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► **To cite this version:**

Ellen M Leitman, Christian B Willberg, Ming-Han Tsai, Huabiao Chen, Søren Buus, et al.. HLA-B*14:02-Restricted Env-Specific CD8+ T-Cell Activity Has Highly Potent Antiviral Efficacy Associated with Immune Control of HIV Infection. *Journal of Virology*, 2017, 91 (22), pp.e00544-17. 10.1128/JVI.00544-17 . pasteur-02549962

HAL Id: pasteur-02549962

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Submitted on 21 Apr 2020

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1 **HLA-B*14:02-RESTRICTED ENV-SPECIFIC CD8+ T-CELL ACTIVITY**

2 **HAS HIGHLY POTENT ANTIVIRAL EFFICACY**

3 **ASSOCIATED WITH IMMUNE CONTROL OF HIV INFECTION**

4
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66

67 **Short Title:** HLA-B*14:02-Restricted Env-Specific CD8+ T-cells in HIV Control

68

69 **ABSTRACT**

70 Immune control of human immunodeficiency virus type 1 (HIV) infection is typically associated
71 with effective Gag-specific CD8+ T-cell responses. We here focus on HLA-B*14, that protects
72 against HIV disease progression, but the immunodominant HLA-B*14-restricted anti-HIV
73 response is Env-specific (ERYLKDQQL, 'HLA-B*14-EL9'). A subdominant HLA-B*14-restricted
74 response targets Gag (DRYFKTLRA, 'HLA-B*14-DA9'). Using HLA-B*14/peptide-saporin
75 conjugated tetramers, we show that HLA-B*14-EL9 is substantially more potent at inhibiting viral
76 replication than HLA-B*14-DA9. HLA-B*14-EL9 also has significantly higher functional avidity
77 ($p < 0.0001$) and drives stronger selection pressure on the virus than HLA-B*14-DA9. However,
78 these differences were HLA-B*14 subtype-specific, applying only to HLA-B*14:02 and not HLA-
79 B*14:01. Furthermore, the HLA-B*14-associated protection against HIV disease progression is
80 significantly greater for HLA-B*14:02 than for HLA-B*14:01, consistent with the superior antiviral
81 efficacy of the HLA-B*14-EL9 response. Thus, although Gag-specific CD8+ T-cell responses
82 may usually have greater anti-HIV efficacy, factors independent of protein specificity, including
83 functional avidity of individual responses, are also critically important to immune control of HIV.

84

85

86 **IMPORTANCE**

87 In HIV infection, although CTL play a potentially critical role in eradication of viral reservoirs, the
88 features that constitute an effective response remain poorly defined. We focus on HLA-B*14,
89 unique among HLA associated with control of HIV in that the dominant CTL response is Env-
90 specific, not Gag. We demonstrate that Env-specific HLA-B*14-restricted activity is substantially
91 more efficacious than the subdominant HLA-B*14-restricted Gag response. Env
92 immunodominance over Gag, and strong Env-mediated selection pressure on HIV, are only
93 observed in subjects expressing HLA-B*14:02, and not HLA-B*14:01. This reflects increased
94 functional avidity of Env response over Gag, substantially more marked for HLA-B*14:02.
95 Finally, we show that HLA-B*14:02 is significantly more strongly associated with viraemic control
96 than HLA-B*14:01. These findings indicate that, although Gag-specific CTL may usually have
97 greater anti-HIV efficacy than Env responses, factors independent of protein specificity,
98 including functional avidity, may carry greater weight in mediating effective control of HIV.

99 **INTRODUCTION**

100 Spontaneous durable control of HIV is observed in a rare subgroup (<1%) of infected individuals
101 known as 'elite controllers' (1). Non-progressive HIV infection is associated with expression of
102 certain HLA class I molecules (2, 3), such as HLA-B*57 and HLA-B*27 alleles (1, 4, 5). An
103 important mechanism underlying the HLA associations with HIV disease outcome is related to
104 the particular HIV-specific epitopes presented by different HLA class I molecules. In particular,
105 'protective' HLA molecules typically present broad Gag-specific epitopes to CD8+ T-cells,
106 whereas disease-susceptible alleles such as HLA-B*35:01 and HLA-B*58:02 present Nef- and
107 Env-specific epitopes, respectively, eliciting CD8+ T-cell responses that are typically associated
108 with poor immune control of HIV (6-10).

109
110 Factors contributing to improved immune control in association with broad Gag and not Nef or
111 Env responses include the sequence conservation especially of the capsid protein, because the
112 cost to viral replicative capacity of Gag escape mutants is often significant (11-14). In contrast,
113 Env escape mutants, for example, are typically tolerated by the virus without significant impact
114 on viral replicative capacity (15). In addition, Gag capsid proteins are much more abundant than
115 Env trimers in mature virions (1,000-1,500 per virion versus 10-20, respectively) (16) and Gag
116 epitopes can be presented within two hours of HIV gaining entry into the target cell, prior to *de*
117 *novo* HIV protein synthesis (17). Hence, HIV-infected cells can be killed by Gag-specific CD8+
118 T-cells before new virion production (17, 18). In contrast, Nef- and Env-specific CD8+ T-cell
119 responses kill virus-infected target cells only after *de novo* synthesis of viral proteins (17-20) and
120 therefore following Nef-mediated HLA class I downregulation (21, 22). Nonetheless, Gag-
121 specific CD8+ T-cell responses are not equally efficacious (6, 23, 24), and there is evidence
122 from the SIV/monkey model that certain non-Gag epitopes, for example within Nef and Vif, are
123 important for immune control (25).

124

125 Furthermore, it is clear that several other factors than HIV protein specificity can play an
126 important role in efficacy of an epitope-specific response. These include functional avidity (26,
127 27), polyfunctionality (28), lytic granules (29) and proliferative capacity (30).

128
129 To investigate further the potential role of non-Gag-specific CD8+ T-cell responses in control of
130 HIV infection, we focused here on HLA-B*14, where the dominant HIV-specific CD8+ T-cell
131 response is in Env (31, 32). The association between HLA- B*14 and immune control of HIV has
132 not been well-studied to date (33), since most studies of elite controllers have focused on those
133 expressing HLA-B*27 or B*57 (26, 29, 30, 34-38). Although HLA-B*14 is not as strongly
134 associated with HIV disease progression as HLA-B*27 or HLA-B*57, nonetheless large studies
135 have consistently shown a significant protective effect (3, 39-41). In addition to the dominant
136 Env-specific CD8+ T-cell response, HLA-B*14-positive individuals also make a subdominant
137 Gag-specific CD8+ T-cell response (42). We set out to investigate the role of these two
138 specificities in HLA-B*14-mediated suppression of HIV and to understand the mechanisms
139 underlying the observed differential antiviral activity among HLA-B*14-restricted CD8+ T-cell
140 specificities.

141

142 **MATERIALS AND METHODS**

143 ***Study subjects***

144 Adult chronically HIV-infected ART-naïve subjects studied here were enrolled in the following
145 cohorts: (i) Thames Valley cohort, UK (n=30) (18); (ii) Gateway cohort, Durban, South Africa
146 (n=17) (43); (iii) the SCOPE (Study of the Consequences of Protease Inhibitor Era) cohort, San
147 Francisco, USA (n=2) (44). Subjects from all cohorts provided written informed consent and the
148 study was approved by the institutional boards of the University of Oxford, the University of
149 KwaZulu-Natal and the University of California, San Francisco. HLA typing was performed using
150 a locus specific PCR amplification strategy and a heterozygous DNA sequencing methodology

151 for the HLA class I exon 2 and 3 amplicons. HIV plasma viral load measurements were done
152 using the Roche Amplicor version 1.5 assay with COBAS Amplicor (Thames Valley, Gateway
153 and SCOPE cohorts) or using the Abbott RealTime HIV assay (SCOPE cohort). CD4+ T-cell
154 counts were enumerated by flow cytometry using standard clinical protocol. Median viral load of
155 these study subjects was 9,700 copies/ml (IQR 555-31,500); median CD4+ T-cell count was 527
156 cells/mm³ (IQR 420-711).

157
158 To analyse the associations between the expression of HLA-B*14:01 and HLA-B*14:02 with
159 immune control of HIV, viral load and HLA data of the ART-naïve chronically HIV-infected
160 subjects (n=5,004; Caucasians: n=3,259; African Americans: n=1,745) were used. These
161 subjects were from the cohorts from the following studies:

- 162 • AIDS Clinical Trial Group (ACTG) Study (<https://actgnetwork.org>);
- 163 • International HIV Controllers Study (3) (<http://www.hivcontrollers.org>);
- 164 • Multicenter AIDS Cohort Study (MACS) (45) (<https://statepi.jhsph.edu/macs/macs.html>);
- 165 • Multicenter Hemophilia Cohort Study (MHCS) (46)
166 (<https://biolincc.nhibi.nih.gov/studies/mhcs>);
- 167 • The Study of the Consequences of Protease Inhibitor Era (SCOPE) (47)
168 (<https://hiv.ucsf.edu/research/scope.html>);
- 169 • Swiss HIV Cohort Study (48) (www.shcs.ch).

170 Viraemic controllers were defined as individuals with viral load <2,000 copies/ml; non-controllers
171 were defined as individuals with viral loads >10,000 copies/ml.

172

173 ***Tetramer generation and staining***

174 Peptide-MHC tetramers conjugated to fluorophores were generated as previously described,
175 using streptavidin-PE or APC (49). Cytotoxic saporin-conjugated tetramers were produced by

176 the same method using streptavidin-SAP (Advanced Targeting Systems) to tetramerise peptide-
177 MHC monomers according to the published approach (50). Briefly, these modified tetramers are
178 coupled to a toxin, ribosome-inactivating protein saporin (SAP), that can selectively kill antigen-
179 specific cells of interest and thereby evaluate the contribution of a particular CD8+ T-cell
180 specificity to viral inhibition (50-54). Efficiency of tetramerisation was confirmed by staining with
181 anti-mouse Ig κ beads (BD Biosciences) with an anti-HLA antibody, followed by tetramer
182 staining. For staining with the fluorescently-conjugated tetramers, PBMC or expanded CD8+ T-
183 cells ($0.5-1 \times 10^6$ cells per stain) were washed with PBS, incubated with relevant tetramers for 20-
184 30 minutes at room temperature in a 96 U-bottom plate, washed again, further incubated with
185 fluorochrome-conjugated antibodies for 15 minutes at room temperature and fixed in 2%
186 formaldehyde solution at 4°C. For staining with SAP-conjugated tetramers, cells were incubated
187 with tetramers for 30 minutes at room temperature, washed, fixed and permeabilised with BD
188 Cytofix/Cytoperm kit (BD Biosciences) and then incubated with a secondary anti-SAP antibody
189 (Alexa Fluor 488, Advanced Targeting Systems) as previously published (50). Controls included
190 cells incubated with no tetramer, HLA-mismatched SAP-conjugated tetramers and free
191 unconjugated SAP. All samples were acquired within 6 hours of staining on MACSQuant
192 Analyser 10 (Miltenyi Biotec). Negative gates were set up using staining with no tetramer or with
193 HLA-mismatched tetramers. Samples were analysed in FlowJo version 9.7.6 (Tree Star, Inc.)
194 and hierarchically gated on singlets, lymphocytes, live cells and CD3+CD4- cells around CD8+
195 tetramer-specific cell populations; in viral inhibition assays, cells were gated on live CD4+GFP+
196 populations.

