific CD8+ T cells were sorted from available donors and CD8+ T-cell clones were established for functional characterization. We chose to investigate functionality at the clonal level as this approach allows high-resolution TCR sequence characterization and exact TCRA/TCRB pairing determination parallel to functional characterization. This is something that cannot be readily addressed ex-vivo by Vβ-staining. CTL clones that grew sufficiently and fulfilled the following criteria were included in our analyses: (i) purity of at least 98% as judged by tetramer staining; (ii) single Vβ clonality as judged by flow cytometry; (iii) single clonality as judged by TRAV and TRBV sequencing (fully resolved single chromatogram); and (iv) single clinical origin (same donor) (Supporting information Fig. S1B). A total of two distinct clonotypes (one dominant TRAV13.1/TRBV19 and one subdominant TRAV39/TRBV20.1) that fulfilled all these criteria were identified in a donor B35_7 and used to determine differences in cytotoxic degranulation potential as determined by CD107a upregulation, ability for IFN-γ, TNF-α, and IL-2 secretion as well as tetramer avidity at limiting antigen dilutions. Additional TCR clonotypes from donors 35_3 and 35_4, were also used to confirm our observations (Table 2). Characterization of the TRAV/TRBV sequences expressed by the two CTL clones from donor 35_7 revealed a sequence profile consistent with the bulk clonotyping results for the highly dominant clone (Supporting information Fig. S1B).

Of note, the NY9-specific CD8+ T-cell response in this donor was extremely oligoclonal, a finding confirmed both by bulk TCR clonotyping as well as in vitro screening (Table 1 and Supporting information Fig. S1B). At high peptide concentration (10 to 10^{-5} M) CD107a mobilization, IFN-γ secretion, as well as TNF-α secretion did not differ significantly between the two TCR clonotypes irrespective of how functionality was defined (mobilization of CD107a alone, mobilization of CD107a, and secretion of IFN-γ or mobilization of CD107a in combination with IFN-γ and TNF-α secretion) (gating strategy as shown in Supporting information Fig. S2A). A slightly higher ability for IL-2 production was recorded for the subdominant clone at 10 and 1 μM. However, this was not statistically significant (Fig. 5A and C). At limiting antigen dilutions (10^{-5} to 10^{-6} M), however, the two profiles diverged and antigen-specific secretion of IFN-γ and TNF-α ceased faster in the subdominant clone. Since high-avidity CD8+ T cells bind preferentially under conditions of limiting tetramer availability [36], we also employed a tetramer dilution assay to measure the functional avidity. Consistent with the intracellular cytokine staining results, the dominant TRAV13.1/TRBV19 TCR retained its ability to bind the NY9: B*3501 complex at lower concentrations (Fig. 5B). To confirm that our findings were not biased due to in vitro culture conditions, additional available pure TRAV13.1/TRBV19 lines were also tested to account for any CTL line-specific variation. Assessment of these additional TRAV13.1/TRBV19 CD8+ T-cell clones (n = 3) revealed a consistent profile with statistically significant differences between the dominant clones and subdominant CTL clone for tetramer avidity (p = 0.0090), CD107a mobilization (p = 0.0096), IFN-γ secretion (p = 0.0005), as well as TNF-α secretion (p = 0.0057) (Fig. 5C). To ensure reproducibility, TCR clonotypes recovered from donors B35_3 and B35_4, were also examined (Fig. 5D, Table 2, Supporting information Fig. S2B). These represented dominant/subdominant pairs with TCRB representation frequencies in the NY9-specific CD8+ T-cell pool either at the boundary of subdominance as in the case of donor 35_3 (D: TRAV8-1/TRBV5-1: 47%; S: TRAV29/TRBV7-9: 37%) or differing >40% as in the case of donor 35_4 (D: TRAV12-3/TRBV7-2: 41%, S: TRAV24/TRBV4-3: <1%) (Table 2). Dominance was defined based on the individual TCRB chain expressed throughout as the latter was deemed an indicator less amenable to variation compared to TCR-alpha chains, It has been estimated that approximately 10% of human αβ T cells can express dual

