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ORIGINAL ARTICLE

Analysis of the CD8$^+$ T cell anti-HIV activity in heterologous cell co-cultures reveals the benefit of multiple HLA class I matches

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Abstract CD8$^+$ T lymphocytes can reduce the production of human immunodeficiency virus 1 (HIV-1) by CD4$^+$ T cells by cytotoxic and non-cytotoxic mechanisms. To investigate the involvement of human leukocyte antigen (HLA) class I compatibility in anti-HIV responses, we co-cultured primary CD8$^+$ T cells, isolated from the peripheral blood of HIV-1-infected individuals, with panels of autologous and heterologous acutely HIV-1-infected primary CD4$^+$ T cells. Altogether, CD8$^+$ T cell anti-HIV activity was evaluated in more than 200 co-cultures. Marked heterogeneity in HIV-1 replication levels was observed among the co-cultures sharing a common CD8$^+$ T cell source. The co-cultures that exhibited greater than 50% reduction in HIV production were found to have significantly increased numbers of matching HLA class I alleles (Yates chi-square = 54.21; $p < 0.001$). With CD8$^+$ T cells from HIV controllers and asymptomatic viremic individuals, matching HLA-B and/or HLA-C alleles were more predictive of strong anti-HIV activity than matching HLA-A alleles. Overall, HLA class I genotype matches were more closely associated with CD8$^+$ T cell anti-HIV activity than supertype pairings. Antibodies against HLA class I and CD3 reduced the CD8$^+$ T cell anti-HIV activity. Stimulated CD8$^+$ T cells exhibited increased anti-HIV activity and reduced dependency on HLA compatibility. These findings provide evidence that the maximal suppression of HIV replication by CD8$^+$ T cells requires the recognition of multiple epitopes. These studies provide insight for HIV vaccine development, and the analytic approach can be useful for the functional characterization of HLA class I alleles and tentative HLA class I supertypes.

Keywords HIV-1 · CD8$^+$ T cells · Antiviral immunity · HLA class I · Supertype · Elite controllers

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Introduction

Thirty-five years ago, the human immunodeficiency virus 1 (HIV-1) was identified as the causative agent of acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi et al. 1983). Since then, many studies have found that protection from HIV-1 infection and disease progression is associated with strong CD8$^+$ T cell antiviral responses (Freel et al. 2011). These responses include the CD8$^+$ T cell non-cytotoxic anti-HIV response (CNAR) (Walker et al. 1986) and the killing of virus-infected cells by antigen-specific cytotoxic T lymphocytes (CTL) (Walker et al. 1987). The relative contributions of these two mechanisms to CD8$^+$ T cell-mediated anti-HIV immunity have not been fully determined (Davenport and Petravic 2010).

Besides CTL activity (Addo et al. 2003; Ho et al. 1993; Makadzange et al. 2010; Migueles et al. 2008; Walker et al. 1987), primary CD8$^+$ T cells from HIV-1-infected individuals can potently suppress the replication of divergent HIV strains in CD4$^+$ T cells without eliminating the infected cells (Mackewicz and Levy 1992; Mackewicz et al. 1998; Walker et al. 1991a; Walker et al. 1986; Walker et al. 1991b; Wiviott et al. 1990). Although maximal suppression levels are generally observed in cell-to-cell contact assays, CD8$^+$ T cells can secrete factors that reduce HIV replication levels. These

soluble factors include the $CD8^+$ cell antiviral factor (CAF) that inhibits HIV transcription (Mackewicz et al. 1995; Walker and Levy 1989), a variety of β-chemokines that block virus attachment (Cocchi et al. 1995), and possibly other cytokines and cellular products (Cocchi et al. 2012; DeVico and Gallo 2004; Levy 2015). In comparison to the cell culture conditions generally required for measuring cytotoxic activity (Ho et al. 1993; Migueles et al. 2008), the non-cytotoxic suppression of HIV-1 replication can be observed with relatively low input ratios of *ex vivo* $CD8^+$ T cells (Levy 2003). Nonetheless, both HIV-1-specific CTL (Makadzange et al. 2010) and anti-HIV-1-non-cytotoxic $CD8^+$ T cells (Killian et al. 2011) exhibit a "memory cell" phenotype (e.g., $CD45RA^-$, $CD27^+$, $CCR7^-$, $PD\text{-}1^+$ cells). Moreover, IL-2, IL-15, and CD3/CD28 co-stimulation enhance the anti-HIV activity (Barker et al. 1997; Castelli et al. 2004), while IL-4 and IL-10 reduce this $CD8^+$ T cell response (Barker et al. 1995). These attributes suggest that inhibition of HIV-1 production is carried out by memory $CD8^+$ T cells having a Th1 profile and perhaps that the same cells can mediate both cytotoxic and non-cytotoxic activity.