197

198 ***Selective depletion of antigen-specific CD8+ T-cells using cytotoxic tetramers***

199 Antigen-specific CD8+ T-cells were selectively depleted using cytotoxic saporin-conjugated
200 tetramers (tet-SAP) as described previously and confirmed in our laboratory (50). First, CD8+ T-
201 cells within PBMC were expanded using a monoclonal CD3.4 antibody bi-specific for CD3 and

202 CD4 (the NIH AIDS Reagent Program) which simultaneously eliminates CD4+ T-cells and
203 expands CD8+ T-cells (55-57). Expanded CD8+ T-cells were cultured in R10 medium (RPMI,
204 10% fetal calf serum (Sigma), 1% L-glutamine (Sigma) and 1% penicillin-streptomycin (Sigma)),
205 supplemented with 50 U/ml human premium grade IL2 (Miltenyi Biotec) (R10/50) for 10-14 days
206 to achieve >90% purity. Expanded CD8+ T-cells were then treated with tet-SAP (5-10 nM) for 2
207 hours at 37°C, washed three times with R10 and cultured in R10/50 for 24-48 hours before using
208 them as effector cells in viral inhibition assays (see below). Control treatments included HLA-
209 mismatched tet-SAP, free saporin or no treatment. Depletion efficiency was confirmed by
210 tetramer staining prior to viral inhibition assay set-up. Tet-SAP-mediated depletion was pre-
211 validated by depleting antigen-specific cells using PE-conjugated tetramers and anti-PE
212 magnetic beads (StemCell Technologies).

213

214 ***Generation of polyclonal epitope-specific CD8+ T-cell lines***

215 Epitope-specific CTL lines were generated as previously described (18) with modifications.
216 Briefly, fresh PBMC were peptide-pulsed ($2-3 \times 10^6$ PBMC/peptide at 200 µg/ml final
217 concentration) for 1 hour and fed with fresh R10/50 2-3 days for 14-21 days. Specificity was
218 tested by tetramer staining. To remove non-specific cells, tetramer-positive cells were sorted on
219 MoFlo XDP (Beckman Coulter) and expanded in R10/50 supplemented with monoclonal OKT3
220 antibody (eBioscience) at 0.1 µg/ml. At the time of the initial set-up and every 10-14 days after,
221 peptide-pulsed irradiated HLA-matched B cells and irradiated feeder PBMC from three HIV-
222 negative donors were added to the sorted cells at a 1:1:1 ratio. Specificity and purity of
223 expanded CD8+ T-cells was confirmed by tetramer staining immediately before using them as
224 effector cells in viral inhibition assays.

225

226 ***Generation of CD8+ T-cell clones***

227 Epitope-specific clones were generated as previously described (38). Briefly, PBMC were
228 stained with fluorescently labeled tetramers. Tetramer-specific single cells were sorted on MoFlo
229 XDP (Beckman Coulter) directly into U-bottom 96-well plates (single cell/well) in R10/50
230 containing monoclonal OKT3 antibody (eBioscience) at 0.1 µg/ml. Twice a week half of the
231 media was replaced with fresh R10/50. After 2-3 weeks, cells were tested for their specificity by
232 tetramer staining. Epitope-specific clones were transferred to 48-well and then to 24-well plates;
233 at the time of transfer and/or every 14-21 days, clones were restimulated with monoclonal OKT3,
234 peptide-pulsed irradiated HLA-matched B cells and irradiated feeder PBMC from three HIV-
235 negative donors.

236

237 ***Viral inhibition assays***

238 To evaluate anti-HIV suppressive capacity of *ex vivo* unstimulated CD8+ T-cells or stimulated
239 epitope-specific CD8+ T-cells, we modified previously described viral inhibition assay (58). We
240 used an HIV-permissive T1 cell line untransfected or transfected with HLA-B*14:02 gene
241 (provided by Otto Yang; this cell line also expresses HLA-A*02, HLA-B*05, HLA-B*06) (59) as
242 target cells. Effector cells were: (i) 'zapped' CD8+ T-cells, from which Env-EL9- or Gag-DA9-
243 specific cells were selectively depleted using tet-SAP as described above; (ii) CTL lines; and (iii)
244 CD8+ T-cell clones. For the initial setup, target cells were infected with pre-titrated NL4-3-GFP
245 by spinoculation for 1 hour, incubated at 37°C for 1 hour, repeatedly washed and further cultured
246 with or without effector cells in duplicate or triplicate. Every 2-3 days, cultures were fed and
247 stained to assess live CD4+ GFP+ cells. % GFP+ uninfected target cells served as a
248 background, subtracted from all values. HIV-suppressive capacity was calculated at the time of
249 the peak of viral growth as follows (58): suppressive capacity = $\log_{10}(\%GFP+ \text{ infected target}$
250 $\text{ cells without effector cells} / \%GFP+ \text{ target cells with effector cells})$.

251

252 The viral inhibition assays shown were done using CTL lines and clones that were generated
253 from 3 subjects.

254

255 ***Antibodies***

256 Antibodies used: α CD3-Brilliant Violet 421 (UCHT1), α CD4-APC (OKT4), α CD4-FITC (OKT4)
257 and α CD8-PE/Cy7 (RPA-T8) (BioLegend); α HLA-APC (G46-2.6) (BD Biosciences); and LIVE-
258 DEAD fixable near-IR marker (Life Technologies); polyclonal chicken α Saporin-Alexa Fluor 488
259 (Advanced Targeting Systems).

260

261 ***Interferon- γ ELISPOT assays***

262 Freshly isolated or cryopreserved PBMC were screened in interferon- γ ELISPOT assays to
263 quantify CD8+ T-cell responses to a set of 410 overlapping 18-mer peptides spanning the HIV-
264 proteome (6) and HLA-restricted optimal epitopes listed in the Los Alamos A-list of optimal HIV
265 CTL epitopes (60). ELISPOT assays were performed as previously described (61, 62). Spots
266 were counted using an automated ELISPOT reader (AID ELISPOT v4.0, Autoimmun
267 Diagnostika, Germany). Positive responses had to be at least three times the mean number of
268 spot-forming colonies (SFC) in the four control wells and >50 SFC/million PBMC after
269 background subtraction. HIV peptides were produced by Schafer-N.

270

271 ***Measurement of functional avidity***

272 Functional avidity, or antigen sensitivity, was defined as the concentration of an exogenous
273 peptide required to elicit half-maximal cellular response. Functional avidity of CD8+ T-cells within
274 PBMC was assessed in *ex vivo* interferon- γ ELISPOT assays by incubating 10^5 PBMC per well
275 with serial peptide dilutions over a range of seven \log_{10} units in triplicate. The peptides used
276 were wildtype Gag-DA9 and Env-EL9. ELISPOT assays were performed as described above.

277

278 ***Site-directed mutagenesis of NL4-3***

279 Y301F, K302R, and Y301F/K302R mutations of HIV Gag sequence as well as K588Q and
280 K588R mutations in Env sequence were introduced respectively into the HIV subtype B NL4-3
281 plasmid by using QuikChange Lightning site-directed mutagenesis kit (Agilent technologies)
282 along with custom-designed mutagenesis forward and reversed primers. The forward primers
283 are shown as follows (mutated codons shown in bold): 5'-C CTG GCT GTG GAA AGA TAC CTA
284 **CAG** GAT CAA CAG CT-3' (Env K588Q); 5'-GAC TAT GTA GAC CGA TTC **TTT** AAA ACT CTA
285 AGA GCC GAG-3' (Gag Y301F); 5'-T AGA GAC TAT GTA GAC CGA TTC TAT **AGA** ACT CTA
286 AGA GCC G-3' (Gag K302R); 5'-A GAC TAT GTA GAC CGA TTC **TTT AGA** ACT CTA AGA
287 GCC GAG CAA G-3' (Gag Y301F/K302R). All mutations were confirmed by sequencing.

288

289 ***Virus production and replication kinetics***

290 All plasmids were maxipreped according to manufacturer's instruction (HiSpeed® plasmid Maxi
291 Kit, Qiagen, Hilden, Germany). To generate mutant viruses, the mutated NL4-3 Gag-Pro
292 amplified purified PCR products with the BstE II (New England Biolabs, Ipswich, MA) linearized
293 pNL4-3Δgag-protease were transfected into GFP reporter GXR cells via electroporation in a
294 BioRad GenePulsar II using 0.4 cm cuvettes at 300 Volts, 500μF, and infinite resistance (14).
295 Virus propagation was then monitored by flow cytometry (LSRII, BD Biosciences) to detect GFP-
296 expressing infected cells for two weeks in culture with GXR cells. Virus culture supernatants
297 were harvested when 30% cells were GFP-positive. Viruses were aliquoted and stored at -80°C
298 until use. All mutations were confirmed again by extracting viral RNA from the harvested
299 supernatant and sequencing. Nucleotide similarity reached 99.99%. Along with WT as positive
300 controls and two negative controls without viruses, NL4-3 mutant viruses were incubated with
301 GXR cells in a 24-well plate for determination of viral titres, as previously described (63). A low
302 MOI (0.01%) was set as the lowest threshold for determining the amount of virus required for
303 inoculation. The GFP+ expression was measured by flow cytometry from day 2 to 7 before it

304 reached the saturated 30-40%. The viral replication capacity was defined by the semi-log
305 calculation of the mean slope of exponential growth in Excel. This was further calibrated to the
306 normalised value relative to the wild type NL4-3 respectively. All assays were done at least in
307 triplicate.

