Supporting Information

Pillai et al. 10.1073/pnas.1111573109

The Swiss HIV Cohort Study

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SI Materials and Methods

Subjects. Nineteen HIV/HCV-coinfected individuals from the Swiss HIV Cohort Study (SHCS) who underwent IFN-α/riba treatment were studied retrospectively (Table S1). The SHCS is a nationwide, clinic-based prospective cohort study with continuous enrolment and at least semi-annual study visits (www.shcs.ch) (1). It has been approved by ethical committees of all participating institutions, and written informed consent has been obtained from participants. All subjects had PBMC and plasma samples available before, during and after IFN-α/riba treatment, were not on antiretroviral therapy during the entire study period (all were ART-naïve), and had detectable HIV-1 RNA at baseline.

Specimens. Our study included blood plasma, cryopreserved PBMC (3×10^6 cells/sample) and CD4+ T-cells negatively selected from the cryopreserved PBMC using the EasySep CD4+ T Cell Enrichment Kit per manufacturer's protocol (Fig. S2).

Expression Profiling. Total RNA was extracted from PBMC and CD4+ T-cells using Trizol, followed by DNase treatment using Promega RQ1 RNase-Free DNase and clean up using the Qiagen RNeasy Plus Mini Kit. DNase-treated clean RNA was transcribed into cDNA using random primers and SuperScript III Reverse Transcriptase. 12 ng of each sample cDNA were amplified with Qiagen QuantiTect Probe PCR Master Mix on an ABI PRISM 7900HT Sequence Detection System. TaqMan primers/probes were used to evaluate APOBEC3G, APOBEC3F, BST-2, and ISG15 expression. Additional TaqMan primers/probes for a panel of five housekeeping genes were included [glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ribosomal protein L13a (RPL-13A), ubiquitin conjugating enzyme (UbcH5b), elongation factor 1-alpha (EF1-α), and metastatic lymph node 51 (MLN51)]. RPL-13A was identified as the most stably expressed gene from a total of five housekeeping genes using the GeNorm algorithm (2). Therefore, raw cycle threshold numbers of amplified gene products were normalized to the housekeeping gene, RPL-13A, (ribosomal protein L13a) to control for cDNA input amounts. Fold induction was determined using the comparative cycle threshold (CT) method (2).

HIV-1 *env-nef* Clonal Sequencing. Genomic DNA was extracted using the Trizol method. The HIV-1 *env-nef* region (532 bp) was amplified by nested PCR using previously published primer sequences and PCR cycling conditions (3). Degenerate versions of these primers were used to ensure that hypermutant and non-hypermutant sequence variants would be amplified with similar efficiency. Replicate PCR products were proportionately pooled (to minimize the probability of template resampling) and were cloned into the pCR4-TOPO vector using the TOPO TA Cloning Kit (Invitrogen). Approximately 20 clones per timepoint per individual were chosen for sequencing. Sequencing was performed in both directions using universal M13 primers.

HIV-1 *vpu* Population Sequencing. Nine individuals were chosen for *vpu* analyses based on sample availability. Viral RNA was extracted and purified from 1 mL plasma using the QIAmp viral RNA kit (Qiagen) after an initial concentration step (ultracentrifugation at 53,000 × *g*). Reverse Transcription was performed using SuperScript II Reverse Transcriptase (Invitrogen) using a gene-specific primer to generate the cDNA. A 40 μL reaction mixture including 15 μL RNA template, 2 μL reverse transcription primer (RT-21), 4 μL 10X RT Buffer, 8 μL 25 nM MgCl2, 4 μL 0.1 M DTT, 2 μL 10 mM dNTPs Mix, 2 μL SuperScript II RT Enzyme, 2 μL RNaseOUT (40U/μL), and 1 μL diethylpyrocarbonate (DEPC) water was added into a 0.6 mL tube, mixed thoroughly, incubated for 1 h at 42 °C, then for 15 min at 70 °C. The *vpu* region was amplified by nested PCR using degenerate primers Vpu-out-F 5'-CAA GCA GGA CAT AAC AAG GTA G-3', Vpu-out-R 5'-TAC AAT TTC TGR RTC CCC TCC TG-3', Vpu-in-F 5'-GGY TTA GGC ATY TYY TAT GGC AGG AAG AAG-3', Vpu-in-R 5'-CGA RTG RRR TTA ACT TTA CAC ATG-3'. PCR was performed using the Expand High Fidelity PCR System (Roche) according to the manufacturer instructions. Generated PCR products were gel purified using QIAquick Gel Extraction Kit (Qiagen), and sequenced in both directions using the nested amplification primers.