| ID   | Designation | TRAV | CDR3 sequence (aa)   | TRAJ | TRBV | CDR3 sequence (aa) | TRBJ | TRBV Freq <sup>a</sup> |
|------|-------------|------|----------------------|------|------|---------------------|------|------------------------|
| B35_3 Dominant | 8-1 | C A V N A N A G F Q J L V F | 8-1 | 5-1 | C A S T F E A G G P Y N E Q F F | 2-1 | 47         |
| Subdominant | 29 | C A S E Q G G S E K L V F | 57-1 | 7-9 | C A S S L Y Q G A G T E A F | 1-1 | 37         |
| B35_4 Dominant | 12-3 | C A M D T G T A S K L T F | 44-1 | 7-2 | C A S S L S P G W N E Q F F | 2-1 | 82         |
| Subdominant | 24 | C A S G Y Q N Q F V F | 26-1 | 4-3 | C A S S E T G G Y S P L H F | 1-6 | <1        |
| B35_7 Dominant | 13-1 | C A A N R H D K V I F | 50-1 | 19 | C A S S P H Q H D T Q Y F | 2-3 | 94         |
| Subdominant | 39 | C A V D F G G T S Y G K L | 52-1 | 20-1 | C A S A N Q G N G G E L F F | 2-2 | <1        |

<sup>a</sup> As determined by bulk TCR clonotyping analysis.
Figure 4. Aa motifs in TCRA and TCRB CDR3s regions of expanded TCR clonotypes. (A) Shared TRAC aa motifs in the seven donors studied as determined by multiple alignments of CDR3 amino-acid sequences. (B) TCRB aa sharing as determined by multiple alignments of CDR3 amino-acid sequences. Both (A) and (B) motifs are denoted as clusters. Common shared motifs are denoted by red squares whereas identical “public” sequences by a blue square. (C) CDR3 WebLogo representations of TRAC CDR3 clusters and (D) TCRB CDR3 clusters. Coloring represents the chemical properties of each aa: polar = green; neutral = purple; basic = blue; acidic = red and hydrophobic = black. Tables show the aa sequences corresponding to each cluster, their TRAV and TRAJ usage as well as their relative frequency (count) in the intradonor pool of NY9-specific clonotypes.
Figure 5. Antigen sensitivity and tetramer avidity profiles of dominant and subdominant NY9-specific TCR clonotype pairs. (A) Representative example of functional sensitivities displayed by NY9-specific CD8+ T-cell clones expressing the TRAV13.1/TRBV19 and TRAV39/TRBV20.1 TCRs as determined by intracellular staining (ICS) in experimental replicates. Data shown are from one TRAV13.1/TRBV19 and one TRAV39/TRBV20.1 clonotype. Each pie chart represents the background adjusted polyfunctional profile of the CTL clone as determined by the combined expression of CD107a+, IFN-γ, TNF-α, and IL-2.

© 2021 The Authors. European Journal of Immunology published by Wiley-VCH GmbH www.eji-journal.eu
TCRA chains on their surface [37]. Analysis of these additional pairs revealed similar functional trends to those observed for donor 35_7 (Fig. 5D). Differences in functional sensitivity were more pronounced for TCR clonotypes whose frequencies corresponded to higher representation skewing in the CD8+ T-cell pool (>-40%) (Supporting information Fig. S2B). Taken together, our results suggest that CD8+ T-cell clonotypes that are preferentially expanded in the NY9 pool may be endowed with better functional avidity and antigen sensitivity at limiting antigen dilutions.

**Dominant NY9-specific CD8+ T-cell clonotypes cross-recognize variants with higher functional avidity**

Viral control in HIV-1 infection has previously been linked to the dominant selection of TCR clonotypes with increased flexibility and, thus, potential for variant cross-recognition [38]. We therefore asked if the dominant hierarchy observed in our samples could also arise in part from an increased cross-reactive potential. To test this hypothesis, the generated clonotypes from donor 35_7, were assessed for their ability to recognize known HIV-2 variants including the common NPVPVGSIIV variant. We found that the dominant TRAV13.1/TRBV19 TCR had a superior cross-recognition capacity in 51Cr release assays compared with the subdominant TRAV39/TRBV20.1 TCR (six of six vs two of six variants tested) (Fig. 6A and B). The difference in cross-recognition between the two clonotypes was especially true for the more common NPVPVGSIIV variant. To ensure replication of our findings, we also extended our analysis to the TCR clonotypes recovered from donors 35_3 and 35_4. Our analysis revealed similar patterns of cross-reactivity to those found in donor 35_7 for four of six variants tested (index NPVPVGNIY, NPIPVGNIY, NPVPVGSI, and NPVPVRNIY), in particular for donor B35_4, whose dominant and subdominant TCR clonotype representation frequencies were more closely matched to those of donor 35_7 (Fig. 6B, Table 2, Supporting information Fig. S3A). Taken together, our results suggest that expanded CD8+ T-cell clonotypes in the NY9 pool may be endowed with increased TCR flexibility, especially at limiting antigen concentrations.