The $CD8^+$ T cell non-cytotoxic anti-HIV activity arises during the acute stage of HIV-1 infection (Mackewicz et al. 1994; Streeck and Nixon 2010) and correlates with a slower course of disease progression (Castelli et al. 2002; Ferbas et al. 1995; Gomez et al. 1994). In comparison to $CD8^+$ T cells from most uninfected individuals, those from HIV-1-infected subjects exhibit greatly increased non-cytotoxic anti-HIV activity (Killian et al. 2011; Killian et al. 2005a; Rosok et al. 1997). This anti-HIV activity rapidly declines following antiretroviral therapy (Killian et al. 2009; Stranford et al. 2001; Wilkinson et al. 1999), likely due to decreases in the levels of cognate antigens, $CD8^+$ T cell activation, and the frequency of circulating memory $CD8^+$ T cells that accompany drug-suppressed virus replication (Giorgi et al. 1998). Notably, studies of SIV-infected rhesus macaques suggest that $CD8^+$ T cell-mediated non-cytotoxic activity, rather than CTL activity, is largely responsible for controlling virus replication in the animals (Andrieu et al. 2014; Klatt et al. 2010; Wong et al. 2010).

Human leukocyte antigen (HLA) class I is expressed on all nucleated cells and functions to display intracellular protein fragments on the cell surface, particularly for recognition by $CD8^+$ T cells (Abbas and Lichtman 2009; Marsh et al. 2000). The HLA class I-A, I-B, and I-C alleles are highly polymorphic, and individual HLA molecules present distinct peptides that are 8–12 amino acids long (Falk et al. 1991; Natarajan et al. 1999). Memory $CD8^+$ T cells are antigen-experienced clonal populations of cells that rapidly respond to a restricted array of HLA class I-bound peptides (Killian et al. 2002). However, divergent HLA class I molecules can be clustered into groups called "supertypes" that present peptides having similar motifs and potential cross-reactivity with clonal $CD8^+$ T cell populations (Sidney et al. 2008). Here, we report studies showing that inhibition of HIV-1 production by bulk $CD8^+$ T cells that have not been artificially stimulated in vitro (hereafter referred to as unstimulated) is directly associated with the number of matching HLA class I alleles on the $CD4^+$ target cells, suggesting that the maximal suppression of HIV replication requires the recognition of multiple HIV epitopes. We also show that the anti-HIV activity of $CD8^+$ T cells that have been stimulated in vitro is less dependent on HLA compatibility.

Materials and methods

Study subjects The HIV-1-infected subjects (n = 26; Table 1) who participated in this study were men reporting sex with men as their primary risk for having acquired HIV-1 infection. Most HIV-1-infected participants were asymptomatic, not receiving antiretroviral therapy, and had normal $CD4^+$ T cell counts (>400 $CD4^+$ T cells/μl). Included were 11 HIV controllers who exhibited low viral loads in the absence of antiretroviral therapy (6 elite controllers who had <75 HIV-1 RNA copies/ml of plasma and 5 low viremia controllers who had <650 HIV-1 RNA copies/ml). Blood from healthy HIV-negative donors was obtained from a local blood bank (Blood Centers of the Pacific, San Francisco, CA). Each participant signed informed consent forms, and the study received approval from the Committee for Human Research at the University of California San Francisco (UCSF).

Measurement of the $CD8^+$ T cell anti-HIV response To measure $CD8^+$ T cell anti-HIV activity, incremental numbers of $CD8^+$ T cells were co-cultured with autologous (i.e., from the same donor) or heterologous (i.e., from a different donor) acutely HIV-1-infected $CD4^+$ T cells and the ensuing level of HIV replication was measured (Fig. 1) (Killian et al. 2009). Briefly, whole blood was collected from each subject by venipuncture into sodium heparin vacutainer tubes (BD). The blood was separated over Ficoll (Sigma) to obtain peripheral blood mononuclear cells (PBMCs), and purified into populations of primary $CD8^+$ T and $CD4^+$ T cells using immunomagnetic beads (Miltenyi). The purities of the isolated $CD8^+$ T and $CD4^+$ T cell populations were consistently >95% in routine flow cytometric assessments (data not shown). The freshly isolated $CD8^+$ T cells were resuspended in freezing medium (90% fetal calf serum (FCS), 10% dimethyl sulfoxide, *v*/*v*) and cryopreserved in liquid nitrogen. The $CD4^+$ T cells were resuspended (3×10^6 cells/ml) in growth medium [RPMI 1640 medium supplemented with FCS (heat-inactivated at 56 °C for 30 min, 10% *v*/*v*), penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and recombinant human IL-2 (100 U/ml; Invitrogen)] and stimulated for 3 days in the presence of phytohemagglutinin-leucoagglutinin (PHA-L, 3 μg/ml, Sigma) in a 37 °C

Table 1 HLA class I and II genotypes, clinical, and demographic features of the HIV-infected subjects studied

