Direct identification of HLA-presented CD8 T cell epitopes from transmitted founder HIV-1 variants

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Funding information
United States Agency for International Development

Abstract
Cytotoxic T lymphocytes (CTLs) are a critical arm of the immune response to viral infections. The activation and expansion of antigen specific CTL requires recognition of peptide antigens presented on class I major histocompatibility complex molecules (MHC-1) of infected cells. Methods to identify presented peptide antigens that do not rely on the pre-existence of antigen specific CTL are critical to the development of new vaccines. We infected activated CD4+ T cells with two HIV-1 transmitted founder (TF) isolates and used high-resolution mass spectrometry (MS) to identify HIV peptides bound on MHC-1. Using this approach, we identified 14 MHC-1 bound peptides from across the two TF isolates. Assessment of predicted binding thresholds revealed good association of the identified peptides to the shared HLA alleles between the HIV+ donors and the naïve PBMC sample with three peptides identified through peptide sequencing inducing a CD8 T-cell response (p < 0.05). Direct infection of naïve CD4 cells by HIV TF isolates and sequencing of MHC-I presented peptides by HPLC-MS/MS enables identification of novel peptides that may be missed by alternative epitope mapping strategies and can provide valuable insight into the first peptides presented by an HIV-infected CD4 cell in the first few days post infection.

KEYWORDS
biomedicine, immunoproteomics, infectious diseases, mass spectrometry—LC-MS/MS, technology

1 INTRODUCTION

There is a growing body of evidence demonstrating the importance of cytotoxic T lymphocyte (CTL) responses in controlling HIV infection, in both natural human infection and pre-clinical animal vaccine studies. Highly functional CTL responses to specific HIV epitopes are correlated with slow disease progression in HIV-infected individuals [1, 2].

The earliest CD8 T-cell response during acute natural HIV-1 infection is directed to the transmitted/founder (TF) variants and contribute to the decline in plasma viraemia [2]. Findings from non-human primates have also demonstrated the value of a vaccine-induced T-cell response in conferring protection against both infection and the clinical progression of disease [3, 4]. In rhesus monkeys, vaccines that stimulate CTLs lead to superior control of simian immunodeficiency virus (SIV) or SIV-HIV hybrid viruses after subsequent challenge [5]. Current strategies of identifying suitable HIV-1 immunogens including the use of mosaic, conserved or networked epitopes are based on either...
comparative analysis of HIV sequences or ELISpot based epitope mapping [6]. However, computational analysis does not account for the complexity of the antigen processing and presentation pathways, while ELISpot approaches can only identify already known epitopes for which there are pre-existing cognate CD8 T cells. Furthermore, these strategies are not designed to identify epitopes that can be found in HIV-1 Tf variants.

A critical component of inducing an effective adaptive, CD8 T-cell immune response is the presentation of ‘self’ peptides in the context of major histocompatibility complex (MHC) class I molecules. The down regulation of peptide presentation is a key strategy by which viruses avoid immune detection and a number of HIV-1 gene products have been implicated to interfere with MHC class I antigen presentation with the strongest evidence favouring roles for HIV-1 Nef and Tat in this process [7].

The identification of HIV epitopes recognized by the immune system has historically been achieved through ELISpot epitope mapping strategies. For these strategies, synthetic peptide libraries are required, which are limited by their predicted or idealized designed epitopes and the cost, a major factor in particular if an autologous peptide strategy is required. This can in part be overcome by using consensus peptides or cohort-specific potential T-cell epitope pools [8]; however, both of these strategies rely on algorithms that mine only existing epitopes. Further drawbacks for both autologous and predictive peptides are that neither is subject to the post-translational modifications that can occur within vivo peptide presentation [9, 10].