**Discussion**

CD8+ T-cell response breadth and magnitude do not necessarily predict the outcome of HIV infection. Hence, in the quest for protective correlates the qualitative attributes of an immunological response have emerged as potential drivers of virological control during the chronic stage of infection [39]. Previous studies in our lab have shown that targeting of the viral protein Gag in donors chronically infected with HIV-2 inversely correlates with viral load [15]. Furthermore, our lab has shown that Gag-specific CD8+ T-cells in HIV-2 infection display an early-differentiated phenotype with limited TCRB family mobilization [35]. In this study, we explored the clonotypic nature of a Gag-specific CD8+ T-cell response by performing a detailed molecular characterization of expanded clonotypes. We studied their TCRA/TCRB CDR3 usage and corresponding functionality in donors chronically infected with HIV-2 using assessments of avidity (strength of TCR: pMHC interaction), sensitivity (ability of a T-cell to respond to a certain amount of cognate antigen), and cross-reactivity paired with cytotoxicity. We show that in donors who remain asymptomatic for a median of 14 years in the absence of antiretroviral therapy, the response to a Gag epitope is focused and “private” in nature. “Public” TCRs have been shown to dominate responses toward immunodominant epitopes in a number of chronic viral infections including HIV-1, EBV, CMV, and HSV-2 [22, 40–43]. However, the relative contribution of these “public” TCRs to viral control remains debatable with some studies associating their presence with a beneficial outcome [24] and others with an increased probability for viral escape [26]. Our results suggest that in the case of the NY9-response, and in the context of undetectable HIV-2 plasma viremia, CD8+ T-cell recruitment into the antigen-specific pool is driven by structural constraints, as exemplified by the presence of shared aa motifs and the diverse, “private” CDR3 profiles observed. These findings are in line with studies reporting an increased CD8+ T-cell clonal diversity in HIV-2 infection compared with HIV-1 [44]. However, earlier studies did not comprehensively address the clonality of tetramer-specific CD8+ T-cells at the molecular level or corresponding TRAV CDR3 sequences. Conversely, the detection of a limited number of public clonotypes (one TCRA and one TCRB) in our samples could mean that public TCRs once existed in our donors but became extinct over time due to exhaustion and/or clonal turnover [34]. Due to the cross-sectional nature of our study, however, confirmation of the latter hypothesis was not possible. Further studies exploring HIV-2 TCR selection in longitudinal samples are therefore warranted.

In keeping with earlier work in the field [7, 12, 27], our data also indicate that recruited NY9-specific CD8+ T cells are highly functional and able to mobilize CD107α and secrete IFN-γ,
Figure 6. Dominance is associated with a better capacity for HIV-2 variant cross-recognition. (A) The ability of TRAV13.1/TRBV19 and TRAV39/TRBV20-1 CTL clones to recognize targets (BCLs) exogenously loaded with decreasing amounts of the index NY9 epitope as well as common NY9 variants was measured in standard 4 h ⁵¹Cr release assays. The assays were performed on day 11 postrestimulation. A 5:1 E:T ratio was used. Data represent averages from three different experiments. Error bars are means (±SD). (B) Cross-reactivity EC₅₀ for all TCR clonotype pairs examined. Each cultured symbol represents the average experimental replicates for a given TCR clone (clonal pairs as shown in Table 2) while different colors denote individual donors. Adjusting for nonspecific background effects, cross-reactivity was considered present when specific lysis was >20% (cut-off). Missing data points denote complete absence of cross-reactivity or cross-reactivities for which an EC₅₀ value could not be calculated (<2 data points).
TNF-α—and to a lesser extend IL-2—in response to antigenic stimulation. Single origin CTL clonotypes displayed comparable functional avidities and sensitivities at peptide concentrations >0.1μg/mL. However, when antigen concentration became limiting, differences did emerge among the different clonotypes studied. More specifically, clonotypes represented at higher frequencies in the NY9-pool (dominant) had higher functional avidities for the NY9 epitope and more sustained cytokine production at low peptide concentrations compared with low frequency ones (subdominant). In addition, high-frequency clonotypes displayed broader cross-recognition of HIV-2 variants. It has been proposed that highly avid CTLs might be better at limiting virus in both the early and chronic stages of infection [34, 45–47]. Nevertheless, a high functional or structural avidity alone does not necessarily always translate into a better antiviral efficacy. [48]. For example, Mamu-A*01 macaques vaccinated with SIVmac239 Gag and challenged with SIVsmE660, fail to control viral replication in vivo despite a high frequency of highly avid GagCM9-specific CD8+ T cells [49]. One explanation could be that highly avid TCR interactions may decrease functional responsiveness by triggering an upregulation of inhibitory receptors [50] and an increased rate of apoptosis [51,52]. However, when clonotypic functionality was assessed in our samples, no impairment was seen among highly avid clonotypes. Hence, our results reiterate that high sensitivity is an important element of an effective antiviral response in HIV-2 infection, especially when accompanied by polyfunctionality, which has also been associated with HIV control [53]. Cross-reactivity for homologous and heterologous antigens has also been associated with better outcomes in HIV-1 infection. In a study by Mothe et al., HIV-1 controllers were shown to raise Gag p24 responses that were characterized by an increased cross-reactivity for epitope variants compared to noncontrollers [54]. In addition, earlier studies in our lab had implicated the GagNY9 epitope in cross-reactive responses [55]. In this study, we found that clonotypes expressing dominant TCRs, such as TRAV13.1/TRBV19 TCR, possessed an increased capacity for variant recognition at limiting antigen dilutions. It is plausible that this family is inherently more cross-reactive, as “public” and “private” TRBV19 (Vβ17) receptors have been implicated in homologous and heterologous cross-reactive responses in infections as diverse as EBV, HIV-1, and influenza A [32, 54,56]. The fact that lysis of the target cells occurred at low E/C0 values and in a concentration-dependent manner further supports a potential physiological significance of this cross-reactivity. However, a previous study by Jennes et al., indicated that such cross-reactive responses have a low overall frequency in vivo and, therefore, their true contribution to natural cross-protection may be lower than that estimated in vitro [57]. Even so, the detection of HIV-2-specific CD8+ T cells bearing TCRs with a potential for cross-recognition warrants further attention and study as such CD8+ T cells may have a greater potential to limit escape variants within HIV-2 hosts before selective fixation, especially at distal sites of active replication such as lymphoid sites.