Subject	HLA class I genotype (HLA A, B, C)	HLA class II genotype (DQB1, DRB1)	Age (years)	$CD4^+$ cells/μl	Viral load[a] (HIV RNA copies/ml)	HAART[b]	CCR5 genotype	Race/ ethnicity
s01	1101/2501, 0801/1402, 0701/0802	0301/0501, 0102/1104	63	568	28,622	N	$^+/^+$	C
s02	0301/3201, 1402/4403, 0401/0802	0302/0501, 0102/0402	56	502	4220	N	$^+/^+$	C
s03	0301/2902, 0702/4403, 0702/1601	0201/0602, 0701/1501	35	795	<75	N	$^+/^+$	C
s04	2601/3002, 1401/1503, 0210/0802	0201/0201, 0701/0901	56	445	<75	Y	$^+/^+$	AA
s05	0205/3201, 5703/5703, 1800/1800	0501/0502, 13/1602	56	1609	<75	N	$^+/^+$	AA
s06	0201/0301, 4402/5101, 0501/1402	N/A	55	410	<75	N	+/+	C
s07	0201/1101, 4402/5201, 0501/1202	0301/0602, 0101/0103	50	242	40,000	N	$^+/\Delta 32$	C
s08	0101/2301, 5001/5701, 0602/0602	0201/0603, 0301/1301	59	484	<75	Y	$^+/^+$	C
s09	0101/3201, 0801/2705, 0201/0701	0201/0501, 0101/0301	69	966	<75	Y	$^+/^+$	C
s10	0201/0301, 0702/4402, 0501/0702	0301/0402, 0408/0804	47	730	650	N	$^+/^+$	C
s11	0101/2402, 5101/5701, 0602/1506	0301/0303, 0701/1101	48	948	14,097	N	$^+/^+$	C
s12	0201/3002, 0801/2705, 0102/0701	0202/0602, 0701/1501	63	722	75,000	N	$^+/\Delta$ 32	C
s13	0301/3201, 5101/5701, 0602/1506	0301/0301, 1101/1101	53	892	103	N	$^+/^+$	C
s14	1101/3301, 1402/2702, 0202/0802	0302/0501, 0102/0403	50	575	<75	N	$^+/^+$	C
s15	2402/2902, 1501/4403, 0303/1601	0201/0603, 0701/1301	47	454	2000	N	$^+/^+$	C
s16	0101/2402, 0801/5701, 0602/0701	0203/0302, 0307/0307	59	1301	<75	Y	$^+/^+$	C
s17	0201/0301, 1402/4001, 0304/0802	0501/0604, 0102/1302	46	529	3401	N	$^+/^+$	C
s18	1101/1101, 3802/5401, 0102/0702	0301/0401, 0405/1202	53	1001	434	N	$^+/^+$	A
s19	0201/2402, 1501/1507, 0303/0303	0301/0302, 0401/1305	42	513	246	N	$^+/\Delta$ 32	C
s20	0103/3201, 3508/7301, 0401/1505	0301/0502, 1101/1501	48	769	<75	N	$^+/\Delta$ 32	C
s21	3101/3201, 1402/4002, 0802/1502	0201/0501, 0102/0701	55	784	3160	N	$^+/^+$	AA/H
s22	0201/0301, 0801/5101, 0304/1502	0201/0501, 0101,0301	60	711	178	N	$^+/^+$	C
s23	0201/3301, 1402/3701, 0602/0802	0301/0501, 0102/1103	57	675	<75	N	$^+/^+$	C
s24	0201/0301, 3801/4402, 5101/1203	0301/0603, 0103/1301	61	524	<75	Y	$^+/\Delta$ 32	C
s25	0301/2601, 3503/4402, 0401/0704	0301/0602, 1101/1501	59	870	<75	Y	$^+/^+$	C
s26	0102/0201, 0801/2705, 0102/0701	0201/0301, 0103/0301	61	555	10,000	N	$^+/^+$	C

C Caucasian, *AA* African American, *A* Asian, *H* Hispanic, *N/A* not available

[a] Value for viral load test result closest to specimen date; some viral load measurements were self-reported

[b] HAART, combination antiretroviral therapy, yes/no (Y/N). $CD4^+$ T cells, but not necessarily $CD8^+$ T cells, were included from the study subjects on HAART

humidified incubator. Subsequently, 10^7 stimulated $CD4^+$ T cells were washed with RPMI medium and resuspended in 1 ml of HIV-1_{SF33} (10,000 $TCID_{50}$/ml in PBMC) for 1 h at 37 °C with periodic mixing. HIV-1_{SF33} is a syncytium-inducing (SI), CXCR4-tropic (X4) isolate that has been maintained in primary cells since its isolation, exhibits rapid replication kinetics with a high degree of cytopathicity, and is not sensitive to β-chemokine-mediated antiviral effects (Mackewicz et al. 1997). The acutely HIV-1-infected $CD4^+$ T cells were then washed and cryopreserved as described above. Thawed $CD4^+$ T cells were resuspended at 10^6 cells/ml of growth medium, and 100-μl aliquots were placed into wells of a flat-bottom 96-well tissue culture plate (Falcon 3072, BD) in triplicate. Co-cultures were established by adding thawed $CD8^+$ T cells (100 μl) to wells containing acutely infected $CD4^+$ T cells at 1:1 and 0.5:1 $CD8^+$ T cell to $CD4^+$ T cell input ratios. In all experiments, unstimulated $CD8^+$ T cells were co-cultured with the acutely HIV-infected $CD4^+$ T cells. In some experiments, parallel co-cultures were established with $CD8^+$ T cells that had been stimulated in vitro for 3 days with either phytohemagglutinin (PHA; Sigma) or anti-CD3 antibody (BD) conjugated to magnetic beads (Dynal).