308

309 ***Amplification and sequencing of proviral DNA***

310 Genomic DNA was extracted from whole blood QIAmp reagents (QIAGEN, UK), following
311 manufacturer's protocol. For the subjects from the SCOPE cohort whole blood was unavailable
312 and DNA was extracted from cryopreserved PBMC using QIAmp DNA Mini Kit following
313 manufacturer's protocol (QIAGEN). HIV Gag and partial Env (containing HLA-B*14-Env-EL9
314 ⁵⁸⁴ERYLKDQQL⁵⁹² epitope) segments were amplified by nested PCR, as previously described
315 (64), using: Gag-specific primers 5'-CTCTAGCAGTGGCGCCCGAA-3', 5'-
316 TCCTTTCCACATTTCCAACAGCC-3' for the first round (product size 1,418 base pairs; HXB2
317 coordinates 627-2045) and 5'-ACTCGGCTTGCTGAAGTGC-3', 5'-
318 CAATTTCTGGCTATGTGCCC-3' for the second round (product size 1307 base pairs; HXB2
319 coordinates 696-2003); Env-specific primers 5'-GGAGATATAAGACAAGCACATTG-3', 5'-
320 CCCTGTCTTATTCTTCTAGGT-3' for the first round (product size 1579 base pairs; HXB2
321 coordinates 7194-8773) and 5'-GTGGAGGAGAATTTTTCTATTG-3', 5'-
322 CTATCTGTTCCCTTCAGCTACTGC-3' for the second round (product size 1349 base pairs; HXB2
323 coordinates 7357-8707). PCR products were purified using QIAquick PCR Purification Kit
324 (QIAGEN, UK) according to the manufacturer's instructions. All sequencing was done using
325 BigDye Terminator v3.1 Ready Reaction mix (Applied Biosystems) as previously described (64)
326 and analysed using Sequencher v4.8 (Gene Codes Corp.). We generated maximum-likelihood
327 trees of all sequences, using Mega6.06-mac software and FigTree v1.4.2, to exclude the
328 possibility of contamination with laboratory viral strains. HIV subtypes were further confirmed

329 with NCBI (website) and REGA (website) HIV genotyping tools. GenBank accession numbers
330 MF445302-MF445379.

331

332 ***Statistical analysis***

333 Statistical analyses were performed in GraphPad Prism for Mac OSX, 5.0c (GraphPad
334 Software). We used paired t-test to compare differences between remaining infected target cells
335 without effector cells to those with effector cells at the peak of viral replication; Kruskal-Wallis
336 test with Dunn's post-test (for >2-group analysis) or Mann-Whitney U test (for 2-group analysis)
337 to analyse differences in HIV suppressive capacity; Fisher's Exact Test to analyse differences in
338 recognition of Env-EL9 versus Gag-DA9 epitopes and in autologous sequences of Env-EL9
339 versus Gag-DA9 epitope; Mann-Whitney U test for differences in magnitude and functional
340 avidity of Env-EL9 versus Gag-DA9 CD8+ T-cell responses, in frequency of tetramer-specific
341 cells in HLA-B*14:01- versus HLA-B*14:02-positive subjects and in viral load and CD4+ T-cell
342 counts; Spearman correlation to analyse the correlation between response magnitude and
343 functional avidity; ANOVA with Dunnett's Multiple Comparison Test for differences in viral
344 replicative capacity of different viral constructs. Functional avidity (EC50) was calculated in
345 Prism using a dose-response function.

346

347 To analyse associations between HLA class I expression and HIV immune control, SAS 9.2
348 (SAS Institute) was used. Genotype frequencies on individual HLA-B alleles were computed
349 using PROC FREQ. To calculate OR and 95% CI for viraemic controllers versus non-controllers
350 with adjusting by HLA-B*27 and HLA-B*57 PROC LOGISTIC was used; OR<1 indicates
351 protection. Presence versus absence of all individual HLA-B alleles that have a frequency equal
352 to or greater than HLA-B*14:01 were included in the models with stepwise selection.

353

354 **RESULTS**

355 **Higher antiviral potency of B*14:02-Env-EL9 versus -Gag-DA9 CD8+ T-cell response**

356 The starting point for this study was an 'elite controller' subject, Subject-1, who first tested HIV-
357 positive in UK in 2011, having previously had two negative tests in 2005 and 2008 (Fig 1A).

358 Since the positive HIV test, Subject-1 maintained an undetectable viral load (<40 copies/ml) and
359 healthy and stable CD4+ T-cell counts (median 1,555 cells/mm³, IQR 1,345-1,788). Viral
360 sequencing revealed that she was infected with subtype B virus. HLA genotyping showed that
361 she was HLA-B*14:02/HLA-C*08:02 homozygous and also expressed another HLA molecule,
362 HLA-A*74:01, associated with slow disease progression (65).

363
364 To investigate the role HLA-B*14:02-restricted CD8+ T-cell responses might play in immune
365 control of HIV, we first screened PBMC in this individual for HIV-specific IFN- γ ELISPOT
366 responses using overlapping peptides spanning the entire HIV proteome (39) together with
367 previously defined HIV-specific epitopes (66). The HLA-B*14:02-restricted responses dominated
368 overall, the highest magnitude responses being to the HLA-B*14:02-restricted Env-EL9
369 (⁵⁸⁴ERYLKDQQL⁵⁹²) (31) and its corresponding overlapping peptide Env-366
370 (⁵⁷⁹RVLAIERYLKDQQLGIW⁵⁹⁶) (Figs 1B,C). The next highest optimal peptide response was
371 towards the HLA-B*14:02-Gag-DA9 epitope (²⁹⁸DRFYKTLRA³⁰⁶) (42).

372
373 To test the hypothesis that suppression of HIV in this patient was mediated principally by HLA-
374 B*14:02-restricted CD8+ T-cell activity, we next compared the antiviral potency of Env-EL9- and
375 Gag-DA9-specific CD8+ T-cells. From this same elite controller ('Subject-1') bulk CD8+ T-cells
376 were first expanded with the bi-specific CD3.4 antibody (55-57), and then depleted of selected
377 CD8+ T-cell specificities using cytotoxic saporin-conjugated tetramers (see Methods) (Fig 1D).
378 The ability of the bulk or depleted CTL to inhibit viral replication *in vitro* was then evaluated using
379 T1 cells expressing HLA-B*14:02 as CD4+ T-cell targets and the B clade NL4-3 as the test
380 strain of HIV (Figs 1E,F). Removal of the Env-EL9 specificity substantially reduced the HIV-

381 suppressive capacity of the expanded CD8+ T-cells (22% of target cells infected versus 0.001%
382 (Fig 1E); and suppressive capacity was reduced by 26-fold (bulk CD8: 3.85 log₁₀ versus Env-
383 EL9-depleted CD8: 0.15 log₁₀) (Kruskal-Wallis p=0.02; Fig 1F). In contrast, depletion of Gag-
384 DA9-depleted cells made little impact. This result suggests that the presence of Env-EL9
385 specificity represents the majority of CD8+ T-cell-mediated control of viral suppression in
386 Subject-1, and that the Gag-DA9 specificity does not contribute significantly.

387
388 A potential caveat of this finding in this study subject is the unequal frequency of Env- and Gag-
389 specific CD8+ T-cells, with Env-specific cells being nearly 20-fold more frequent than Gag-
390 specific cells (Fig 1D). To address this matter, we adopted two approaches. First, we repeated
391 targeted depletion experiments using cells from another chronically B-clade infected HLA-
392 B*14:02-positive controller (Subject-2, VL=80 copies/ml, CD4=1,355 cells/mm³), who had equal
393 frequencies of Env-EL9- and Gag-DA9-specific CD8+ T-cells (Fig 1G, left panels). As with
394 Subject-1, elimination of the Env-EL9 specificity resulted in preservation of 25% of infected
395 target cells at the peak of viral replication compared to only 1% in bulk CD8+ T-cell coculture
396 (Fig 1H), representing a 16-fold reduction (1.6 log₁₀ versus 0.1 log₁₀) of suppressive capacity of
397 the Env-EL9-depleted CTL (Kruskal-Wallis p=0.03; Fig 1I). In contrast, Gag-DA9 depletion had
398 only a minor effect, thus supporting the notion that Env-EL9-specific CTL mediate the major
399 antiviral efficacy of the two HLA-B*14:02-restricted responses.

400
401 In the second approach, we tested antiviral efficacy of Env-EL9- and Gag-DA9-specific CD8+ T-
402 cells directly by generating epitope-specific CTL lines and clones. In the experiments using
403 peptide-specific lines (Figs 2A-C), once again Env-EL9-specific CTL were significantly more
404 potent at suppressing viral replication at the same effector to target ratio 1:100 (p=0.02; Figs
405 2B,C). Similarly, Env-EL9-specific CTL clones were more potent inhibitors of viral replication
406 than were Gag-DA9 clones. This was particularly evident at the lower effector to target ratios

407 (1:1000) (Figs 2D-F). Taken together, these experiments suggest that Env-EL9-specific CD8+ T-
408 cells are more efficacious at suppressing HIV replication than Gag-DA9-specific cells.

409

410 ***Higher functional avidity, antigen recognition, and magnitude of EL9 versus DA9***

411 In order to further investigate this observed antiviral superiority of Env-EL9-specific cells over
412 Gag-DA9-specific cells, we next examined functional avidity of the two specificities (determined
413 by the peptide concentration required for 50% maximal recognition or EC50), response
414 magnitude and frequency of epitope recognition in a larger number of HLA-B*14-positive
415 subjects (n=30). Among all HLA-B*14-positive subjects, functional avidity, or antigen sensitivity
416 (EC50), of the Env-EL9 response was >24-fold higher than that of the Gag-DA9 response
417 (median 0.84 versus 20.3 μ M, $p < 0.0001$; Fig 3A, left panel). This difference was significant
418 among both HLA-B*14:01-positive (median 3.7 versus 21.3 μ M, $p = 0.003$) and HLA-B*14:02-
419 positive subjects (median 0.3 versus 19.8 μ M, $p < 0.0001$; Fig 3A, right panel). However, Env-EL9
420 functional avidity was 12-fold higher in HLA-B*14:02-positive subjects than in HLA-B*14:01-
421 positive subjects ($p = 0.005$; Fig 3A, right panel).

422

423 The magnitude of the Env-EL9 response was also >9-fold higher than that of the Gag-DA9
424 response among all HLA-B*14-expressing subjects among responders ($p = 0.003$; Fig 3B, left
425 panel). This difference was only significant among HLA-B*14:02-positive subjects (Fig 3B, right
426 panel). Additionally, HLA-B*14:02-positive subjects had a significantly higher magnitude of the
427 Env-EL9 response compared to the HLA-B*14:01-positive subjects ($p = 0.03$). Interestingly, the
428 magnitude of both Env-EL9 and Gag-DA9 responses was negatively correlated with EC50 (i.e.
429 positively with functional avidity) (Env-EL9: $r = -0.73$, $p = 0.0002$; Gag-DA9: $r = -0.88$, $p < 0.0001$; Fig
430 3C), indicating that cells with higher functional avidity mounted a response of greater magnitude.