There are however some limitations in our study including the small number of HLA-B*3501 donors available, their virological profiles (asymptomatic individuals with low/undetectable plasma viral load) that made viral sequencing difficult and the limited number of single origin “pure” dominant/subdominant clones that could be established in the context of such highly oligoclonal and hierarchical responses. It should also be noted that all donors used for the generation of clonotypes, for which plasma was available for sequencing (B35_4, B35_7), harbored the WT sequence of the virus in their plasma, and no variation was detected among them when sequencing was performed (Supporting information Fig. S3B). Therefore, in this cross-sectional study it was not possible to examine the direct contribution of established TCR clonotypes to viral control. Still, in the light of the previous association of Gag responses in HIV-2 infection with lower viremia [12,15] and given the favorable functional profile of the detected clones, such a contribution does not seem unlikely. Further studies adequately powered to address such questions are therefore warranted.

In conclusion, we have performed a detailed analysis of the clonotypic nature of a Gag-restricted CD8+ T-cell response in HIV-2 infection that has enabled the identification of distinct features associated with the selection and expansion of Gag-specific TCR clonotypes in HIV-2 infection. Our findings offer insights into some of the qualitative parameters that may, potentially, contribute to viral control and set a roadmap for future studies exploring viral control in HIV-2 infection.

Materials and methods

Study participants

Study participants were recruited from the Caio community-cohort in Guinea-Bissau. They were of the Manjako ethnic group, carriers of the HLA-B*3501 allele, HIV-2 infected, ART-naive, and had detectable IFN-γ ELISpot responses to the HIV-2 gag epitope NPVPGNIY (NY9). Ethical approval for the study was obtained from the Gambia Government/MRC Laboratories Joint Ethics Committee, the Ministry of Health of Guinea-Bissau, the Oxford Tropical Research Ethics Committee, and Oxford Research Ethics Committee.

Peripheral blood mononuclear cell (PBMC) isolation

Peripheral blood was collected by venipuncture into EDTA-containing vacutainer tubes (BD Biosciences) and PBMCs were separated using density centrifugation over Ficoll–Hypaque (Lymphoprep; Nycomed Pharma AS, Oslo, Norway). Cells were resuspended in freezing medium (FCS+10%DMSO) and cryopreserved until use.