To measure HIV replication levels in the cultures, 100-μl aliquots of the cell culture supernatant from each well were collected on days 3 and 6 of culture, centrifuged at 16,000×*g* for 1 h at 4 °C, and the resulting virus pellets were assayed for reverse transcriptase (RT) activity (Hoffman et al. 1985). In ongoing cultures, the supernatant removed for measurement of HIV levels was replaced with an equal volume of fresh growth medium. To enumerate the $CD8^+$ T cell anti-HIV activity in each culture,

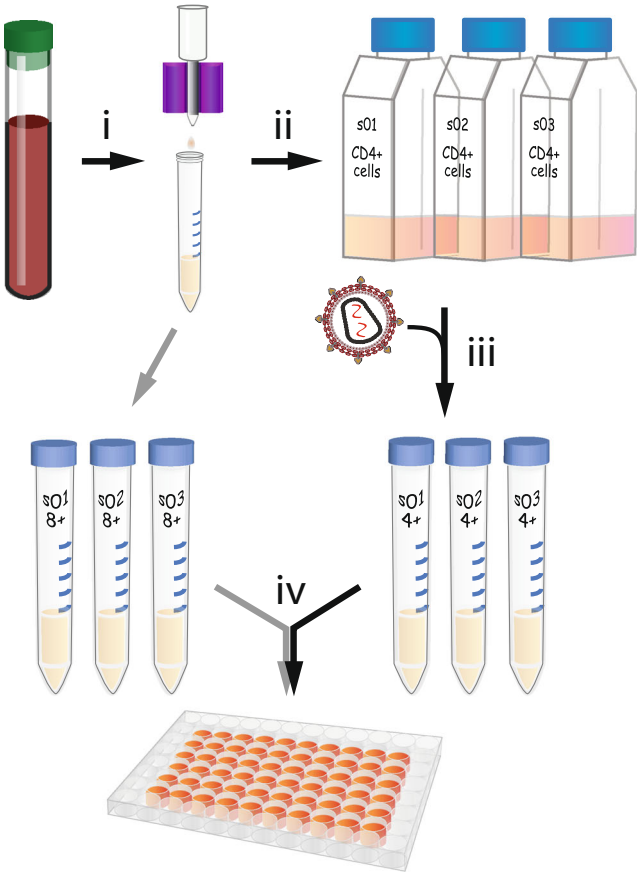

Fig. 1 Overview of the approach. Whole blood was collected from HIV-1-infected study participants, and peripheral blood mononuclear cells (PBMCs) were then isolated using a Ficoll gradient and manipulated as follows: *i* the resulting PBMCs were labeled with immunomagnetic beads and processed to obtain purified populations of $CD8^+$ and $CD4^+$ T cells; *ii* the purified $CD4^+$ T cells were stimulated with PHA-L for 3 days and then were *iii* acutely infected for 1 h with HIV-1_{SF33}; *iv* co-cultures were established by plating $CD8^+$ T cells and acutely HIV-1-infected $CD4^+$ T cells together in a combinatorial manner. Performing panels of co-cultures was facilitated by cryopreserving freshly isolated $CD8^+$ T cells and acutely HIV-infected $CD4^+$ T cells

the percent decrease in virus replication was calculated as the ratio of RT activity in the co-culture containing $CD8^+$ T cells to the RT activity in the corresponding culture containing only $CD4^+$ T cells.

$$\textit{Anti-HIV activity} = \left(1 - \left[\frac{\text{RT } \textit{activity in co-culture}}{\text{RT } \textit{activity in } CD4^+ \textit{ cells alone}}\right]\right) \times 100$$

HLA-class I and CD3 blocking experiments To determine the need for HLA class I binding, a purified monoclonal antibody specific for human HLA-A,B,C (clone W6/32, azide-free, AbD Serotec, Raleigh, NC) (Slingluff et al. 1994; Ware et al. 1995) or matched isotype control antibody (IgG2a, AbD Serotec) was evaluated for the potential ability to block the $CD8^+$ T cell anti-HIV activity. The antibodies (final concentration of 50 μg/ml) were added daily during the first 3 days of cell culture. To determine the need for T cell receptor (TCR) complex binding, anti-CD3 antibody (final concentration of 1 μg/ml; clone SK7; Becton Dickinson, BD) was added immediately after the $CD8^+$ T cells and $CD4^+$ T cells were plated.

HLA typing High-resolution (allele level) HLA class I and II genotyping was carried out by using the sequence-based typing method as recommended by the 13th International Histocompatibility Workshop (Thio et al. 2003; Tilanus and Group 2000). The HLA class I and II genotypes of the study subjects are provided in Table 1. HLA class I and class II supertypes were assigned to the four-digit HLA class I alleles based on previously defined clusters (Doytchinova and Flower 2005; Sidney et al. 2008). The 12 possible HLA class I supertypes were A01, A02, A03, A24, B07, B08, B27, B44, B58, B62, C01, and C02. The 9 possible HLA class II supertypes were DQ1, DQ2, DQ3 and DR1, DR2, DR3, DR4, DR5, and DR9.

Statistical analyses All data were compiled in an Access database (Microsoft). The associations between HLA class I and II genotypes and $CD8^+$ T cell anti-HIV activity were assessed using χ^2 tests corrected for continuity and Mann–Whitney rank-sum tests. The Cochran–Armitage test for trend was used to assess for the presence of an association between categories of antiviral activity and categories of matching numbers of HLA genotypes. Results from experiments with anti-HLA class I antibodies were compared using Student's *t* tests. HLA linkage disequilibrium was evaluated using an online calculator made available by the Los Alamos National Laboratory (http://www.hiv.lanl.gov). Excel (Microsoft), Total Access Statistics X.8 (FMS), and R (R-Core-Team 2013) were used to perform the statistical analyses, and SigmaPlot 11.2 (Systat) was used create the graphs.