431

432 Together, these data demonstrate that the greater antiviral potency of the HLA-B*14-Env-EL9-
433 specific response observed above is also associated with higher functional avidity and response
434 magnitude compared with the Gag-DA9-specific response.

435

436 ***Differential Env-EL9 and Gag-DA9 selection pressure in B*14:01 versus B*14:02***

437 To further understand the differences between Env-EL9- and Gag-DA9-specific CD8+ T-cell
438 function, we next investigated what selection pressure is imposed on the virus by these two
439 responses (Fig 4). Consistent with previously published data describing HLA-associated
440 polymorphisms from analysis of 3,754 HLA-typed treatment-naïve persons (67, 68) within the
441 Env-EL9 epitope (Fig 4A), K588Q is strongly selected among both HLA-B*14:01- and HLA-
442 B*14:02-positive persons, and variants at Env-588 and at other residues within Env-EL9 are
443 observed more commonly in HLA-B*14:02-positive subjects (although here these differences
444 between HLA-B*14:01- and HLA-B*14:02 did not reach statistical significance; Fig 4B). These
445 Env-EL9 sequence data indicate stronger selection pressure imposed on the virus by the HLA-
446 B*14:02-EL9 response than by the HLA-B*14:01-restricted EL9 response.

447

448 To put these Env-EL9 variants arising in HLA-B*14-expressing individuals into the context of
449 variation within this epitope overall, the frequency of Env-EL9 variants in B- and C-clade
450 infection in all subjects (<https://www.hiv.lanl.gov>) is 50% and 34%, respectively, the most
451 frequent variants being K558R (29% and 9% in B- and C-clade sequences, respectively),
452 K588Q (8% and 16%). Thus, K588R is relatively common, and, as shown in Fig 4A, is not an
453 HLA-B*14:01 or HLA-B*14:02 footprint, and indeed is not selected without accompanying
454 variants in any of the subjects studied here; whereas K588Q is selected in 17/35 (49%) HLA-
455 B*14+ve subjects studied here, and in 17/25 (68%) of those whose autologous virus encoded
456 Env-EL9 variants.

457

458
459 For the Gag-DA9 epitope, as shown in previous large cohort studies (67-69), the most frequent
460 K302R variant is only selected in HLA-B*14:01-positive subjects ($p=0.01$). Thus there was
461 significantly more variation in HLA-B*14:02-positive subjects in the Env-EL9 epitope than in
462 Gag-DA9 ($p=0.001$; Fig 4B), consistent with this being the dominant response among HLA-
463 B*14:02-positive subjects. Among B*14:01-positive subjects the Env-EL9 epitope was not
464 targeted significantly more than the Gag-DA9, and correspondingly there was no significant
465 difference in the selection of variants within Env-EL9 and Gag-DA9 in these subjects.

466
467 ***Selection of K588Q and not K588R is an escape variant in HLA-B*14-positive subjects***
468 The Env-EL9 sequence data shown above confirm previous studies showing that K588Q at
469 position 5 (P5) in the epitope and binding in the D pocket of the HLA-B*14 peptide-binding
470 groove (70) is an HLA-B*14 footprint but K588R is not (67). This is surprising, given the relatively
471 frequent occurrence of Arg/Lys substitutions as a mechanism by which HIV can escape
472 recognition by other CTL specificities. Indeed, the HLA-B*14:01-associated escape variant
473 within Gag-DA9 is a case in point, K302R being the substitution characteristically selected at P5
474 in the epitope.

475
476 In order to address this question, the ability of Env-EL9-specific CD8+ T-cells to cross-react with
477 the K588Q and with the K588R variants were analysed in 8 HLA-B*14-positive subjects for
478 whom samples were available. In 6 of 8 subjects the autologous variant was K588Q, and in the
479 remaining two autologous virus encoded wildtype Env-EL9. The pattern of cross-recognition
480 observed was quite distinct for the two variants K588Q and K588R. For K588Q, in all 8 subjects,
481 the frequency of cross-reactive cells, double-stained by EL9-wt and EL9-K588Q tetramers, was
482 substantially lower than the frequency of EL9-wt-specific CD8+ T-cells (Fig 5). In all cases,
483 including the 6 in whom the K588Q variant had been selected, EL9-wt-specific responses were

484 readily detectable, and in 5 of 8 cases greater in magnitude than the EL9-K588Q-specific
485 response. By contrast, in most of these subjects (6 of 8), the frequency of CTL cross-reactive for
486 EL9-wt and K588R was higher than that of EL9-wt-specific CTL. Indeed, in 6 of 8 of these
487 subjects the K588R variant-specific response was higher than the EL9-wt-specific response.

488
489 These results would explain why K588R is not selected as an escape mutant, since the EL9-wt-
490 specific response typically cross-reacts strongly with the K588R variant. By contrast, the K588Q
491 variant is not cross-recognised and therefore this mutant would carry a selective advantage for
492 the virus.

493
494 ***Impact of Gag-DA9 and Env-EL9 escape mutants on viral fitness and HIV outcome***
495 Previous studies have indicated that the most effective CTL responses are those capable of
496 driving the selection of escape mutants that significantly reduce viral replicative capacity (11-14).
497 These are more likely in p24 Gag, which is highly conserved as opposed to Env, which is highly
498 variable. We next, therefore, investigated the impact on viral replicative capacity of the most
499 common escape mutants in p24 Gag-DA9 and Env-EL9 (Figs 6A,B). In contrast to the general
500 observation of the high cost of p24 Gag mutants, the K302R mutant had little impact on viral
501 replicative capacity (VRC), consistent with previous studies of this variant (71). The Env EL9
502 mutant marginally but not significantly decreased VRC. Thus, in this particular case, the fitness
503 cost resulting from the selection of escape mutants within the capsid protein appears to have
504 little impact on viral replicative capacity.

505
506 HLA-B*14-positive subjects who had wild type Env-EL9 had lower viral loads (median 9,068
507 versus 21,546 copies/ml, $p=0.05$; Fig 6C) and higher CD4+ T-cell counts (median 606 versus
508 455 cells/mm³, $p=0.005$). Again, this difference was exclusive to HLA-B*14:02-positive subjects
509 among whom individuals with wild type Env-EL9 had lower VL versus those with a variant

510 (median 80 versus 20,003 copies/ml, $p=0.04$; Figs 6D,E), and a trend towards higher CD4+ T-
511 cell counts (median 1,340 versus 410 cells/mm³, $p=0.2$), although there were only three subjects
512 with wild type sequence.

513
514 Taken together these results suggest that the Env-EL9 response is highly effective and
515 contributes to successful immune control of HIV, whereas Gag-DA9 is not.

516
517 ***Stronger association of HLA-B*14:02 than HLA-B*14:01 with HIV immune control***
518 On the basis of the findings above, if the B*14-Env-EL9 response makes a significant
519 contribution to immune control of HIV, one would predict greater protection against HIV disease
520 progression in association with HLA-B*14:02 compared with HLA-B*14:01. To test this
521 hypothesis, we first compared the frequency of HLA-B*14:01 with that of HLA-B*14:02 in
522 viraemic controllers (viral load <2,000 copies/ml) versus non-controllers (viral load >10,000
523 copies/ml) derived from several previously well-described cohorts (see Methods). Although there
524 was a trend towards HLA-B*14:02 being more protective than HLA-B*14:01 among HLA-B*14-
525 positive Caucasians (self identified as 'white'; $n=285$) and HLA-B*14-positive African Americans
526 (self-identified as 'black'; $n=104$), in neither group was the difference statistically significant
527 (Table 1). We then extended the analysis to all controllers and non-controllers in our cohorts
528 (whites, $n=3,259$; blacks, $n=1,745$) by performing a regression analysis with stepwise selection
529 that included all HLA-B alleles having a frequency equal to or greater than HLA-B*14:01 (HLA-
530 B*14:01 frequency in whites: 2.42%; in blacks: 1.60%) (Table 2). HLA-B*14:02 showed a
531 significant independent protective effect in both whites (OR = 0.44, $p = 2e-7$ in whites) and
532 blacks (OR = 0.54, $p = 2e-2$ in blacks), but HLA-B*14:01 did not in either group. These results
533 are consistent with previous data where HLA-B*14:02 was shown to confer protection in a
534 logistic regression model that included all HLA class I alleles with phenotypic frequency >2%
535 (33) and with our immunological findings.

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DISCUSSION

This study investigated HIV control mediated by HLA-B*14. We showed that the HLA-B*14-restricted Env-EL9-specific CD8+ T-cell response was more efficacious against HIV than Gag-DA9-specific response. In association with this, Env-specific response had significantly greater function avidity, was more frequently targeted and was of higher magnitude than the Gag-specific response. We demonstrated that the functional avidity for Env-EL9 was significantly higher for responses restricted by HLA-B*14:02 than by HLA-B*14:01. Finally, we showed a significantly stronger association between HLA-B*14:02 and protection against HIV disease progression compared with the protection mediated by HLA-B*14:01.

Higher anti-HIV efficacy of Env- compared to Gag-specific CD8+ T-cells responses is unusual and, in fact, unreported until the current study. Numerous studies have suggested that Gag-specific CD8+ T-cell responses are associated with better disease outcome in HIV infection, are more efficacious in control of HIV than other specificities and are often dominant responses in elite controllers (6, 10, 18, 72-83). However, our result is consistent with studies showing that non-Gag responses can also mediate viral control. In the macaque model of elite control, Env-, Nef- and Vif-specific CD8+ T responses have been shown to be dominant and efficacious in viral clearance (25, 84, 85). In HIV infection, a dominant HLA-B*57-restricted Nef response was present in an elite controller, although antiviral efficacy of that response has not been evaluated (86). Similarly, HLA-B*27:02, allele associated with better protection than HLA-B*27:05 (P. Goulder, unpublished data), restricts dominant CD8+ T-cell response in Nef, although as above its efficacy remains to be determined. Furthermore, effective elimination of infected cells by Env-specific CD8+ T-cells, including HLA-B*14-Env-EL9-restricted cells, has been reported in HIV infection (87, 88). Our finding of the superior Env-specific antiviral efficacy is also consistent with a recent study of HLA-B*57/27-negative HIV elite controllers indicating that potent cytotoxic

562 capacity (measured by granzyme B expression and infected cell elimination) of HIV-specific
563 CD8+ T-cells as opposed to the identity of the targeted epitope is the determining factor in
564 mediating successful control of infection (89).