Direct identification of peptides presented by HLA class I and II molecules associated peptides by LC-MS/MS can be an invaluable tool both for enabling T-cell epitope discovery, in addition to informing vaccine design and delivery strategies [11]. The use of LC-MS/MS enables the direct characterization and quantitation of HLA-restricted peptides derived from both HLA Class I and Class II MHC complexes [12]. This technique has been used to characterize the HLA class I-associated immunopeptidome in C1866 cell line and primary human CD4 cells infected with HIV-1 for identification of viral presented antigens that were broadly recognized by HLA-matched HIV-1-infected individuals [13]. Furthermore, strategies to distinguish co-precipitating proteins from HLA-bound ligands were established in the context of HIV-1 [14] and identification of viral peptides containing a protein splice junction was reported [15]. It was recently shown that detection of HIV-1 viral peptides early in infection (6 h) is possible using LC-MS/MS and can lead to identification of non-immunogenic, early presented peptides (Yang et al. 2021 accepted in Cell Reports). Analysis of human cells infected with MVA HIVcons vaccine successfully detected HLA Class I associated peptides derived from conserved HIV-1 regions [11, 16]. Env-specific peptides derived from HIV-infected cells were recently observed in antigen presenting cells [17].

Utilizing a sequential elution step with anti-MHC Class I antibodies, it is possible to create a fractionation of MHC Class I associated peptides that can then be processed for identification using LC-MS/MS. Additional resolution of the MHC class can also be achieved using monoclonal antibodies specific for each subclass. Moreover, the flow through from these affinity columns can be used to detect and quantify the amount of expressed HIV antigens as described recently for Vaccinia virus-infected dendritic cells [18, 19].

In this study, we set out to identify novel HIV-1 epitopes presented on MHC-I from TF HIV-1 variants using HPLC-MS/MS sequencing from an HIV negative, HLA-matched PBMC donor, and confirm immunogenicity of the identified peptides using ELISpot analysis with PBMCs isolated from the HIV patients from which the founder viruses were derived (Figure 1). Use of TF variants that have not undergone immune evasion mutations and that possess traits that confer increased ability to be transmitted at the population level would yield vaccine relevant epitopes.

2 | RESULTS

2.1 | Kinetics of CD4+ T cell infection by primary HIV-1 isolates

For optimal detection of MHC bound peptide by LC-MS/MS sequencing, it is imperative to ensure that a large percentage of cells are productively infected [12]. The large number of cells required for this technique can pose a logistical challenge in standard vaccine or epidemiological studies. To determine whether commercially sourced samples with compatible HLA profiles can be used as a surrogate donor, we assessed the fraction of polyclonally expanded and activated CD4 T cells that were productively infected following spinoculation over a period of 168 h to determine the optimal time for achieving maximum infection. Infection was defined by the detection of soluble p24 antigen in culture supernatant and quantification of the fraction of CD4 T cells that expressed intracellular p24 antigen detectable by flow cytometry. p24 quantification in supernatant was evaluated by ELISA at a time course of 24–168 h post infection with intracellular p24 quantified at 24, 48 and 148 h post infection.

Initial experiment using expanded CD4 cells infected with the lab adapted HIV-1 isolate CH070 in the absence of DEAE-Dextran resulted in detected p24 concentration in the supernatant of approximately 400 pg/mL 24 h after spinoculation was consistent throughout all the sampled time points up to 168 h post infection and yielded low levels percentages p24+ cells at 168 h post infection (Figure S1).
The addition of Dextran to target cells prior to infection has been shown to significantly increase infectivity. To assess whether Dextran could improve infectivity, activated CD4 cells were cultured for 24 h in the presence of 10 μg/mL of DEAE-Dextran prior to infection. On this occasion, the expanded cells were infected with the TF HIV strains R880F and R463F. To maximize cell number available for peptide sequencing, a single time point of 144 h post infection was assessed.