Results

$CD8^+$ T cells exhibit marked heterogeneity in their ability to decrease HIV production by differing infected $CD4^+$ T cells This study began with the observation that unstimulated (i.e., without in vitro stimulation) $CD8^+$ T cells from HIV-1-infected individuals can exhibit heterogeneity in their ability to reduce HIV-1 replication in parallel co-cultures containing $CD4^+$ T cells from different healthy HIV-1 seronegative blood bank donors (range = 0 to 99% decrease in HIV production at 1:1 $CD8^+$ T cell to $CD4^+$ cell input ratios; Fig. 2a). To further investigate the generalizability of this $CD8^+$ T cell anti-HIV activity, HIV replication levels were measured in panels of

autologous and heterologous co-cultures that contained unstimulated $CD8^+$ T cells and acutely HIV-infected $CD4^+$ T cells from various HIV-1-infected donors. The $CD4^+$ T cells from HIV-1-infected donors were used for two reasons: they allowed for assessments of autologous co-cultures, and HLA-typing data were available for these cells. Unstimulated $CD8^+$ T cells from each of the 23 asymptomatic HIV-1-infected subjects (including 3 subjects receiving HAART) were co-cultured with acutely HIV-infected $CD4^+$ T cells from 2 to 20 different HIV-1-infected donors. Altogether, the $CD4^+$ T cells isolated from 25 HIV-1-infected donors were used among 231 co-culture experiments. The unstimulated $CD8^+$ T cells from the asymptomatic HIV-1-infected subjects potently reduced HIV replication in autologous $CD4^+$ T cells (e.g., generally greater than 95% suppression at 1:1 $CD8^+$ T cell to $CD4^+$cell input ratios). In comparison, at 1:1 $CD8^+$ T cell to $CD4^+$ T cell input ratios, unstimulated $CD8^+$ T cells from HIV-negative subjects were not observed to exhibit greater than 50% reduction in HIV replication in any co-cultures, including those containing autologous $CD4^+$ T cells infected in vitro (data not shown).

When the $CD8^+$ T cells from the HIV-1-infected subjects were co-cultured with acutely HIV-infected heterologous $CD4^+$ T cells derived from HIV-1-infected individuals, marked variation was again observed in the $CD8^+$ T cell antiviral activity (Fig. 2b). Maximal $CD8^+$ T cell anti-HIV activity was consistently observed in co-cultures containing autologous $CD4^+$ T cells. The $CD8^+$ T cells in all of the autologous co-cultures exhibited greater than 50% reduction in HIV production at 1:1 and 0.5:1 $CD8^+$ T cell to $CD4^+$ T cell input ratios. In many cases, $CD8^+$ T cells that were able to reduce HIV production in autologous $CD4^+$ T cells by greater than 98% inhibited HIV replication by less than 25% in heterologous $CD4^+$ T cells at 1:1 cell input ratios. Overall, 91 of the 209 (43.5%) heterologous co-cultures exhibited greater than 50% reduction in HIV production at 1:1 $CD8^+$ T cell to $CD4^+$ T cell input ratios. Furthermore, the variable ability of the $CD8^+$ T cells to decrease HIV production by greater than 50% was consistently observed within each panel of co-cultures containing a shared source of $CD8^+$ T cells (e.g., across rows in Fig. 2b). These results demonstrate that the observed level of $CD8^+$ T cell anti-HIV activity is highly dependent on a feature(s) of the HIV-infected $CD4^+$ T cells.

$CD8^+$ T cell-mediated reduction in HIV replication is strongly associated with parity of HLA class I genotype Among the 26 HIV-1-infected subjects included in this study, 62 unique HLA class I alleles (15 HLA-A, 25 HLA-B, and 22 HLA-C) were present. The most prevalent HLA class I alleles were A0201 (allele frequency = 0.19), A0301 (0.17), C0802 (0.14), A3201 (0.12), B0801 (0.12), B1402 (0.12), and C0602 (0.12). The most prevalent HLA class I haplotypes were A0201/B4402/C0501, A0101/B5701/C0602, and A0101/B0801/C0701, each present in 4 of 26 study subjects. These allele and haplotype frequencies are consistent with those reported for US populations (Cao et al. 2001), although B0702 appears to be underrepresented in our study, while the C0802 frequency is elevated. Genotype homozygosity at the HLA-A, HLA-B, or HLA-C locus was rare in this study group.

To evaluate the relationships between the $CD8^+$ T cell-mediated reduction in HIV replication and the HLA class I and II relatedness of the co-cultured $CD4^+$ T cells, these factors were co-analyzed. Anti-HIV activity was categorized as either (i) <50% decrease or (ii) >50% decrease and then evaluated (Table 2). Only $CD8^+$ T cells that demonstrated the ability to reduce HIV replication by >50% in at least one co-culture at a 1:1 $CD8^+$ T cell to $CD4^+$ T cell input ratio were included in the analyses. This criterion for inclusion was used to avoid the analysis of $CD8^+$ T cells that lacked any appreciable anti-HIV activity, such as those from HIV-1-infected individuals who are receiving antiretroviral therapy and/or have advanced to disease (Killian et al. 2005a; Killian et al. 2009; Stranford et al. 2001).