HIV-1 Vpu Phenotypic Analysis. Plasmids, antibodies, and reagents: pcDNA3.1 (Invitrogen, Carlsbad, CA) was used as an empty vector control. The pcDNA3.1-based plasmid expressing codon optimized Vpu (pVphu) were provided by Dr. Klaus Strebel (NIAID, Bethesda, MD). Mutations in pVphu were introduced using the Stratagene (La Jolla, CA) QuikChange kit and confirmed by nucleotide sequencing. The plasmid expressing GFP (pCG-GFP) was provided by Jacek Skowronski (Case Western Reserve University, Cleveland, OH). The murine monoclonal antibody to BST-2/HM1.24/CD317 was a gift from Chugai Pharmaceutical Co. (Kanagawa, Japan). An isotype control antibody, IgG2a, and a secondary goat anti-mouse IgG antibody conjugated to allophycocyanin (APC) were obtained from BioLegend (San Diego, CA). Cells and transfections: The HeLa cells used to evaluate BST-2 surface down-regulation, clone P4.R5, were obtained from Dr. Ned Landau (New York University, New York, NY); these cells are a derivative of clone P4 and were maintained in DMEM supplemented with 10% FBS, penicillin/streptomycin, and puromycin. Cells were transfected with Lipofectamine 2000 (Invitrogen) according to the supplied directions. For experiments evaluating BST-2 surface down-regulation, 100 ng of pVphu or pcDNA3.1 along with 50 ng of pCG-GFP were used to transfect 0.5 million HeLa P4.R5 cells in wells of a 12-well plate as previously reported (4). The next day, the cells were stained for surface BST-2 and analyzed by flow cytometry as described (4).

Sequence and Statistical Analysis. Phylogenetic reconstruction and BLAST were used to inspect sequences for inter-individual contamination or contamination with HIV-1 lab strains. Sequences were aligned using Multalin (5) and analyses were performed using Hypermut (www.hiv.lanl.gov) (6) and the HyPhy software

package (www.hyphy.org) (7). HIV-1 subtype was determined by applying the REGA HIV-1 subtyping tool (8) to *pol* sequences that were previously generated for drug resistance genotyping at the SHCS. A battery of non-parametric statistical tests (Spearman's rank correlation coefficient, Mann-Whitney U test, Paired Wilcoxon) was applied to gene expression and genetic data using GraphPad Prism v5.0c. In cases where a (parametric) Pearson's r test was used, the Kolmogorov-Smirnov test was implemented beforehand to determine that the data were distributed normally.

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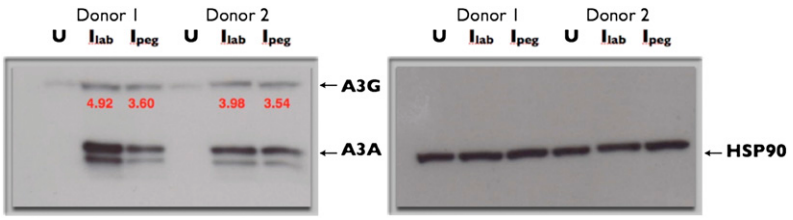

Fig. S1. Pegylated IFN-α induces apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3 (APOBEC3G) expression in vitro. Primary monocytes were isolated from two uninfected donors and were plated for 1 wk at 500,000 cells/mL. Cells were left untreated (U) or were treated with either laboratory-grade nonpegylated IFN-α (Ilab) or clinical grade pegylated IFN-α (Ipeg; equal molarities) for 2 d. APOBEC3G and HSP90 (loading control) were measured by Western blotting. APOBEC3G was also measured by real-time PCR to ensure that mRNA copy number reflects protein concentration (RT-PCR fold induction with respect to untreated control is listed on each lane in red).