No detectable p24 expression was observed in CD4 cells cultured with and without Dextran in both pre-infection samples and in the uninfected control samples at 144 h post infection (Figure 2A). Following infection with either R880F or R463F in the presence of Dextran, a significant increase in p24 concentration was observed in the supernatant 144 h after infection against uninfected cells (Figure 2A, p < 0.001). Interestingly, there was also a significant difference in p24 production detected between the different viruses (R880F—23,130 pg/mL vs. R463F—66,185 pg/mL, Figure 2A, p < 0.05), which reflects intrinsic differences in the viral replicative capacity of these two viruses [20]. Concurrent assessment by p24 intracellular flow cytometry demonstrated that 39.1% and 51.5% of both R880F and R463F infected cells, respectively, were alive, CD3+, CD4+ and p24+ (Figure 2B and Figure S1) indicating productive viral infection at sufficient frequency to proceed with peptide sequencing.

### 2.2 Identification of HIV-1 peptides presented on MHC using LC-MS/MS

Infected expanded CD4 cells (~1 x 10^7) derived from sample CTL-CP1 were pelleted and resuspended in lysis buffer before immunoprecipitation of HLA-I complexes and elution of HLA-bound peptides. Peptides were then further purified and subjected to LC-MS/MS analysis. Following sequencing, 14 HIV peptides derived from both viruses (8–11 amino acids) were identified (Table 1). One peptide (GQWWHQNF) corresponds to a sequence within a peptide previously identified by Yue et al. [20] and a second peptide (RVMGTQMN) is also contained within a responsive 15mer although the exact epitope had not yet been established [20]. Responses to the additional 12 peptides were not identified by Yue et al. through their autologous mapping, potentially highlighting limitations of mapping with predefined libraries using
TABLE 1  HIV-1 derived peptide sequences identified by LC-MS/MS from CD4 (CTL-CP1) cells infected with transmitted founder viruses

| Peptide      | TFV      | Gene | AA position   | MS score (−10lgP) | HLA allele | Rank binding score |
|--------------|----------|------|---------------|-------------------|------------|--------------------|
| SQVHQTNIM<sup>a</sup> | R880F    | GAG  | 368–377       | 23.85             | B*15:03    | 0.088              |
|              |          |      |               |                   | C*02:10    | 1.299              |
| KVSQNYPIM    | R880F    | GAG  | 127–135       | 17.45             | C*02:10    | 1.346              |
|              |          |      |               |                   | C*15:05    | 0.969              |
| LEITTHSF     | R880F    | ENV  | 374–381       | 22.24             | B*18:01    | 0.018              |
|              |          |      |               |                   | B*15:01    | 0.767              |
| DELSKLVEM    | R880F    | VPU  | 7–15          | 25.76             | B*18:01    | 0.007              |
|              |          |      |               |                   | B*15:01    | 0.767              |
| GQVVHQNLF    | R463F    | GAG  | 139–146       | 21.54             | B*15:03    | 0.365              |
|              |          |      |               |                   | B*18:01    | 1.759              |
| KIDAWEKIRLR  | R463F    | GAG  | 12–22         | 37.71             | A*74:01    | 0.067              |
|              |          |      |               |                   | A*03:01    | 1.165              |
| RVMGTQMNY    | R463F    | ENV  | 2–10          | 38.83             | A*03:01    | 0.037              |
|              |          |      |               |                   | A*15:03    | 0.214              |
|              |          |      |               |                   | C*02:10    | 0.188              |
| ATNAIGDIRK   | R463F    | ENV  | 310–320       | 37.85             | A*03:01    | 0.437              |
|              |          |      |               |                   | A*15:03    | 0.188              |
|              |          |      |               |                   | C*02:10    | 0.188              |
| RIIKILYQSSK<sup>a</sup> | R463F    | REV  | 17–27         | 33.2              | A*03:01    | 0.210              |
|              |          |      |               |                   | A*15:03    | 0.214              |
|              |          |      |               |                   | C*02:10    | 0.188              |
| ILGSGTKK<sup>b</sup> | R463F    | REV  | 115–123       | 31.43             | A*03:01    | 0.408              |
|              |          |      |               |                   | A*15:03    | 0.878              |
| SIILGSGTKK<sup>c</sup> | R463F    | REV  | 114–123       | 28.83             | A*03:01    | 0.205              |
|              |          |      |               |                   | A*15:03    | 1.960              |
| SSIILGSGTKK<sup>c</sup> | R463F    | REV  | 113–123       | 18.43             | A*03:01    | 0.365              |
|              |          |      |               |                   | A*15:03    | 1.441              |
| FLRENLAF     | R463F    | POL  | 16–23         | 15.29             | A*03:01    | 0.408              |
|              |          |      |               |                   | A*15:03    | 0.878              |
|              |          |      |               |                   | C*02:10    | 1.423              |
| GQMIHQPLSPRTLNAWVK | R880F    | GAG  | 141–158       | NA                | NA         | NA                 |
|              |          |      |               |                   | NA         | NA                 |
| RKAKIIRDY    | R880F    | POL  | 997–986       | NA                | B*15:03    | 0.090              |
|              |          |      |               |                   | B*15:03    | 0.090              |
| LYCVHQRIEVK  | R880F    | GAG  | 85–95         | NA                | NA         | NA                 |
|              |          |      |               |                   | NA         | NA                 |
| DYGMAGGDCCVAGRQDE | R463F    | POL  | 8–25          | NA                | NA         | NA                 |
|              |          |      |               |                   | NA         | NA                 |
| FQHGLGISY    | R463F    | TAT  | 38–47         | NA                | B*15:03    | 0.004              |
|              |          |      |               |                   | B*18:01    | 0.223              |
|              |          |      |               |                   | C*02:10    | 0.041              |