Among the 209 heterologous co-cultures, the proportion of co-cultures having at least one HLA class I matching allele was significantly elevated among those exhibiting greater than 50% reduction in HIV production (84 vs. 31%; Yates $\chi^2 = 54.21$, $p < 0.001$). The presence of some co-cultures having a single HLA class I match in the absence of a strong reduction of virus replication is consistent with previous reports showing that $CD8^+$ T cells from HIV-1-infected individuals only respond to a fraction of the theoretically recognizable epitopes (Addo et al. 2003; Killian et al. 2005b). In this regard, a direct monotonic trend (Fig. 3a) was evident between the number of HLA class I matching alleles and the magnitude of the $CD8^+$ T cell-mediated reduction in HIV production ($R_S = 0.663$, $p < 0.001$). Thus, strong $CD8^+$ T cell anti-HIV activity was associated with an increased probability of presentation of an immunogenic epitope. These results demonstrate that maximal $CD8^+$ T cell anti-HIV activity is strongly associated with HLA class I compatibility between $CD8^+$ T cells and HIV-infected $CD4^+$ T cells.

$CD8^+$ T cell anti-HIV activity is more closely associated with HLA class I than HLA class II Additional analyses were performed to investigate the potential involvement of HLA class II-restricted $CD8^+$ T cell responses (Ranasinghe et al. 2016). Among the cells included in this study, there were 12 unique HLA-DQ alleles and 21 unique HLA-DR alleles. A direct monotonic relationship was observed (Fig. 3b) between $CD8^+$ T cell antiviral activity and the frequency of matching HLA class II alleles ($R_S = 0.402$, $p < 0.001$). However, in comparison to the relationship with HLA class I matches, the Spearman correlation coefficient for the relationship with HLA class II matches was lower. Sensitivity analyses, based on logistic regression models for predicting percent suppression, did not reveal any improvement over HLA class I

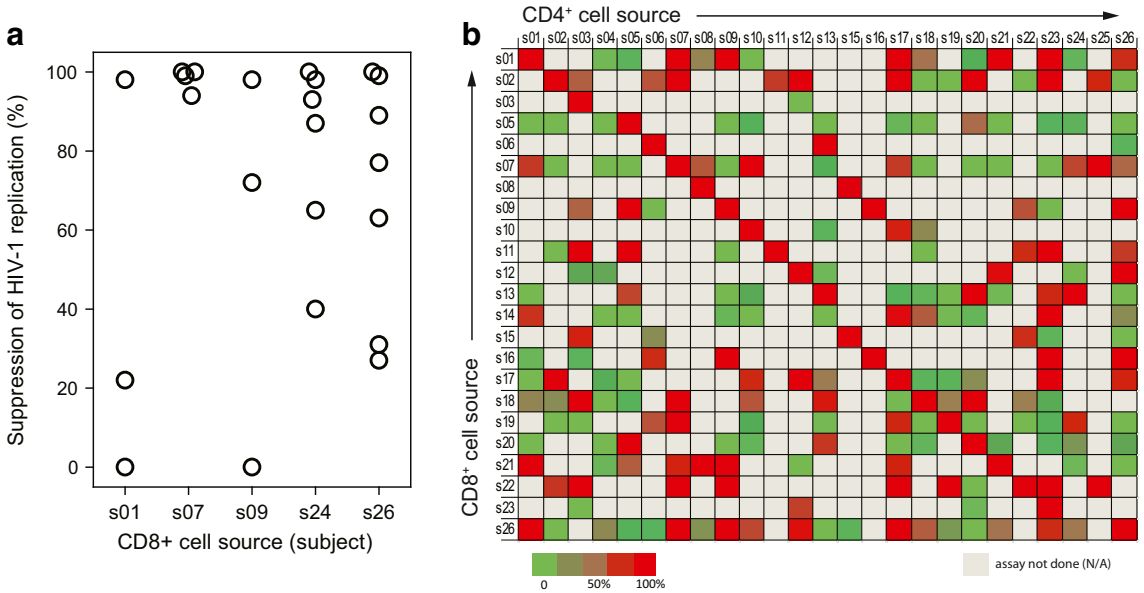

Fig. 2 $CD8^+$ T cells from HIV-1-infected individuals exhibit marked heterogeneity in their ability to reduce HIV production by $CD4^+$ T cells from different individuals. **a** Shown are the antiviral activities of $CD8^+$ T cells from five HIV-1-infected study participants when co-cultured at 1:1 cell input ratios with acutely HIV-1-infected $CD4^+$ T cells from different HIV-negative blood donors. **b** Shown is an ordered (by subject number) heat map representation of anti-HIV activity in more than 200 co-cultures containing variable $CD8^+$ T cells and acutely HIV-infected $CD4^+$ T cells from HIV-1-infected study participants (1:1 cell input ratio). Individual rows share $CD8^+$ T cells from a common study subject, while individual columns have $CD4^+$ T cells from a common study subject. *Bright green* and *bright red squares* indicate low (<30%) and high (>70%) level of suppression, respectively. *Lightly shaded areas* indicate data not available (N/A; co-cultures not performed) (color figure online)

