and TGFβ 1 (10 ng/ml)(Peprotech) for one week. Differentiated Langerhans cells were matured with 1 μg/ml Peptidoglycan (Sigma) overnight. Cells were pulsed with HIV-1 peptide pools (10 μM total) for 3 h and irradiated (3000 rad). Fresh donor PBMC were prepared by Ficoll-Paque (Amersham Biosciences) centrifugation. Donor PBMC were plated at a density of 10^7cells/ml together with 5×10^4/ml peptide loaded irradiated donor Langerhans cells in 24 well culture plates in complete DMEM medium with 10 μM HIV-1 peptide pools. Cultures were fed with 10 u/ml IL-2 (BD biosciences) five days after stimulation and re-stimulated with peptide loaded irradiated (3000 rads) fresh donor PMBC every week. Replicate 96 well plates were pulsed with 1 μCi per well ^{3}H Thymidine after 4 days of culture, harvested after 18 h, and ^{3}H Thymidine incorporation was detected using a β Counter (Perkin Elmer 1450 LSC).

CTL analysis

A0201$^+$ TAP- deficient T2 hybridoma target cells (ATCC) or A2 supertype transfected 721.221 cell lines were plated at a density of 10^6 cells/ml in 24 well plates. Cells were pulsed with 10 μM A0201-restricted HIV-1 peptide pool or 10 μM A0201-restricted HTLV-TAX as negative control peptide for 18 hours at 37°C. Cells were washed twice with DMEM media (20% fetal calf serum, L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), 2-ME (50 μM). (DMEM media). and pulsed with 100 μC of ^{51}Cr for 90 minutes at 37°C. Target cells were washed three times with serum free OptiMEM media (Gibco) to remove excess ^{51}Cr and plated with sorted HIV-1 specific CD8$^+$ T cells at 30:1, 10:1, 3:1 and 1:1 ratios. After four hours of incubation 50 μl of culture supernatant were mixed with liquid scintillation cocktail (Perkin Elmer Optiphase supermix) and analyzed for ^{51}Cr release using a Luminescence Counter (Perkin Elmer 1450 LSC). Percent specific chromium release was calculated using the formula [(Experimental release- spontaneous release)/(maximum release in 5% Triton-x100- spontaneous release)]. To analyze CTL responses to naturally processed HIV-1 epitopes, TAP sufficient T1 hybridoma target cells T1 (A0201+, B5101$^+$, CD4$^+$) were infected with 0.5 MOI HIV-IIIB per cell for 3 hours. Cells were washed and plated at a density of 10^6/ml in DMEM media. 2–3 days later, HIV-IIIB infected T1 cells were used in ^{51}Cr release assay as described above. Intracellular p24 staining by FACS was used to demonstrate that essentially all target cells were HIV-IIIB-infected.

IFNγ-ELISPOT assay on HIV-1 infected donor cells

CD8$^+$ T-cell responses to pools of HIV-1 epitopes were quantified by gamma interferon (IFN-γ) ELISpot assay as follows. Peripheral blood mononuclear cells (PBMC) isolated from HIV-1 infected patients were plated at 100,000 per well with peptide pools at a final concentration of 10 μM in anti-interferon gamma mAb 1-D1K (Mabtech, Stockholm, Sweden) coated polyvinylidene 96-well plates (Millipore.MA) and processed as previously described [21]. For each individual peptide, the assay was run in triplicate. Negative and positive controls were obtained by incubating individual PBMC with medium alone (negative control) and phytohemagglutinin (PHA) as a positive control for naive patients. For the assessment of general immunocompetence of HIV-1 infected patients, a CEF peptide pool was used as an internal positive control. The CEF pool consists of optimal T cell epitopes for CMV/EBV and Influenza viruses provided by the NIH reagent program. Only HIV-1 patients whose blood yielded positive CEF and/or PHA responses were chosen as subjects for the present study.

The number of specific IFN-γ secreting T cells was determined with an automated ELISPOT reader (AID, Strassberg, Germany), calculated by subtracting the average negative control value and expressed as the number of spot-forming cells (SFC) per 10^6 input cells. Negative controls were approximately 50 SFC per 10^6 input cells for HIV-1 infected patients and around 40 for naïve subjects. A response was considered positive if there were 50 SFC per 10^6 input cells and the activity was at least three times as great as the mean background activity.

IFNγ– production on HIV-1 specific T cell lines generated from naive donors

HIV-1 peptide pool-specific T cell lines were generated as described above. Two weeks after the second stimulation, 25,000 per well HIV-1 specific T cells were plated into anti-interferon gamma mAB 1-D1K (Mabtech) coated polyvinylidene 96-well plates (Millipore) with 25,000 per well irradiated donor PBMCs and stimulated with 10 μM of each HIV-1 peptide pool. Plates were processed and spot forming cells were calculated as described above.

HLA-A 0201-binding assay

A0201$^+$ TAP- deficient T2 hybridoma cells (ATCC) were plated at a density of 10^6 cells/ml in 24 well plates. Cells were pulsed with 10 μM A0201 restricted HIV-1B peptides, 10 μM A0201restricted HTLV-TAX (LLFGYPVYV) positive control peptide and 5 μg/ml β 2 microglobulin (BD biosciences) for 18 hours at 37°C in serum free AIM5 media (Gibco). A0201 expression was determined by flow cytometry (FACSAria) using FITC-conjugated BB7.2 mAb (BDbiosciences). Mean cell fluorescence (MCF) intensities were normalized to HTLV-TAX positive control peptide using the formula [(MCF.sample- MCF.control)/(MCF.HTLV-TAT-MCF.control)].

DimerX staining

A2:Ig fusion protein DimerX (BD Biosciences) was passive loaded with individual A0201 restricted HIV-1B peptides