565
566 The data presented here are consistent with previous studies suggesting that an important factor
567 contributing to antiviral efficacy of CD8+ T-cell responses is high functional avidity (26, 27, 90,
568 91). Functional avidity of the Env-EL9 response was >24-fold higher than that of the Gag-DA9-
569 specific response and correlated strongly with response magnitude; the Env-specific response
570 was also more frequently targeted than the Gag-specific response (Fig 3). Thus, HLA-B*14-Env-
571 EL9 CD8+ T-cells with higher antigen sensitivity than HLA-B*14-Gag-DA9 cells would be
572 expected to be more efficacious in controlling viral replication.

573
574 These qualitative differences between Env- and Gag-specific CD8+ T-cells were only significant
575 among HLA-B*14:02-positive individuals (Fig 3). In the case of functional avidity, the difference
576 between the Env- and Gag-specific responses was significant even among HLA-B*14:01-
577 positive individuals, although less markedly so. However, the avidity of the Env-EL9 response
578 was still 12-fold higher in the HLA-B*14:02-positive subjects compared to the HLA-B*14:01-
579 positive subjects. These observations suggest that despite restriction of the same epitopes by
580 these two closely-related HLA-B*14 molecules, HIV-specific HLA-B*14:02-restricted CD8+ T-
581 cells are qualitatively different from HIV-specific HLA-B*14:01-restricted CD8+ T-cells, and the
582 difference is primarily determined by the superior function of HLA-B*14:02-restricted Env-EL9-
583 specific CD8+ T-cells.

584
585 It has previously been proposed that an important mechanism by which HLA class I molecules
586 influence rates of HIV disease progression is related to the specificity of the particular HIV
587 epitopes that are presented (8). As described above, factors other than the specificity are

588 important. However, the demonstration here of the HLA-B*14-EL9 response as both
589 immunodominant among HLA-B*14-positive subjects and efficacious in suppressing HIV is
590 consistent with previous observations of HLA-B*14 being associated with protection against
591 rapid HIV disease progression (3, 39, 41). The substantially higher functional avidity of this Env-
592 EL9 response among HLA-B*14:02- than HLA-B*14:01-positive subjects is also consistent with
593 the findings here that HLA-B*14:02 is significantly more protective against HIV disease
594 progression than HLA-B*14:01.

595
596 These studies have focused on the two principal HLA-B*14-restricted HIV-specific responses.
597 We have not considered other HLA-B*14-restricted HIV-specific responses since these are the
598 only 2 that drive selection pressure on the virus (67). The labour-intensive nature of the work
599 and the consequently small number of subjects studied here limits our concluding definitively
600 that in all cases HLA-B*14-restricted Env-EL9-specific CD8+ T-cells inhibit HIV replication more
601 effectively than HLA-B*14-restricted Gag-DA9-specific responses. In addition, sample availability
602 precluded our comparing the capacity of HLA-B*14:01-restricted CTL clones compared with
603 HLA-B*14:02-restricted CTL clones to inhibit viral replication.

604
605 Structurally, HLA-B*14:01 and HLA-B*14:02 differ only at the position 11 (P11), with a serine
606 and an alanine, respectively (92). However, P11 is unlikely to explain the difference in HIV
607 control between the two HLA molecules, because of its 'buried' location in the α 1 domain of HLA
608 near the C pocket (93, 94) where it does not contribute to interactions between HLA domains or
609 with a peptide or TCR, is not solvent-accessible and is of low variability (95, 96). The peptide-
610 binding motif for HLA-B*14:02 has previously been determined (70). Studies identifying HLA-
611 B*14-restricted epitopes reported only 2-digit HLA, thus not showing an HLA-B*14:01 motif
612 explicitly (31, 42). However, due to lack of significant structural differences the peptide-binding
613 motif is likely to be the same for both molecules.

614
615 On the other hand, the 'buried' P11 may alter the confirmation of the nearby α 1 residues or
616 affect upstream peptide processing (92). Of note, HLA-B*14:02 and HLA-B*14:03 differ by a
617 single amino acid in the HLA sequence at P156 but share only ~30% of their peptides (97). This
618 single difference at P156, essential in D and E peptide-binding pockets (95), may play a role in
619 the differential association of HLA-B*14:02 and HLA-B*14:03 with ankylosing spondylitis (97,
620 98). Previous HIV-specific studies have also shown that one amino acid difference between HLA
621 subtypes, such as HLA-B*35:01 and HLA-B*35:03 (99), or HLA-B*35:01 and HLA-B*35:08
622 (100), HLA-B*42:01 and HLA-B*42:02 (24), and HLA-B*57:02 and HLA-B*57:03 (101), is
623 sufficient to have a significant impact on disease outcome.

624
625 Another distinguishing feature between HLA-B*14:01 and HLA-B*14:02 alleles appears to be in
626 the selection pressure that they exert on the virus. First, the Gag-DA9 K302R mutation was
627 found exclusively in HLA-B*14:01-positive subjects, consistent with previous large cohort studies
628 involving >3500 study subjects (67, 68). This again would point to the lack of antiviral efficacy of
629 HLA-B*14:02-Gag-specific CD8+ T-cells. At the same time, HLA-B*14:02 appeared to have
630 selected Env-EL9 K588Q mutation and there is a hint that this selection is associated with
631 higher viraemia and lower CD4+ T-cell counts (Figs 6C-E). Curiously, however, neither of these
632 mutations had a significant impact on viral replication, although Env-EL9-K588Q tended to have
633 a slightly lower replicative capacity than the wild type, while the opposite was true for Gag-DA9-
634 K302R (Fig 6). This is particularly interesting in the case of the Gag epitope. This epitope (Gag
635 298-306) overlaps with the highly conserved major homology region (Gag 285-304) in the C-
636 terminus of HIV p24 capsid, which is essential for virion assembly and stability (102, 103) and
637 previous reports showed rapid reversion of K302R in the absence of HLA-B*14:01 (69, 104),
638 implying K302R inflicts a significant cost to viral replicative capacity. However, apparent lack of
639 impact of this mutation on viral replication was also recently reported by another group (71).

640
641 It is perhaps surprising that previous large studies investigating the relationship between HLA
642 class I type and HIV disease progression did not identify the difference between HLA-B*14:02
643 and HLA-B*14:01 in terms of the protective effect conferred. In some earlier studies 2-digit HLA
644 typing was employed (Hendel et al 1999) which prevented these analyses being undertaken.
645 Also, HLA-B*14 class I subtypes are not especially prevalent, especially in African populations
646 (the phenotypic frequency of HLA-B*14 is 6.0% versus 8.7% in the present study (Tables 1,2))
647 and hence large study numbers are needed to achieve adequate statistical power. The current
648 analysis involved 3,259 Whites and 1,745 Blacks, and even with these numbers the protection
649 afforded by HLA-B*14:02 in the Blacks was only evident at a p value 0.02.

650
651 In conclusion, these studies indicate that, although Gag-specific CD8+ T-cell responses may
652 usually have greater antiviral efficacy against HIV for the several reasons described above,
653 influences such as functional avidity of individual responses are also critically important factors
654 that may override protein-specificity in contributing to immune control of HIV infection. This
655 finding is relevant to the development of vaccines designed to generate effective antiviral CD8+
656 T-cell responses.

657

658 **ACKNOWLEDGEMENTS**

659 This work was funded by grants from the National Institutes of Health (RO1AI46995 to PJRG),
660 the Wellcome Trust (WT104748MA to PJRG), NIHR research capability funding (to PCM) and
661 the Clarendon Fund (to EML). This project has been funded in whole or in part with federal funds
662 from the Frederick National Laboratory for Cancer Research, under Contract No.
663 HHSN261200800001E (to MC). The MACS is funded primarily by the National Institute of
664 Allergy and Infectious Diseases (NIAID), U01-AI35042 (Johns Hopkins University Bloomberg
665 School of Public Health, Joseph Margolick, PI), U01-AI35039 (Northwestern University, Steven

666 Wolinsky, PI), U01-AI35040 (University of California, Los Angeles, Roger Detels and Oto
667 Martinez MPI), U01-AI35041 (University of Pittsburgh, Charles Rinaldo, PI), and UM1-AI35043
668 (Johns Hopkins University Bloomberg School of Public Health, Lisa Jacobson, PI). The funders
669 had no role in study design, data collection and analysis, decision to publish, or preparation of
670 the manuscript.

671
672 The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS,
673 NIAID, NIH: CD3.4 Bi-specific Monoclonal Antibody (Cat#12278) from Drs. Johnson Wong and
674 Galit Alter.

675
676 The authors have declared that no competing interests exist.

677

678 **FIGURE LEGENDS**

679 **Figure 1. Higher antiviral potency of B*14:02-EL9 versus -DA9 CD8 T-cell response. (A)**

680 HIV-related clinical profile of subject Subject-1; gray area shows time period during which
681 infection occurred. All viral load measurements were undetectable (<40 copies/ml) and are
682 shown below the limit of detection (LOD) of 40 copies/ml for convenience. **(B)** CD8+ T-cell IFN- γ
683 ELISPOT responses to overlapping peptides spanning the entire HIV proteome in Subject-1.
684 Dotted line shows the cutoff magnitude (50 SFC/10⁶ PBMC). **(C)** CD8+ T-cell IFN- γ ELISPOT
685 responses to epitopes restricted by HLA class I alleles expressed by Subject-1. HLA-A*36:01-
686 restricted responses are not shown as these are not defined. Dotted line shows the cutoff
687 magnitude (50 SFC/10⁶ PBMC). **(D-F)** Data for Subject-1; **(G-J)** data for Subject-2. **(D,G)**
688 Tetramer stainings confirming HLA-B*14-02-Env-EL9 (top panels) and HLA-B*14:02-Gag-DA9
689 (bottom panels) CD8+ T-cell responses in bulk (left panels) and tetramer-depleted cultures (right
690 panels). Gated on live CD3+CD4- lymphocytes around CD8+ tetramer+ cells; numbers indicate
691 % of CD8+ cells. **(E,H)** Viral replication (%GFP+ cells) time course in infected T1-HLA-B*14:02-

692 positive target cells with or without effector CD8+ T-cells. Results were compared to T1-HLA-
693 B*14:02 target cells only at the peak of viral replication using paired t-tests. **(F,I)** Suppressive
694 capacity of 'bulk' 'Env-EL9-depleted' or 'Gag-DA9-depleted' effector cells calculated as
695 described in the Methods. Kruskal-Wallis test with Dunn's multiple comparison test. E,F,H,I,
696 Error bars represent s.e.m. *p<0.05, **p<0.01, ***p<0.001. Only significant differences are
697 shown. Legend applies to panels (E), (F), (H) and (I).