Control peptides highlighted identified by Yue et al. [20] in bold italics.

<sup>a</sup>Peptides previously identified by Ternette et al. [13].

ELISpot. Derivations of certain peptides with overlapping or alternative residues may exist but none matched exactly the sequences identified.

The peptides were assessed for their predicted binding against the HLA profiles of the different PBMC samples (Table 1). Five peptides were selected from Yue et al. for their ability to induce responses at 12 months post estimated date of infection and were used as internal control peptides. Assessment of predicted binding used a rank binding threshold of ≤ 2.0. This measurement is an assessment of the affinity of the predicted peptide sequence to bind the associated HLA alleles when compared to 400,000 random peptides [21].

Assessment of predicted binding thresholds for the sequenced and control peptides revealed a good correlation of predicted peptides to the shared HLA alleles with 9/14 peptides predicted to associate to common HLA alleles. Some differences in modelled HLA allele associations were observed with two peptides (LEITTHSF and DELSKLVEM) predicted to associate to HLA-B*18:01 that is not represented in either volunteer 175038 or 175042 (Table S1).
TABLE 2  Phenotypes of PBMC samples used in analysis

|                | SPV$^a$ | CD4/µL$^b$ | HLA-A     | HLA-A     | HLA-B     | HLA-B     | HLA-C     | HLA-C     |
|----------------|---------|------------|-----------|-----------|-----------|-----------|-----------|-----------|
| 175038/R880F  | 5.99    | 655        | Aa02:01   | Aa*03:01  | Ba15:03   | Ba47:01   | Ca02:10   | Ca06:02   |
| 175042/R463F  | 5.83    | 414        | Aa01:01   | Aa30:02   | Ba15:03   | Ba45:01   | Ca02:10   | Ca06:02   |
| CTL-CP1       | NA      | NA         | Aa03:01   | Aa74:01   | Ba15:03   | Ba18:01   | Ca02:10   | Ca15:05   |

$^a$Set point viral load (SPVL) was determined using formula Log10 average vL of time points within first year post enrollment.

$^b$CD4/µL value reflects CD4 count ~510 days post EDI.

FIGURE 3  ELISPOT assessment of peptide specific responses. (A) Volunteer 175038 ELISPOT ~510 days post EDI. (B) Volunteer 1754042 ELISPOT responses ~510 days post EDI. Responses were deemed positive by Student t-test assessment to mock control wells *p < 0.05, ***p < 0.001, ****p < 0.0001. Control peptides highlighted in bold italics.