genotype matches when HLA class II genotype matches were added to the model. Moreover, several (29/87 = 33%; HLA class II data not available for 4/91 co-cultures) co-cultures exhibited >50% reduction in HIV production without having any HLA class II matches. The occurrence of HLA-B*1402 and HLA-DR*0102 was linked in our study population (p = 4.34e−06), with 6 of the 26 HIV-infected individuals expressing both alleles and none expressing only one of these alleles. Thus, at least part of the observed association between $CD8^+$ T cell antiviral activity and the frequency of HLA class II matches is likely due to strong linkage disequilibrium with HLA class I (Ness et al. 1981).

HLA-B and HLA-C matches are associated with a strong $CD8^+$ T cell anti-HIV response In addition to evaluating the relationship between the frequency of HLA class I matches and $CD8^+$ T cell anti-HIV activity, we also assessed potential associations with individual HLA class I loci. Present among the 209 heterologous co-culture experiments were 79 co-cultures that featured a single matching HLA class I allele (Fig. 4a). Among these 79 co-cultures were 35 and 44 co-cultures that exhibited <50 and >50% reduction in HIV production, respectively. The large majority (64/79) of matches were present at the HLA-A locus. Remarkably, none of the 35 co-cultures in this subset that exhibited a <50% decrease had matching HLA-B alleles; 4 of the co-cultures with >50% reduction had matching HLA-B alleles. At the HLA-A locus, the $CD8^+$ T cells from 11 study subjects exhibited differential anti-HIV activity while sharing the same matching allele with variable heterologous $CD4^+$ T cells. The matching alleles were HLA-A*0101, 0201, 0301, 1101, and 3201. At the HLA-B and HLA-C loci, none of the $CD8^+$ T cells exhibited differential anti-HIV activity while sharing the same matching alleles. Notably, the $CD8^+$ T cells from four separate individuals shared a single HLA-C0802 matching allele with variable $CD4^+$ T cells. Despite the identified presence of optimal epitopes in the Nef and Gag sequences of HIV-1_{SF33}, such as AAVDLSHFL and TPQDLNTML, respectively (Llano et al. 2009), none of the co-cultures having only a matching C0802 allele exhibited >50% reduction in HIV production.

While sizable percentages of both anti-HIV activity categories contained only matching HLA-A alleles, relatively few matches were present at only the HLA-B or HLA-C loci, consistent with linkage disequilibrium findings (Hiller et al. 1978). Therefore, we compared the frequencies of matches at the HLA-A locus with those having matches at the HLA-B and/or HLA-C loci among co-cultures exhibiting discordant CD8+ T cell anti-HIV activity (Fig. 4b). Among the co-cultures exhibiting a <50% reduction in HIV production, 25 and 6% had matching HLA-A and HLA-B/C alleles, respectively. Among the co-cultures exhibiting a >50% reduction in HIV production, 59 and 46% had matching HLA-A and HLA-B/C alleles, respectively. Consequently, the relative ratio (RR) of co-cultures having strong CD8+ T cell anti-HIV activity and matching HLA-B/C (RR = 7.7) alleles was more than three times higher than the corresponding ratio for matching HLA-

Table 2 Analyses of $CD8^+$ T cell-mediated reduction of HIV production with respect to HLA class I and II compatibility

	HIV suppression (*N*)		χ^{2a}	*p* value
	<50%	>50%		
Autologous	0	22	–	–
Heterologous	118	91	–	–
Class I genotype matches[b]				
0 matches	81	15	54.21	**<0.001**
1 match	35	44	29.37	**<0.001**
2 or more matches	2	32	39.83	**<0.001**
≥1 HLA-A match	30	56	26.20	**<0.001**
≥1 HLA-B match	2	31	38.09	**<0.001**
≥1 HLA-C match	7	33	28.61	**<0.001**
≥1 HLA-A, HLA-B, and HLA-C matches	0	14	17.07	**<0.001**
Class I supertype matches				
0 matches	3	0	–	–
1 match	20	6	–	–
2 or more matches	95	85	6.11	**0.013**
≥1 HLA-A match	84	81	8.78	**0.003**
≥1 HLA-B match	58	64	8.63	**0.003**
≥1 HLA-C match	102	81	0.12	0.729
≥1 HLA-A -B and -C matches	35	51	13.70	**<0.001**
Class II genotype matches[c]				
0 matches	49	29	1.55	0.213
1 match	59	33	0.00	0.987
2 or more matches	6	25	15.07	**<0.001**
≥1 HLA-DQ	61	55	1.26	0.262
≥1 HLA-DR	10	26	13.18	**<0.001**
≥1 HLA-DQ and HLA-DR matches	6	23	16.24	**<0.001**
Class II supertype matches				
0 matches	13	2	4.68	**0.031**
1 match	20	16	3.23	0.072
2 or more matches	81	69	1.37	0.242
≥1 HLA-DQ	89	76	2.3	0.130
≥1 HLA-DR	93	78	1.94	0.164
≥1 HLA-DQ and HLA-DR matches	81	69	1.37	0.242
Total matching alleles[d]				
Class I genotype	39	129	85.64	**<0.001**
Class II genotype	73	89	9.91	**0.002**