698

699 **Figure 2. Suppressive capacity of epitope-specific Env and Gag CTL lines and clones.**

700 Panels A-C show results for CTL lines, generated by peptide-stimulation of epitope-specific cells
701 from PBMC, sorting them and further expanding before testing in inhibition assays; panels D-F
702 show examples of clones, generated by single-cell sorting of epitope-specific cells and growing
703 them out in culture before testing their antiviral capacity. **(A, D)** Confirmatory tetramer stainings
704 of epitope-specific CTL lines (A) and clones (D). Gated on live CD3+CD4- cells around
705 CD8+tetramer+ cells; numbers indicate % tetramer+ cells (of CD3+CD4-). **(B, E)** Viral replication
706 in infected T1-HLA-B*14:02-positive target cells with or without effector cells. Results were
707 compared to T1-HLA-B*14:02 target cells only at the peak of viral replication using paired t-tests.
708 *p<0.05, **p<0.01, ***p<0.001, ns, not significant (p>0.05). **(C, F)** Suppressive capacity of
709 effector cells. Mann-Whitney U test. B,C,E,F, error bars represent s.e.m. Legend in (B) applies
710 to panels (C), (E) and (F).

711

712 **Figure 3. Higher functional avidity and magnitude of EL9- versus DA9-specific response.**

713 **(A)** Functional avidity (EC50) of Env-EL9 versus Gag-DA9 CD8+ T-cell responses in all HLA-
714 B*14-positive subjects (n=30, left panel) or separately in HLA-B*14:01-expressing (n=16) or
715 HLA-B*14:02-expressing (n=14) subjects (right panel). Lines and numbers indicate median
716 values. Mann-Whitney U tests. **(B)** Magnitude of Env-EL9 or Gag-DA9 responses determined by
717 IFN-γ ELISPOT assay in all HLA-B*14-positive subjects (n=30; left panel) or in HLA-B*14:01-

718 expressing (n=16) and HLA-B*14:02-expressing (n=14) subjects (right panel). Numbers above
719 the bar graphs indicate median values; error bars show interquartile ranges; Mann-Whitney U
720 tests. **(C)** Correlation between response magnitude and functional avidity in HLA-B*14-positive
721 (left), HLA-B*14:01-positive (middle) and HLA-B*14:02-positive subjects. R and p values were
722 obtained by Spearman correlation. Legend for all panels is shown in panel (A).

723
724 **Figure 4. Differential Env-EL9 and Gag-DA9 selection pressure in B*14:01 versus B*14:02.**

725 **(A)** Consensus sequences of Env-EL9 and Gag-DA9 epitopes and polymorphisms associated
726 with HLA-B*14 subtypes; overlapping polymorphisms associated with non-HLA-B*14 alleles are
727 also shown. Data compiled from previously published large cohort studies (67-69). **(B)**
728 Frequency of Env-EL9 and Gag-DA9 wild type and variant sequences in the studied HLA-B*14-
729 positive subjects. Graphs at the bottom compare frequency of subjects with autologous wild type
730 (filled bars) or mutated (empty bars) sequence of Env-EL9 versus Gag-DA9 epitopes. Fisher's
731 Exact Tests. A,B, residues identical to the wild type are presented as '-', non-identical residues
732 are specified.

733
734 **Figure 5. K588Q and not K588R is an escape variant in HLA-B*14-positive subjects.**

735 **(A)** Representative example of responses to EL9 wildtype versus EL9 K588Q variant peptides at
736 different peptide concentrations by IFN- γ ELISPOT assay. Shown example is subject OX018
737 who has autologous EL9 K588Q variant. **(B)** Cross-recognition of EL9 wildtype and EL9 K588Q
738 (top row) versus EL9 wildtype and EL9 K588R (bottom row). Shown example is subject OX018
739 who has autologous EL9 K588Q variant. **(C)** Cross-reactivity data of wildtype EL9-specific cells
740 with K588Q and K588R variants for 8 H:A-B*14-positive subjects determined by tetramer
741 staining.

742

743 **Figure 6. Impact of Gag-DA9 and Env-EL9 escape mutants on viral fitness and HIV**
744 **infection outcome. (A)** Replication kinetics of NL4-3 containing wild type p24 and Env
745 compared to the C-clade version of Gag-DA9 epitope and three other HLA-B*14-associated Gag
746 and Env mutants. Infectivity is expressed as %GFP-positive GXR reporter cells over 7 days after
747 infection. Error bars represent s.e.m. **(B)** Viral replication capacity of the viruses in A. ANOVA
748 with Dunnett's Multiple Comparison Test comparing NL4-3 versus the other viruses. Error bars
749 represent s.e.m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant ($p > 0.05$). Legend as in panel
750 (A). **(C-E)** Viral loads in HLA-B*14-positive (C), HLA-B*14:01-positive (D) and HLA-B*14:02-
751 positive (E) subjects with either wild type or mutated autologous sequences in Env-EL9 and
752 Gag-DA9 epitopes. X-axes indicate patients' autologous epitope sequences. Only significant p
753 values ($p > 0.05$), obtained by Mann-Whitney U test, are shown. Horizontal bars indicate
754 medians. Wt, wild type; var, variant sequence.

755

756 **Table 1. Frequency of HLA-B*14:01 versus HLA-B*14:02 among viraemic controllers and**
757 **non-controllers.**

758

759 **Table 2. HLA-B*14:02 is significantly enriched among viraemic controllers.**

760

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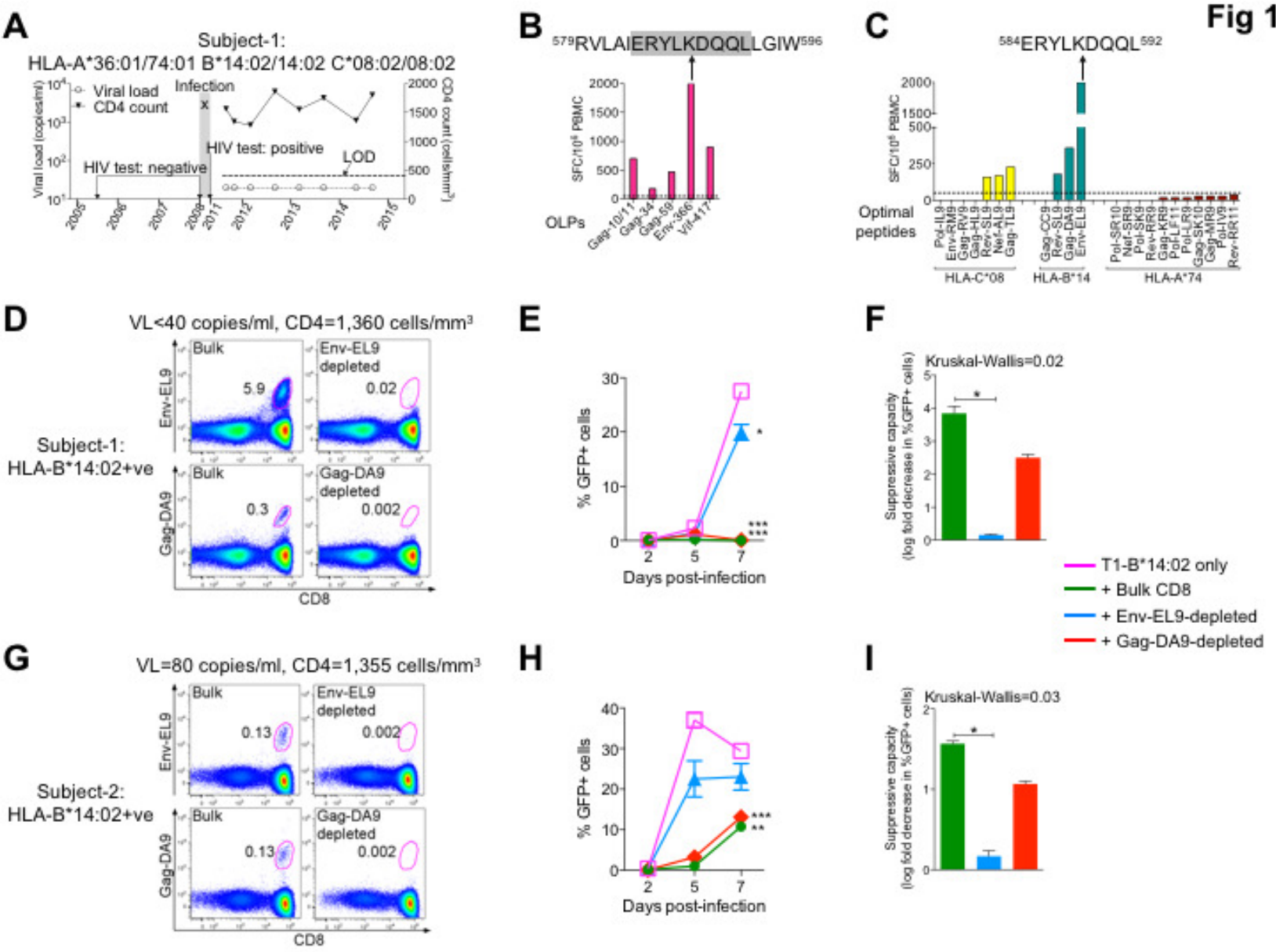