HLA-peptide tetrameric complexes

HLA-B’3501 MHC class I heavy chains were amplified using PCR from cDNA isolated from B’3501-positive subjects using
HLA-B-specific primers with a substitution of the transmembrane and cytosolic regions of MHC class I molecule and a recognition site for BirA biotinylation enzyme as described previously [58]. The modified heavy chains and β2-microglobulin were cloned into a PGMT-7 vector and expressed in BL21(DE3) ployS competent cells (Invitrogen). Monomeric proteins were purified from bacterial inclusion bodies, solubilized in 4 M urea, and refolded in the presence of the Gag NY9 epitope (NPVPVNIY). Refolded monomeric complexes were purified by Fast Protein Liquid Chromatography, biotinylated with BirA (Avidity, CO), and conjugated at a 4:1 molar ratio with PE-labeled extravidin (Sigma-Aldrich) to form tetrameric HLA-peptide complexes (“tetramers”). Tetramers were titrated against appropriate CTL clones prior to use to determine the optimum concentration for maximal staining.

**CD8+ T-cell clone establishment**

Clones were generated from PBMCs by peptide-HLA tetramer sorting and limiting dilution as previously described [59]. Briefly, thawed PBMCs were stained with PE-conjugated NY9 tetramer and CD8-APC (BD Bioscience, clone SK1) and gated NY9+CD8+ T cells were collected and seeded in a 96-well plate at a concentration of 1 cell per well in the presence of 0.2 × 10^6 mixed allogeneic irradiated PBMCs and 0.5 μg/mL PHA. Cloning plates were incubated at 37°C. After 10 days, wells with substantial growth were expanded in 24-well plates using the cloning mix above supplemented by IL-2 (200 U/mL). CTL clonality was confirmed by Vβ-antibody staining as well as by PCR and sequencing as previously described [22]. CTL clones with two or more TRBV populations or with chromatograms that could not be fully resolved in the analysis were characterized “nonclonal” and were excluded from subsequent studies. Assays were performed on day 10 or 11 for optimal performance [60].

**TCR clonotyping**

TCR clonotyping was performed on sorted CD8+ T cells as previously described [61]. Briefly, PBMCs were thawed and stained with the live-dead stain ViViD (molecular probes) as well as with CD8-QD705 (clone 3B5) or CD8-PB (RPA-T8), CD3-H7APC (clone SK7), IFN-γ-PB or IFN-γ-FITC (clone B27), TNFα-Cy7PE or TNFα-APC (clone MAb11), IL2-APC, IL-2 PE or IL-2-BV421 (clone MQ1-17H12), and CD19-BV421 (clone HIB19).

**Antibodies**

The following fluorophore-conjugated monoclonal antibodies were used for immune phenotyping: CD107a-Ahx680, CD107a-APC or CD107-PE (clone H4A3), TCRβv-FITC (IoTest, Beckman Coulter), CD8-QD705 (clone 3B5) or CD8-PE (RPA-T8), CD3-H7APC (clone SK7), IFN-γ-PB or IFN-γ-FITC (clone B27), TNFα-Cy7PE or TNFα-APC (clone MAb11), IL2-APC, IL-2 PE or IL-2-BV421 (clone MQ1-17H12), and CD19-BV421 (clone HIB19).

**Surface and intracellular staining (ICS)**

CD8+ T-cell clones in CTL sensitivity assays were stimulated with 0.5 × 10^5 autologous B cells previously pulsed with 10-fold dilutions of NY9 peptide starting at 10 μg/mL for 6 h at 37°C in the presence of 1 μL GolgiSTOP (Monesens; BD Biosciences), 1 μL GolgiPLUG (Brefeldin A; BD Biosciences) and anti-CD107α antibody to capture surface mobilized events real-time as previously described [62]. *Staphylococcus enterotoxin* B (10 μg/mL) and PBS (no stimulation) controls were also included in this assay. At the end of the incubation, cells were stained with Aqua (Invitrogen) and CD8, permeabilized using Cytofix/Cytoperm (BD Biosciences), washed and stained intracellularly with antibodies against IFN-γ, TNF-α, and IL-2. At the end of the assays, cells were fixed with 1% paraformaldehyde and events were acquired either on a DAKO Cyan flow cytometer (Beckman Coulter) with the program Summit, on an LSRII flow cytometer or a BD Fortessa X20 using BD FacsDiva software. Data were analyzed using Flow Jo (Treestar Inc). All flow cytometry experiments were performed adhering to the “Guidelines for the use of flow cytometry and cell sorting in immunological studies” [63].