2.3 Validation of identified epitopes by ELSpot

To further understand the relationship between the peptides identified by LC-MS/MS sequencing and the predicted HLA association, IFN-γ ELSpot analysis was performed using PBMC samples from the autologous chronic infected HIV patients (175038 and 175042 at ~510 days post estimated date of infection) from which the TF viruses used to infect the CTL-CP1 PBMC sample were derived (see Table 2). Three peptides identified by MHC associated peptide sequencing induced a CD8 T-cell response (Figure 3, p < 0.05). All the 3R880F specific control peptides (3/3) had detectable responses in volunteer 175038 whereas 1/2 R463F specific control peptides elicited a response in volunteer 175042. Among the peptides identified by sequencing that elicited responses, two had been previously identified by mapping strategies (GQWVHQNF and RVMGTQMNY) [20]. Peptide SQVHQTNIM, predicted to be associated to B*15:03 and C*02:10, was not identified by either autologous epitope mapping or listed on the LANL database and so may be a newly identified CD8 T-cell epitope.

3 DISCUSSION

Peptide sequencing directly from MHC of HIV-infected CD4 cells can successfully identify peptides presented throughout the HIV life cycle and can identify peptides that may be missed by traditional epitope mapping strategies. ELSpot analysis of the peptides identified through MHC sequencing revealed that peptides SQVHTQNNIM, GQVWQHQNF and RVMGTQMNY retained functionality (as measured by IFN-γ production) in corresponding donor PBMC samples at approximately 500 days post infection. Peptide SQVHQTNIM (~amino acid position gag 368–377) elicited strong IFN-γ responses that were missed by autologous mapping, though a variant of this has been previously identified using this LC-MS/MS approach (SQVTNSATIM) [13]. Although the current methodologies are reagent and cell intensive, the peptides identified by directly sequencing from acutely infected samples offer an insight into the early epitopes presented to the immune system following HIV-1 infection. Furthermore, the unbiased nature through which these peptides are identified allows for the discovery of novel epitopes whose functional properties can then be analysed.
A further advantage is that this method does not require the use of PBMC samples from HIV-infected individuals to identify peptides presented in the context of different HLA alleles. HIV variants with different phenotypic characteristics (replicative capacity, sequence diversity, clade) can be used to infect PBMC samples derived from HIV naïve individuals. The distribution of HLA allele frequency has been assessed previously including the prevalence of different HLA alleles within a longitudinal African HIV cohort [22].

Few studies have examined the peptidome of HIV-1-infected cells in the context of primary HIV-1 infection. In this case, we carefully selected two TF variants that differ in viral fitness and disease progression of the patients from whom they were derived. This allowed for identification of peptides presented from TF variants to initiate the earliest CTL response to HIV-1, which is what an effective vaccine against HIV-1 should aim to overcome. We identified 10 peptides from variant R463F that was isolated from a rapid progressor and that has been previously shown to replicate rapidly in vitro, compared to only four peptides from R880F that was isolated from a HIV viremic controller and that grows to significantly lower titres in vitro [20]. This association between both in vivo and in vitro viral replication kinetics [20] with the number of epitopes that we identified through a HPLC-MS/MS approach further suggests that epitopes identified may be physiologically relevant. The finding that more epitopes were identified from the rapid progressor whose TF variant grows to higher titres in vitro suggests a role for viral replicative capacity in influencing the quantity of intracellular viral proteins and thus the number of viral peptides that can be loaded onto MHC-1. Application of this methodology to identify epitopes presented by these two patients with divergent clinical outcomes using a longitudinal approach may further identify immune escape patterns that may shed further light on the role of CTL immune escape on driving disease progression.

Through careful selection of viruses with different characteristics and target CD4 cells from individuals with representative MHC Class I HLA alleles, this approach may provide an alternative method to identify those epitopes efficiently presented in both HLA pathways in models of HIV infection. Further evaluation of these peptides against conserved sequences may give further insight into their applicability for vaccine design. Furthermore, application of this approach to study antigen presentation by professional antigen presenting cells such as dendritic cells presenting peptides from more diverse HIV-1 TF isolates could potentially lead to identification of further physiologically relevant HIV-1 epitopes.