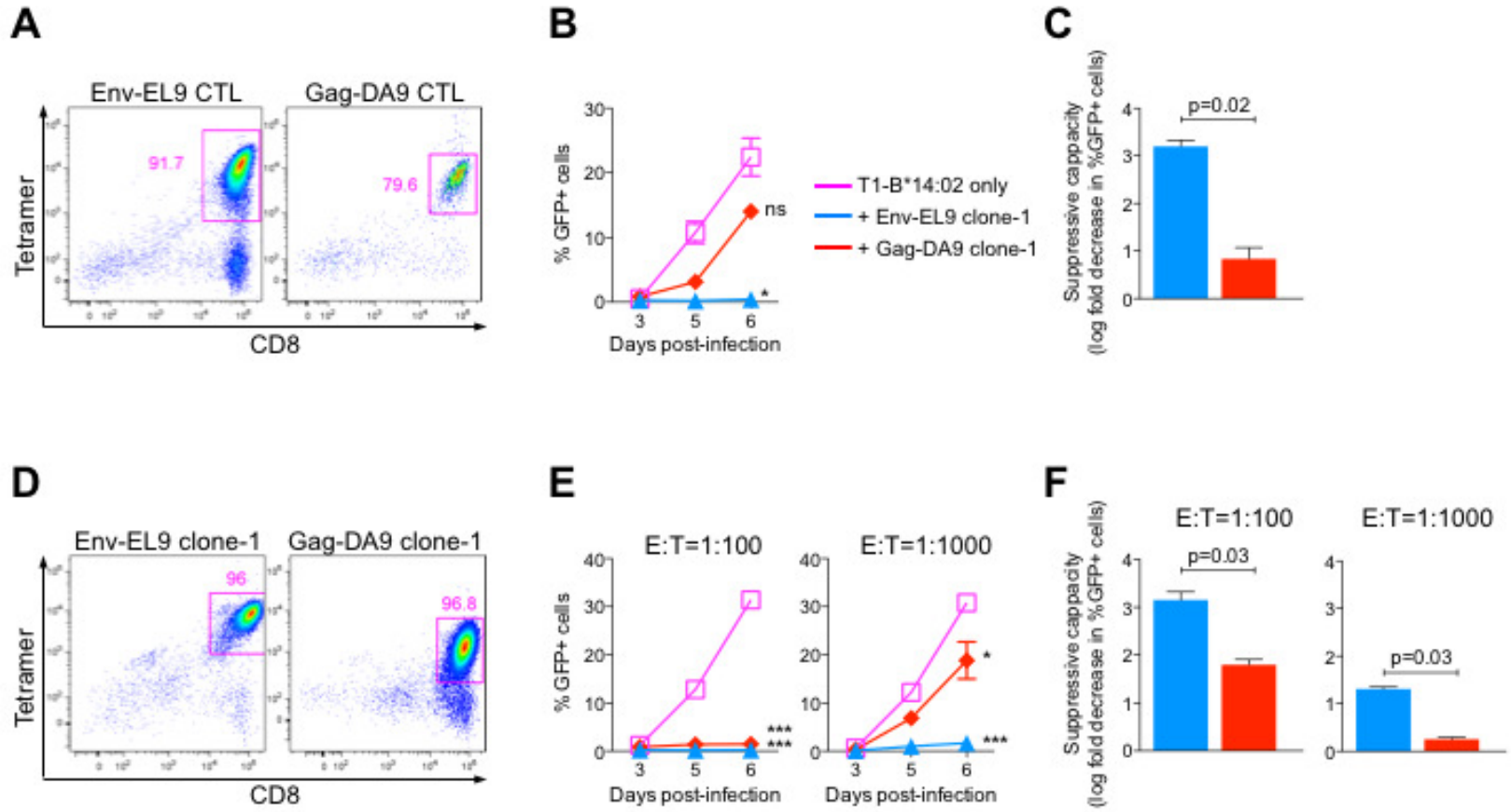
Fig 2

Fig 3

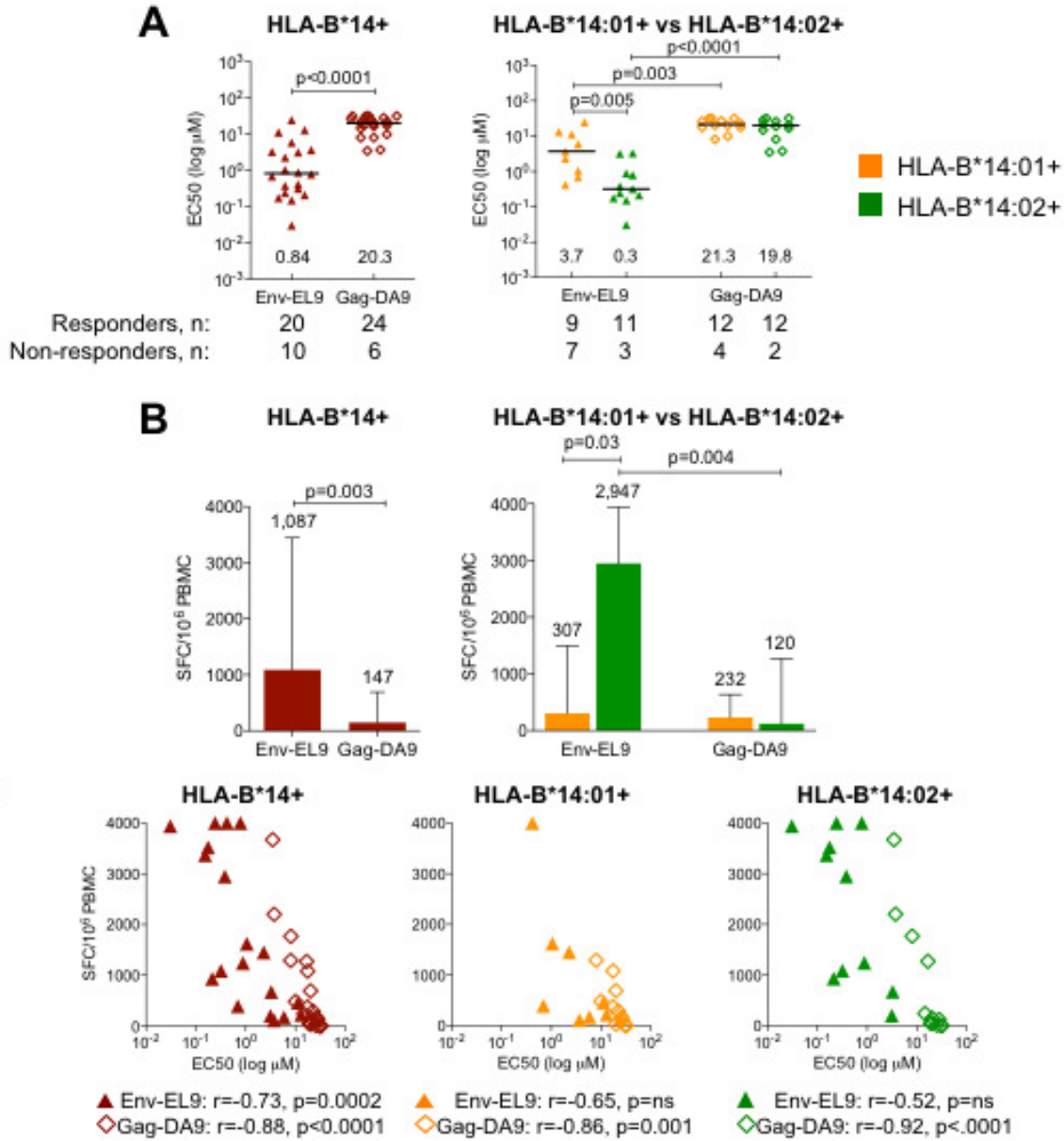


Fig 4

A

Consensus B clade		D	R	F	Y	³⁰² K	³⁰³ T	L	R	A	E	⁵⁸⁵ R	Y	L	⁵⁸⁸ K	D	Q	Q	⁵⁹² L
HLA-associated polymorphisms within DA9 or EL9	B*14:01	-	-	-	-	R	-	-	-	-	-	-	-	-	Q	-	-	-	-
	B*14:02	-	-	-	-	-	V	-	-	-	-	-	-	-	Q	-	-	-	-
	C*03:04 C*03:02/03/04	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	F
		-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-

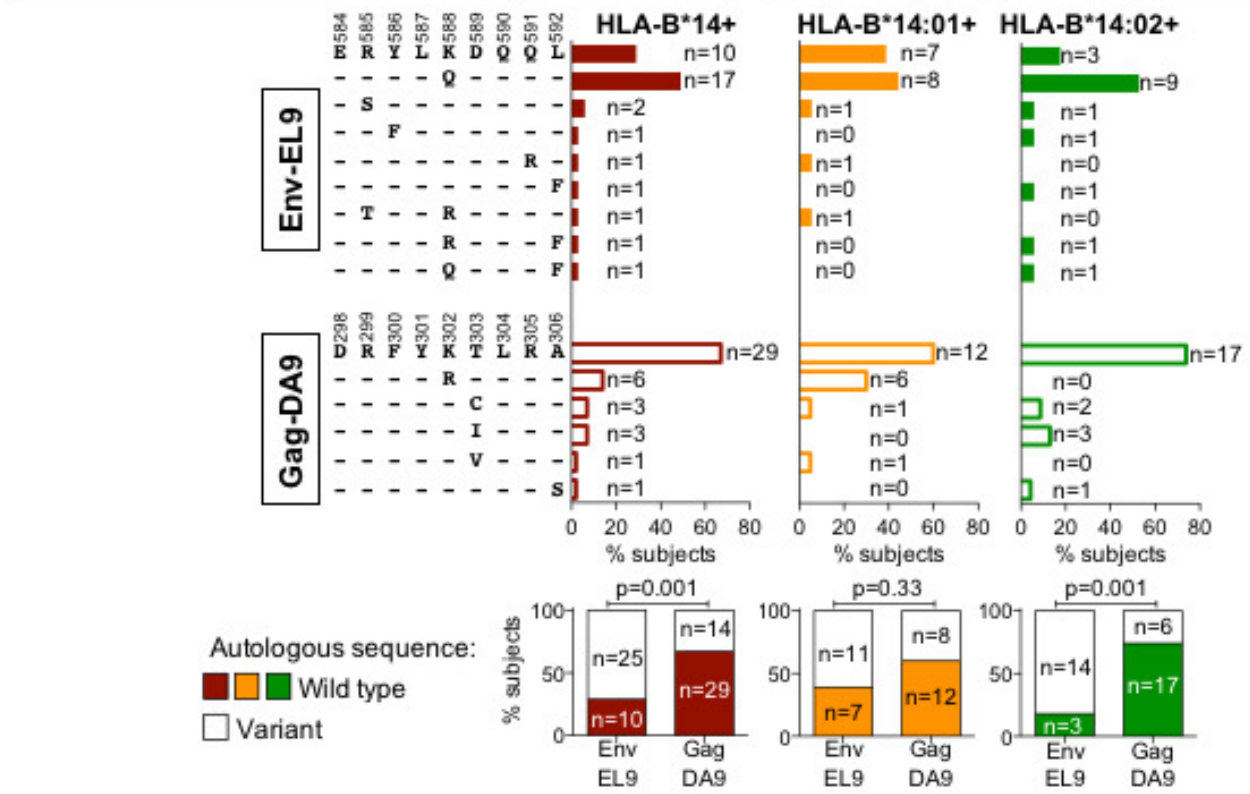
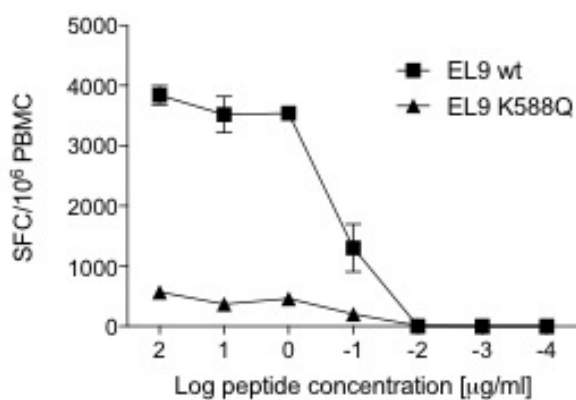
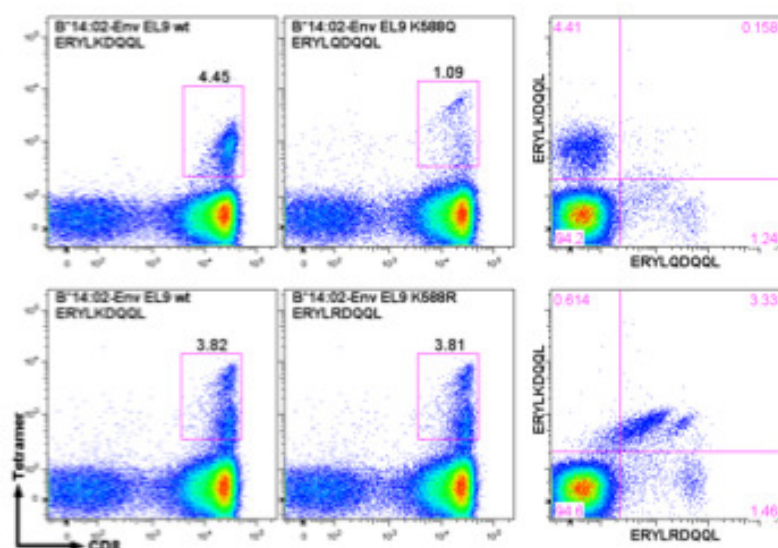


