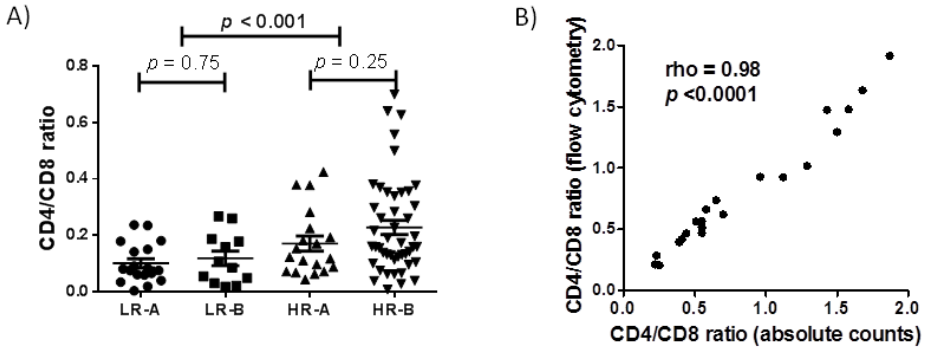
Supplementary Table 1. Clinical data of HIV-subjects from the case:control study.

| **Characteristics** | **LR-subjects (n=19)** | **HR-subjects (n=19)** | ***p*** |
|---|---|---|---|
| Male sex, n/n (%) | 19/19 (100) | 19/19 (100) | 1 |
| Age (years) | 39 [33-54] | 40 [34-49] | 0.8 |
| CD4 counts (cells/μL) | 70 [56-140] | 126 [46-165] | 0.28 |
| CD4/CD8 ratio[A] | 0.08 [0.04-0.15] | 0.12 [0.07-0.22] | **0.026** |
| VL (log HIV RNA copies/mL) | 4.8 [4.2-5.2] | 5.0 [4.6-5.7] | 0.23 |
| Time to diagnosis (months) | 1.0 [0-2.2] | 1.0 [0-4.0] | 0.26 |
| IDU transmission, n/n (%) | 2/19 (10%) | 3/19 (16%) | 0.6 |
| Previous C event, n/n (%) | 5/19 (26) | 6/19 (32) | 0.7 |
| Anti-HVC+[B], n/n (%) | 3/17 (18) | 1/18 (6) | 0.26 |
| X4-viral tropism[C], n/n (%) | 5/16 (31) | 2/17 (12) | 0.17 |

Continuous variables are expressed as median values (IQR) and categorical variables are expressed as number of cases (%). VL, Viral Load; IDU, Intravenous Drug User. Mann-Whitney U and Chi-squared tests were used to analyze continuous and categorical variables, respectively. [A]Calculated using frequencies from flow-cytometry. [B]Data only available from 17 LR-subjects and 18 HR-subjects. [C]Data only available from 16 LR-subjects and 17 HR-subjects.




Supplementary Figure 1

Supplementary Figure 1. Comparison between the CD4/CD8 ratio in the two different groups of LR- and HR-subjects. In the case:control study (LR-A and HR-A), CD4 and CD8 T-cell frequency determined by flow-cytometry were used to calculate the CD4/CD8 ratio, whereas in additional subjects from the Virgen del Rocío Hospital (LR-B and HR-B), clinical data of CD4 and CD8 T-cell absolute counts were used to calculate CD4/CD8 ratio (A). Mann-Whitney U-test was used for comparison. An additional group of HIV subjects, also from the Virgen del Rocío Hospital, had available CD4 and CD8 data from both, absolute values and cytometry values, and the correlation between such paired data of CD4/CD8 ratios was performed (B). Spearman rank test was used for the correlation.

SUPPLEMENTAL MATERIAL

*Supplemental information about subjects and the study design*

LR- and HR-subjects from the case:control study were selected from the Spanish AIDS Research Network (RIS) cohort CoRIS (1) and associated samples were obtained from the RIS-BB (2). Additional LR- and HR-subjects were HIV-infected subjects visiting the Infectious Diseases Service of the Universitary Hospital Virgen del Rocío between 2003 and 2013. The supplementary group of HIV-infected subjects used for the correlation between paired data of CD4/CD8 ratios, calculated by both absolute counts and cytometry frequencies, was also from the Infectious Diseases Service of the University Hospital Virgen del Rocío.

*Laboratory measurements*

For the case:control study, clinical and demographic data from HIV-infected individuals were obtained from CoRIS (1). For subjects from the Virgen del Rocío Hospital, the absolute numbers of CD4 and CD8 T-cells were determined in fresh blood with an Epics XL-MCL flow cytometer (Beckman Coulter) and plasma HIV-1 RNA levels were measured by quantitative PCR (COBAS Ampliprep/COBAS Taqman HIV-1 test, Roche Molecular Systems), according to the manufacturer's instructions. The detection limit for this assay was 40 HIV RNA copies/mL. Plasma samples were also tested for Hepatitis C virus antibodies using an HCV ELISA (Siemens Healthcare Diagnosis).

*Flow-Cytometry*

Briefly, thawed PBMCs were incubated at 4ºC with surface marker antibodies for 20 minutes, followed by fixation and permeabilization according to the manufacturer's instructions (FoxP3/Transcription Factor Staining Buffer, Ebioscience), and

subsequently incubated with intracellular FoxP3 for 30 minutes. Viable cells were identified using LIVE/DEAD fixable Aqua Blue Dead Cell Stain (Life Technologies, USA). Antibodies used were: anti-CD3, anti-CD4, anti-FoxP3, anti-ki67, anti-CD95 (BD Biosciences, USA) and anti-CD45RA (Life Technologies, CA, USA). Isotype controls for FoxP3, Ki67 and CD95 were included in each experiment. Flow cytometry was performed on LSR Fortessa (BD, USA), and a minimum of 1 x $10^6$ total events was collected. Analysis was performed using FlowJo version 9.2 (Tree Star) and data is expressed as frequencies and MFI (Mean Fluorescence Intensity). Regulatory T-cells (Treg) were defined using Miyara´s phenotype for activated/effector Treg (CD4+CD45RA-FoxP3$^{high}$) (3).

*Determination of viral tropism*

V3 loop amplification and sequencing were performed as previously described (4). The V3 sequences were interpreted using the bioinformatics genotypic tropism predictor geno2pheno (G2P), which is freely available online (http://coreceptor.bioinf.mpi-inf.mpg.de/index.php). A clonal version of G2P with false-positive rates (FPR) of 5% (G2P5%clo) was used. HIV-1 variants were classified as R5 or X4, with the latter classification including pure X4 and dual-tropic viruses.

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