**TCR avidity assay**

pMHC/TCR avidity was measured by incubating CD8+ T-cell clones (10^5 cells) with serial twofold dilutions of PE-conjugated NY9 tetramer starting at a final concentration of 10 μg/mL. Stains were performed for 15 min at 37°C after which time cells were washed and stained with CD8. A non-NY9-specific CD8+ T-cell clone, as well as an irrelevant tetramer (B*07 RPM) were also used in the assay as negative controls for background (nonspecific) staining. After staining, cells were fixed using 2% paraformaldehyde and events were acquired on a CyAn Flow Cytometer (Beckman–Coulter) using the program Summit. The analysis of the results was carried out with the program FlowJo (Treestar Inc) and gating was based on the negative control.
**51Cr release assay**

CTL clone cytotoxicity was assessed using standard 51Cr release assays as previously described [22]. Briefly, autologous 51Cr-labelled BCLs were pulsed with peptides (10 μg/mL final concentration) or appropriate titrations of peptides (range 1.28 × 10⁻⁴ to 10 μM) before being added to CTL clones at a 5:1 effector-to-target ratio for 4 h at 37°C. The following HIV-2 peptides were used in the assay: NPVPVGNIY, NPVPVNIY, NPVPVSGIY, NPVPVRNIY, NPVPVRNIY, and SPIPVGNIY. Selected peptides represented all known major variants of the NY9 sequence at the time. Well controls were also included in the assay and comprised target cells in R10 for spontaneous background 51Cr release as well as target cells in 5% Triton-X for maximal 51Cr release. After incubation, plates were read using a Beta counter (PerkinElmer). Assays were performed in duplicate and background 51Cr release was always less than 20%. The percent lysis was calculated from the formula (E-M/T-M) × 100, where E is the experimental release, M is the spontaneous release in the presence of R10 medium, and T is the release in the presence of 5% Triton X.

**Killing assay**

Alternatively, CTL clone cytotoxicity was assessed by coculture of B-cell lines pulsed with the corresponding peptide. Briefly, autologous CFSE-labeled BCLs were pulsed with the corresponding peptides (10 μg/mL final concentration) and appropriate titrations of peptides (range 1.28 × 10⁻⁴ to 10 μM) for 1 h before being added to CTL clones at a 5:1 effector-to-target ratio for 4 h at 37°C. The following HIV-2 peptides were used in the assay: NPVPVGNIY, NPVPVNIY, NPVPVSGIY, NPVPVRNIY, NPVPVRNIY, and SPIPVGNIY. After incubation, cells were stained for CD19-BV421 (Biolegend) and life/dead 7AAD-PE-Cy7 markers (ThermoFisher). Events were acquired on Attune Flow cytometer (ThermoFisher) and data were analyzed using Flow Jo 10 (Treestar Inc).

**Statistical analysis**

Statistical tests were performed using Fisher’s exact test or Mann–Whitney U test on the GraphPad Prism software. Differences were considered statistically significant when p < 0.05. Repertoire diversity, richness, and evenness were calculated using the R package, Vegan. TCR diversity in each sample was determined by calculating the Shannon entropy for each repertoire and the maximum Shannon entropy (from the number of total recovered sequences from each sample) to give a normalized value between 0.0 and 1.0. TCRA and TCRB CDR3 clustering was done by multiple alignments of the CDR3 aa sequences using MUSCLE (EMBL-EBI). The distance between the alignments was then determined with the computational package vegan and clusters were visualized in R. Clusters were defined when the distance between two or more alignments was less than or equal to 0.5. Cluster aa motifs were visualized using WebLogo3.

**Acknowledgments:** We would like to thank Tim Vincent for help with the participant recruitment and administrative support, and Dr. Katherine James for the viral sequencing. This study was supported by MRC Unit funding to the Human Immunology Unit (Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford) and the MRC Laboratories in the Gambia, as well as MRC strategic Grant G0801751. The work was also made possible through kind contributions by the Bodossaki Foundation and the Harold Hyam Wingate Foundation (scholarships to E.M.).

**Author Contributions:** S.R.-J. and T.D. conceptualized and acquired funding for the study. S.R-J, T.D., G. S.-J, C.P., and R.K. supervised the study; E.M. performed the experiments and formal analysis and wrote the manuscript draft. EG-M performed experiments and data analysis; A.J. assisted in the recruitment of participants; Y.P. assisted the production of tetramers; S.D, J.R.A, D.W, G S.-J, and D.D assisted in the clonotyping methodology and analysis; all authors were involved in the review and editing of the final manuscript.