4 | MATERIALS AND METHODS

4.1 | Viruses, PBMCs and peptides

TF HIV virus sequences (provided by Professor Eric Hunter, Emory) and associated PBMC samples derived from two volunteers enrolled within IAVI Protocol C (volunteer Id 175038 (R880F) and 175042 (R463F)) in addition to a commercial PBMC sample (CTL-CP1 – Cellular Technology Limited, Shaker Heights, OH, USA) were used for all experiments. Table 2 describes all volunteer phenotypes.

4.2 | Cell culture and sample infection

HIV negative PBMC sample (CTL-CP1) was thawed and cultured in R10 media supplemented with IL-2 (Sigma 50 U/mL final concentration) and the CD3/CD8 bispecific antibody (Clone) to expand CD4 T-cells. On Day 10 of expansion, DEAE Dextran (Sigma 10 μg/mL final concentration) was added to the media and the cells were cultured for a further 24 h. On Day 11, the cells were centrifuged (200 × g for 10 min) and resuspended at a final concentration of 2.3 × 10⁷ cells/mL R463F and R880F TF HIV Infectious Molecular Clones were added to PBMC samples at an MOI 0.4 (R880F 8.9 × 10⁴ IU, R463F 8.3 × 10⁵ IU) and infections proceeded using a standard spinoculation protocol (1800 × g, 120 min). After spinoculation, the cell pellets were washed twice in PBS and plated at 1.9 × 10⁶/mL and cultured for 6 days in R10 and 50 U of IL-2.

4.3 | Flow cytometry

Determination of HIV infection by flow cytometry was assessed using a five colour intra-cellular panel. Cells were permeabilized using Cytofix/Cytopperm (BD 554722) and assessed for viability using Aqua Fluorescent (Thermo Scientific L34957). The antibodies used for assessment of T-cell populations and HIV infectivity were α-p24-PE (BC KC-RD1 6604667), α-CD3 APC (BD 345767), α-CD4 PeCF594 (Clone RPA T4) and α-CD8 BV451 (BD562429). Cells were fixed for 12+ h in cytofix prior to acquisition on BD Fortessa. Flow cytometry data were analysed using FlowJo software version 9 (Treestar Inc, USA).

4.4 | p24 ELISA

Determination of HIV infection by ELISA was performed using the Perkin Elmer HIV-1 p24 ELISA kit (Cat# NEK050) as per manufacturer’s instructions. Samples were prepared for analysis by resuspending in 50 μL TRITON-X supplemented with R10 media to a final volume of 250 μL.

4.5 | Sample preparation

At 6 days post infection, the infected cells are harvested without enzymatic treatment using a chemical cocktail (kindly provided by Nicola Ternette). Briefly, a 2x lysis buffer solution of 1% Igepal CA-630, 100 mM Tris, pH 8.0, 300 mM NaCl supplemented with complete protease inhibitor from Roche (one tablet/10 mL) was prepared with mass spectrometry (MS) grade dH₂O. Infected cells were washed once with PBS and pelleted at 300 × g for 10 min and resuspended in 5 mL of 2x
Peptide elution and MS

MHC-peptide elution and MS to identify HIV specific peptides was conducted as previously described [12]. Briefly, lysates of infected cells were cleared by two subsequent centrifugation steps at 500 × g for 10 min and 20,000 × g for 30 min. MHC Class I HLA complexes were captured from lysates on Protein A-sepharose beads (Expedeon) cross-linked to W6/32 antibody (5 mg/mL) at gravity flow and washed using sequential runs of 50 mM Tris buffer, pH 8.0 containing first 150 mM NaCl, then 400 mM NaCl, and then, no salt. HLA-peptide complexes were eluted with 5 mM 10% acetic acid (v/v). Affinity column-eluted material was loaded onto a 4.6 × 50 mm ProSwift RP-15 column (Thermo Fisher Scientific) and eluted using a 500 µL/min flow rate over 10 min from 2% to 35% buffer B (0.1% formic acid in acetonitrile) in buffer A (0.1% formic acid in water) using an Ultimate 3000 HPLC system (Thermo Scientific). Detection of eluted peptides was performed using a variable wavelength detector at 280 nm. Fractions up to 12 min that did not contain β2-microglobulin were combined and dried.