Fig 5

A



B



C

	OX018 K588Q	N079 K588Q	H016 K588Q	R117 K588Q	R055 K588Q	0026 K588Q	0145 wt	H006 wt	median
Env EL9 wt-specific	4.4%	6.9%	0.3%	0.6%	0.1%	1.4%	0.5%	2.8%	1.0%
Env EL9 wt total	4.6%	6.9%	0.3%	0.6%	0.1%	2.0%	0.5%	4.5%	1.3%
K588Q-specific	1.2%	0.3%	1.1%	1.7%	0.5%	0.9%	0.3%	1.7%	1.1%
K588Q total	1.2%	0.4%	1.1%	1.7%	0.5%	1.5%	0.3%	3.3%	1.2%
Cross-reactive wt/K588Q	0.2%	0.1%	0.0%	0.0%	0.0%	0.6%	0.0%	1.7%	0.1%
Env EL9 wt-specific	0.6%	0.5%	0.2%	0.1%	0.1%	0.6%	0.3%	0.2%	0.3%
Env EL9 wt total	3.9%	5.8%	0.2%	0.6%	0.2%	1.9%	0.6%	3.7%	1.3%
K588R-specific	1.5%	1.6%	0.1%	0.3%	1.6%	0.1%	0.2%	2.3%	0.9%
K588R total	4.8%	6.8%	0.1%	0.9%	1.7%	1.3%	0.5%	5.8%	1.5%
Cross-reactive wt/K588R	3.3%	5.2%	0.0%	0.5%	0.1%	1.3%	0.2%	3.5%	0.9%

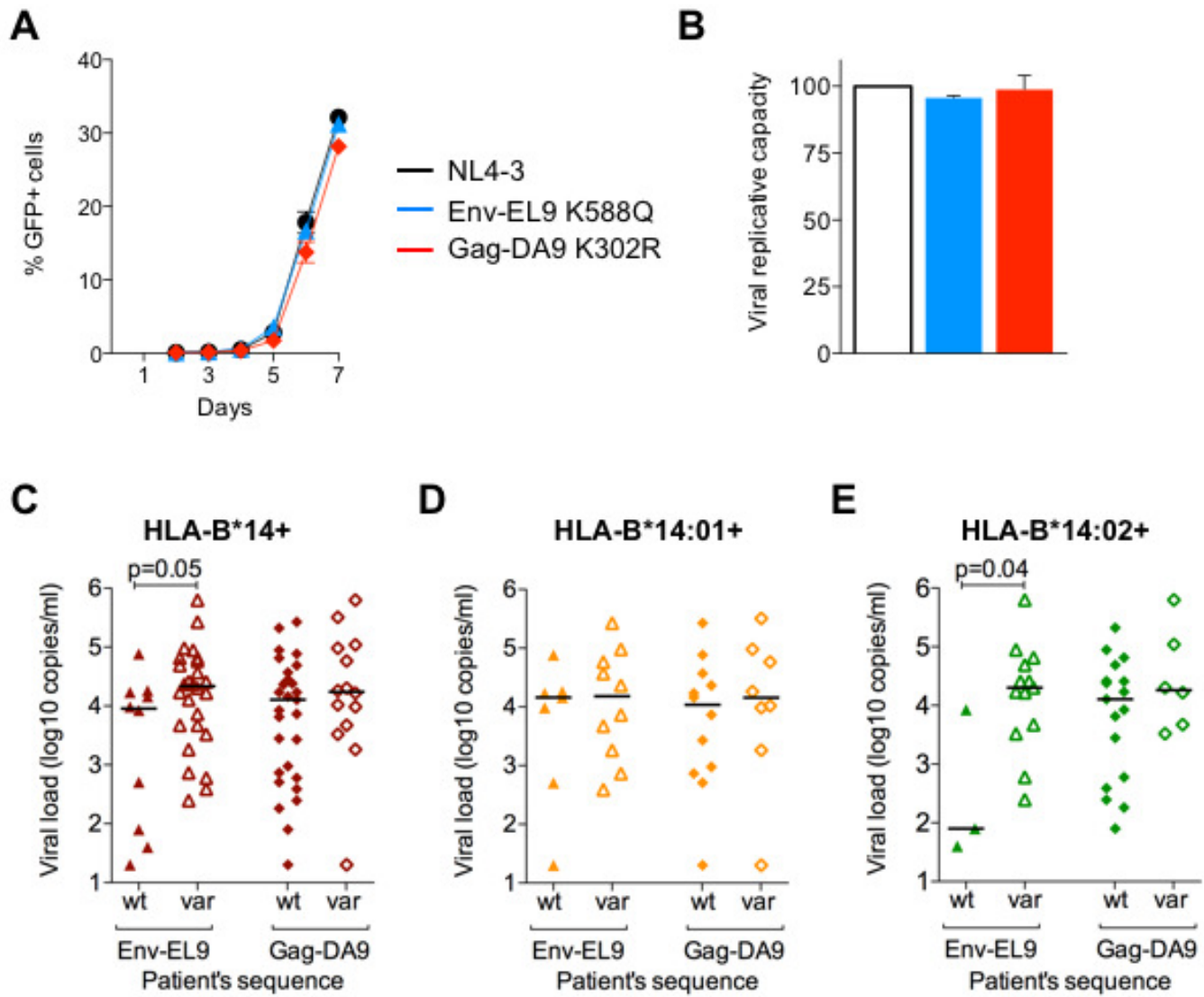
Fig 6

Table 1. Frequency of HLA-B*14:01 versus HLA-B*14:02 among viraemic controllers and non-controllers. ART-naïve chronically HIV-infected subjects were categorised as viraemic controllers (viral loads <2,000 copies/ml plasma) or non-controllers (viral loads >10,000 copies/ml plasma). Logistic regression was used to compute significance (p values), OR and 95%CI for the differences in frequency of HLA-B*14 subtypes in controllers versus non-controllers, with adjusting by HLA-B*57/27 expression. OR, odds ratio; CI, confidence interval; n/a, not applicable.

HLA-B*14+ (n=285) from 2,259 whites					HLA-B*14+ (n=104) from 1,745 blacks					
	Controllers, n	Non- controllers, n	OR (95% CI)	P value		Controllers, n	Non- controllers, n	OR (95% CI)	P value	
			Not adjusted					Not adjusted		
B*14:01	23	54	B*14:01 vs B*14:02	1.41 (0.80- 2.47)	0.23	B*14:01	7	B*14:01 vs B*14:02	1.96 (0.74- 5.17)	0.18
B*14:02	78	130				B*14:02	30			
			Adjusted for B*27, B*57					Adjusted for B*57, B*81		
B*14:01	23	54	B*14:01 vs B*14:02	1.54 (0.86- 2.77)	0.15	B*14:01	7	B*14:01 vs B*14:02	1.52 (0.56- 4.13)	0.41
B*14:02	78	130				B*14:02	30			

Table 2. HLA-B*14:02 is significantly enriched among viraemic controllers. Presence or absence of individual HLA-B alleles that have a frequency equal to or greater than that of HLA-B*14:01 were tested by logistic regression with stepwise selection. OR, odds ratio; CI, confidence interval.

	Whites (n=3,259)			Blacks (n=1,745)			
	Stepwise selection on HLA-B alleles that are of equal or greater frequency as B*14:01			Stepwise selection on HLA-B alleles that are of equal or greater frequency as B*14:01			
	OR	95%CI	P value	OR	95%CI	P value	
B*57:01 vs others	0.25	0.20-0.32	2e-31	B*57:03 vs others	0.15	0.11-0.21	2e-29
B*27:05 vs others	0.34	0.26-0.45	2e-15	B*81:01 vs others	0.20	0.12-0.33	1e-10
B*52:01 vs others	0.40	0.25-0.63	7e-5	B*39:10 vs others	0.22	0.11-0.45	2e-5
B*14:02 vs others	0.44	0.32-0.60	2e-7	B*57:01 vs others	0.41	0.19-0.91	3e-2
B*13:02 vs others	0.47	0.33-0.67	3e-5	B*27:05 vs others	0.44	0.21-0.91	3e-2
B*40:02 vs others	0.48	0.31-0.75	1e-3	B*14:02 vs others	0.54	0.32-0.90	2e-2
B*08:01 vs others	1.66	1.28-2.13	1e-4	B*07:02 vs others	1.45	1.01-2.09	4e-2
B*38:01 vs others	1.66	1.04-2.66	3e-2	B*53:01 vs others	1.51	1.12-2.03	7e-3
B*40:01 vs others	1.76	1.24-2.50	2e-3	B*35:01 vs others	1.92	1.29-2.86	1e-3
B*07:02 vs others	2.04	1.60-2.60	8e-9	B*15:10 vs others	2.27	1.33-3.89	3e-3
				B*58:02 vs others	2.63	1.51-4.59	6e-4
				B*45:01 vs others	4.10	2.36-7.13	6e-7