**Conflict of interest:** The authors declare no financial or commercial conflict of interest.

**Data availability statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Peer review:** The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202048931

**References**

1. Phair, J., Jacobson, L., Detels, R., Rinaldo, C., Saah, A., Schrager, L. and Munoz, A., Acquired immune deficiency syndrome occurring within 5 years of infection with human immunodeficiency virus type-1: the Multicenter AIDS Cohort Study. J. Acquir. Immune Defic. Syndr. (1988), 1992. 5: 490–496. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1560346
2. Essbjörnsson, J., Mansson, F., Kvist, A., da Silva, Z. J., Andersson, S., Fenyo, E. M., Isberg, P.-E. et al., Long-term follow-up of HIV-2-related AIDS and mortality in Guinea-Bissau: a prospective open cohort study. Lancet HIV 2018. 6: E25–E31.
3. Marlink, R., Kanki, P., Thior, I., Travers, K., Eisen, G., Siby, T., Traore, I. et al., Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science 1994. 265: 1587–1590.
4. Whittle, H., Morris, J., Todd, J., Corrah, T., Sabally, S., Bangali, J., Ngom, P. T. et al., HIV-2-infected patients survive longer than HIV-1-infected patients. AIDS 1994. 8: 1617–1620.
HIV-1 infection when CD4 T-cell counts fall. 6981.

Vaccine J. Hum. Virol. HIV-1 infection. 2006.

Racz, P. et al., Maintenance of HIV-specific CD4+ T cells in HIV-2, a naturally contained human retroviral infection. J. Virol. 1994.

Klosterman, J. A. et al., Viral load in patients from West African communities. Vaccine 2007: 5329–5345.

Price, D. A., Asher, T. E., Wilson, N. A., Nason, M. C., Brenchley, J. M., Metzler, I. S., Venturi, V. et al., Public clonotype usage identifies protective Gag-specific CD8+ T cell responses in SIV infection. J. Exp. Med. 2009: 923–936.

Venturi, V., Price, D. A., Douek, D. C. and Davenport, M. P., The molecular basis for public T-cell responses? Nat. Rev. Immunol. 2008: 231–238.

Iglesias, M. C., Almeida, J. R., Fastenackels, S., van Bockel, D. J., Hashimoto, M., Venturi, V., Gostick, E. et al., Escape from highly effective public CD8+ T cell clonotypes by HIV. Blood 2011: 2138–2149.

Allsopp, C. E., Harding, R. M., Taylor, C., Bunce, M., Kwiatkowski, D., Anstey, N. and Brewster, A. J., Interleukin genetic differentiation in Africa: HLA class I antigens in The Gambia. Am. J. Hum. Genet. 1992: 411–421. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1734720.

Yindom, I. M., Leigidowicz, A., Martin, M. P., Gao, X., Qi, Y., Zaman, S. M., Van De Loeff, M. S. et al., Influence of HLA class I and HLA-KIR compound genotypes on HIV-2 infection and markers of disease progression in a Manjak community in West Africa. J. Virol. 2010: 820–828.

Quigley, M. F., Almeida, J. R., Price, D. A. and Douek, D. C., Unbiased molecular analysis of T cell receptor expression using template-switch anchored RT-PCR. Curr. Protoc. Immunol. 2011: Unit10.33. https://doi.org/10.1002/0471142735.im1033s94.

Folch, G. and Lefranc, M. P., The human T cell receptor beta variable (TRBV) genes. Exp. Clin. Immunogenet. 2000: 47–54.

Scaviner, D. and Lefranc, M. P., The human T cell receptor alpha variable (TRAV) genes. Exp. Clin. Immunogenet. 2000: 83–96.

Gillespie, G. M., Stewart-Jones, G., Rengasamy, J., Beatrice, T., Bwayo, J. J., Plummer, F. A., Kaul, R. et al., Strong TCR conservation and altered T cell cross-reactivity characterize a B*57-restricted immune response in HIV-1 infection. J. Immunol. 2006: 3893–3902.

Moss, P. A., Moots, R. J., Rosenberg, W. M., Rowland-Jones, S. J., Bodmer, H. C., McMichael, A. J. and Bell, J. I., Extensive conservation of alpha and beta chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide. Proc. Natl. Acad. Sci. U. S. A. 1991: 88: 8897–8900.

Almeida, J. R., Price, D. A., Papagno, L., Arkoub, Z. A., Sauce, D., Bornstein, E., Asher, T. E. et al., Superior control of HIV-1 replication by CD8+ T cells is reflected by their avidity, polyfunctionality, and clonal turnover. J. Exp. Med. 2007: 204: 2473–2485.

Leigidowicz, A., Onyango, C., Yindom, L. M., Peng, Y., Cotten, M., Jaye, A., McMichael, A. et al., Highly avid, oligoclonal, early-differentiated antigen-specific CD8+ T cells in chronic HIV-2 infection. Eur. J. Immunol. 2010: 40: 1963–1972.