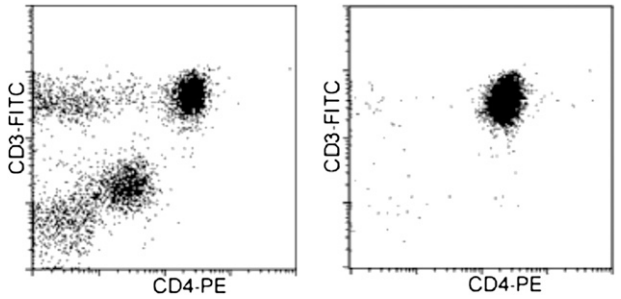

Fig. S2. Purity of negatively selected CD4+ T cells. Flow cytometry in *Left* represents unfractionated peripheral blood mononuclear cells (PBMC), and flow cytometry in *Right* represents cellular population after negative selection (>97% purity).

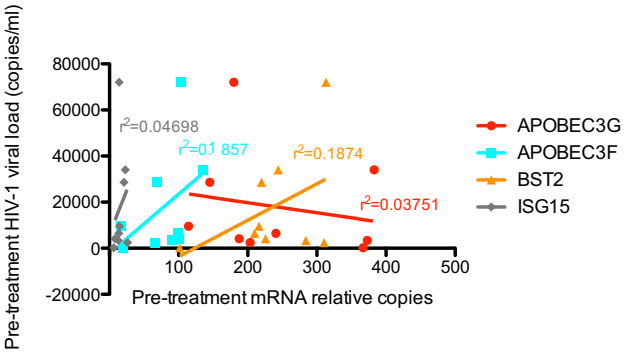

Fig. S3. Relationship between HIV-1 viral load and restriction factor expression pre–IFN-α/ribavirin treatment. Pretreatment HIV-1 viral load was compared with pretreatment mRNA relative copy numbers of APOBEC3G, APOBEC3F, bone marrow stromal cell antigen 2 (BST-2/tetherin/CD317), and IFN-stimulated gene 15 (ISG15) in isolated CD4+ T cells. No significant correlations between viral load and restriction factor expression were identified.

Table S1. Subject characteristics at baseline and IFN-α/riba treatment regimen

Subject	Sex	Age (y)	Risk group*	Duration of HIV-1 infection (y)	CDC disease stage†	HIV-1 subtype	HIV-1 viral load (copies/mL)	CD4+ count (cells/μL)	IFN-α dose‡ *(μg/wk)*	Riba dose‡ *(mg/d)*	Treatment duration (wk)
A	M	38	IDU	11	A	B	43,200	404	120	1,000	51
B	M	45	OTHER	13	B	B	55,215	270	180	1,200	14
C	M	42	MSM	5	A	B	78,886	587	100	800	26
D	F	26	HET	2	A	B	13,100	374	80	800	48
E	M	28	HET	3	A	B	167,500	501	180	800	26
F	F	31	IDU	7	B	B	5,540	463	180	800	25
G	M	33	BLOOD	9	A	B	3,128	259	135	800	48
H	M	32	IDU	5	A	B	62,900	461	180	1,000	16
I	M	32	MSM	6	A	B	4,070	755	180	800	47
J	M	39	IDU	19	A	B	8,620	590	120	1,000	47
K	M	37	HET	4	B	B	34,050	570	50	600	48
L	M	52	MSM	5	A	B	28,600	444	180	800	25
M	F	27	IDU	6	C	B	3,360	510	180	800	23
N	M	42	IDU	18	C	B	183	332	180	1,000	48
O	F	24	HET	4	A	J/B/F¶	6,490	839	135	800	24
P	M	39	HET	19	A	B	4,110	336	180	800	21
Q	F	37	IDU	10	B	B	2,425	599	72	800	47
R	M	27	IDU	4	A	B	72,000	698	100	1,000	47
S	M	30	IDU	7	A	B	9,550	696	180	1,200	48
Median	—	33	—	6	—	—	9,550	501	180	800	47

*BLOOD, transfusion recipient; HET, heterosexual; IDU, i.v. drug use; MSM, men who have sex with men.

†A, asymptomatic, acute infection or persistent generalized lymphadenopathy; B, symptomatic conditions; C, AIDS indicator conditions; CDC, Centers for Disease Control and Prevention.

‡Doses shown are doses at the time of the on-treatment sampling, and in some cases, they reflect dose modifications in response to treatment side effects.

¶*pol* sequence did not correspond to any particular HIV-1 subtype or circulating recombinant form; subregions exhibited homology to subtypes J, B, and F.