Each sample was resuspended in 20 µL buffer A and analyzed both on an Orbitrap Elite (Thermo Scientific) online coupled to an Acquity nano UPLC (Waters) and a TripleTOF 5600 (AB SCiEX) coupled to an Eksigent ekspert nanoLC 400 cHiPLC system. Orbitrap Elite: Peptides were separated on a nano Acquity UPLC system (Waters) supplemented with a 25 cm BEH130 C18 column, 1.7-mm particle size using a linear gradient from 8% to 35% buffer B in buffer A at a flow rate of 250 nL/min for 60 min. Peptides were introduced to an Orbitrap Elite mass spectrometer using a nanoESI source. Subsequent isolation and collision-induced dissociation was induced on the 20 most abundant ions per full MS scan using an isolation width of 1.5 amu. All fragmented precursor ions were actively excluded from repeated selection for 15 s. TripleTOF 5600: Peptides were separated on an ekspert nanoLC 400 cHiPLC system (Eksigent) supplemented with a 15 cm × 75 µm ChromXP C18-CL, 3 µm particle size using a linear gradient from 8% buffer A to 35% buffer B at a flow rate of 300 nL/min for 60 min. Peptides were introduced to TripleTOF 5600 mass spectrometer and collision-induced dissociation fragmentation using ramped collision energy was induced on the 30 most abundant ions per full MS scan using unit isolation width 0.7 amu. All fragmented precursor ions were actively excluded from repeated selection for 15 s.

Raw data were converted to Mascot generic files using mconvert or ProteinPilot 4.5 Sequence interpretation of MS/MS spectra were performed using a database containing all annotated human SwissProt entries including translations of all six reading frames of the sequenced HIV-1 IIIB genome in addition to translations of all known assigned HIV-1 protein coding regions (GenBank:KK925006) or a database containing all annotated human SwissProt entries (02/2013, 20,253 entries) and all HIV-1 entries in NCBI (02/2013, 446,954 entries) with PEAKS 7 and Mascot 2.4. The probability score threshold was defined by decoy database searches implemented in the regarding search engines at a general false discovery rate of 5% [13]. All identified peptides provided as supplementary file. Full data set for HIV and self-peptides available at dataspace.iavi.org.

ELISpot

A single PBMC vial for volunteer 170038 (514 days post EDI) and 175042 (510 days post EDI) was assessed by Human IFN-γ 96 well ELISpot kit (Cellular Technology Limited, Cat# hIFNg-1M/2) as per manufacturer’s instructions. Responses were assessed by Student t-test for difference to mock tested wells (p < 0.05) [23].

Acknowledgements

The authors wish to acknowledge Adam Coleman, Peter Hayes, Jakub Kopycinski, Francesco Lala and Helen Coutinho for their help in assistance in performing the virus infections, flow cytometry and ELISpot assays. This work was funded in part by IAVI and made possible by the support of the United States Agency for International Development (USAID) and other donors. The full list of IAVI donors is available at http://www.iavi.org. The contents of this manuscript are the responsibility of IAVI and do not necessarily reflect the views of USAID or the US Government.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are openly available in the PRIDE partner repository at http://proteomexchange.org, reference number PXD025105.

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SUPPORTING INFORMATION
Additional supporting information may be found online https://doi.org/10.1002/pmic.202100142 in the Supporting Information section at the end of the article.

How to cite this article: Hare, J., Macharia, G., Yue, L., Streetfield, C. L., Hunter, E., Purcell, A., Ternette, N., & Gilmour, J. (2021). Direct identification of HLA-presented CD8 T cell epitopes from transmitted founder HIV-1 variants. Proteomics, 21, e2100142. https://doi.org/10.1002/pmic.202100142