Kedzierska, K., La Gruta, N. L., Davenport, M. P., Turner, S. J. and Doherty, P. C., Contribution of T cell receptor affinity to overall avidity for virus-specific CD8+ T cell responses. Proc. Natl. Acad. Sci. U. S. A. 2005: 102: 11432–11437.
Lichterfeld, M.
51
Ueno, T.
52
Lopes, A. R.
45
Schuldt, N. J.
53
and

Koup, R. A.
47
Lissina, A.

Moysi et al.
Eur. J. Immunol. 2021. 51: 2485–2500

for adoptive immunotherapy. expansion of high- or low-avidity cytotoxic T lymphocytes and efficacy

M. R.
51
D. R.

Ndhlovu, Z. M.

Koup, R. A.
47
Lissina, A.

T cells efficiently eliminate motile

Rimbert, M.

McClurkan, C. L.

Gostick, E.

T cells after early

Betts, M. R., Nason, M. C., West, S. M., De Rosa, S. C., Migueles, S. A., Abra-

Ham, J., Lederman, M. M., HIV nonprogressors preferentially maintain

highly functional HIV-specific CD8+ T cells. Blood 2006. 107: 4781–4789.

Mothe, B., Llano, A., Barrondo, J., Zamarreno, J., Schiainili, M., Miranda,

C., Ruiz-Riol, M. et al., CTL responses of high functional avidity and broad

variant cross-reactivity are associated with HIV control. PLoS One 2012. 7:
e29717.

Rowland-Jones, S. L., Dong, T., Dorrell, L., Ogg, G., Hansauta, P., Krausa,

P., Kimani, J. et al., Broadly cross-reactive HIV-specific cytotoxic

T-lymphocytes in highly-exposed persistently seronegative donors. Immunol. Lett. 1999. 66: 9–14.

Petrova, G. V., Naumova, E. N. and Gorski, J., The polyclonal CD8 T cell

response to influenza M158-66 generates a fully connected network of
cross-reactive clonotypes to structurally related peptides: a paradigm
for memory repertoire coverage of novel epitopes or escape mutants. J. Immunol. 2011. 186: 6390–6397.

Jennes, W., Camara, M., Dieye, T., Mboup, S. and Kestens, L., Higher

homologous and lower cross-reactive Gag-specific T-cell responses in
human immunodeficiency virus type 2 (HIV-2) than in HIV-1 infection. J. Virol. 2008. 82: 8619–8628.

Altman, J. D., Moss, P. A., Goulder, P. J., Barouch, D. H., McHeyzer-Williams,
M. G., Bell, J. I., Mcmichael, A. J. et al., Phenotypic analysis of antigen-
specific T lymphocytes. Science 1996. 274: 94–96.

Sharrock, C. E., Kaminski, E. and Man, S., Limiting dilution analysis of
human T cells: a useful clinical tool. Immunol. Today 2001. 11: 281–286.

Kabatitz, D., Herzog, W. R., Zanker, B. and Wagner, H., Human cyto-
toxic T-lymphocytes. I. Limiting-dilution analysis of alloreactive cytotoxic
T-lymphocyte precursor frequencies. Scand. J. Immunol. 1985. 22: 329–335.

Douek, D. C., Betts, M. R., Brenchley, J. M., Hill, B. J., Ambrozak, D. R., Ngi, K. L., Karandikar, N. J. et al., 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. 2002. 168:
3099–3104.

Betts, M. R., Brenchley, J. M., Price, D. A., De Rosa, S. C., Douek, D. C., Roed-
erer, M. and Koup, R. A., Sensitive and viable identification of antigen-
specific CD8+ T cells by a flow cytometric assay for degranulation. J. Immunol. Methods 2003. 281: 65–78.

Cossarizza, A., Chang, H. D., Radbruch, A., Acs, A., Adam, D., Adam-
Klages, S., Agace, W. W. et al., Guidelines for the use of flow cytometry
and cell sorting in immunological studies (second edition). Eur. J. Immunol. 2019. 49: 1457–1973.

abbreviations:
TCRB: T-cell receptor beta chains
TRBV: T-cell receptor beta variable region

Full correspondence: Prof. Sarah Rowland-Jones, Nuffield Department of
Medicine, University of Oxford, ND3 Research Building, Old Road
Campus, Roosevelt Drive, Oxford OX3 7FZ, United Kingdom.
e-mail: sarah.rowland-jones@ndm.ox.ac.uk

Received: 3/10/2020
Reviewed: 7/6/2021
Accepted: 5/8/2021
Accepted article online: 9/8/2021

© 2021 The Authors. European Journal of Immunology published by
Wiley-VCH GmbH www.eji-journal.eu