[a] Yates chi-square, corrected for continuity. Statistical comparisons exclude results from autologous co-cultures. All data in the table are from co-cultures having a 1:1 $CD8^+$ T cell to $CD4^+$ T cell input ratio

[b] Numbers indicate counts of co-cultures having strict four-digit HLA allelotype matches at the -A, -B, and/or -C loci, ignoring possible HLA class II matches

[c] Numbers indicate counts of co-cultures having strict four-digit HLA allelotype matches, ignoring possible HLA class I matches. HLA class II typing data were not available for one study participant, equating to missing HLA class II data for eight cultures

[d] Numbers indicate sums of strict four-digit HLA allelotype matches. For each co-culture, there are six possible distinct matches for class I (two at HLA-A, HLA-B, HLA-C) and four possible distinct matches for class II (two at HLA-DQ, HLA-DR). The total numbers of possible matching alleles were adjusted to account for homozygosity at each locus

A alleles (RR = 2.4). Thus, HLA-B/C matching alleles are more predictive of strong anti-HIV activity than matching HLA-A alleles (Yates $\chi^2 = 5.07$, $p = 0.024$).

Similar relationships between $CD8^+$ T cell anti-HIV activity and HLA class I compatibility among HIV controllers and asymptomatic viremic individuals To investigate

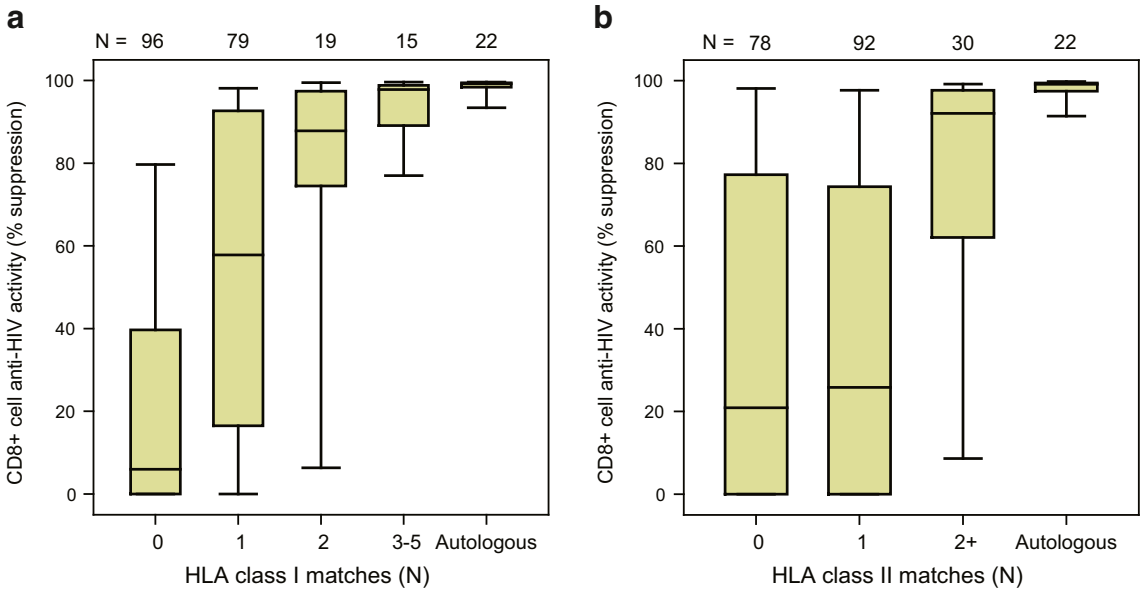

Fig. 3 Direct association between the CD8$^+$ T cell-mediated reduction of HIV production and the HLA class I and class II relatedness of the HIV-infected CD4$^+$ T cells. *Box plots* show the distributions of the CD8$^+$ T cell anti-HIV activity data categorized by **a** the number of HLA class I four-digit allelotype matches (0, 1, 2, 3–5, and autologous) and **b** the number of HLA class II four-digit allelotype matches (0, 1, 2+, and autologous) between the co-cultured CD8$^+$ T cells and the acutely HIV-infected CD4$^+$ T cells (1:1 cell input ratio). The *lower*, *middle*, and *upper boundaries* of each *box* reveal the 25th, 50th, and 75th percentiles, respectively. *Whiskers* indicate the 10th and 90th percentiles. A Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks was performed (excluding the autologous data), and Dunn's method was used to make pairwise multiple comparisons

potential differences in the CD8$^+$ T cells from HIV-1-infected individuals having different viral loads (VL), we compared the patterns of variability in anti-HIV activity in heterologous co-cultures containing CD8$^+$ T cells from HIV controllers (six elite controllers and five viremic controllers; see "Materials and methods" section) with those containing CD8$^+$ T cells from asymptomatic viremic individuals (n = 9, VL ≥2000). The anti-HIV activity exhibited by CD8$^+$ T cells from both HIV controllers and viremic individuals was directly proportional to the number of matching HLA class I alleles, with two or more matching alleles generally being required for maximal control of virus replication (Fig. 5a). Furthermore, for both groups, the

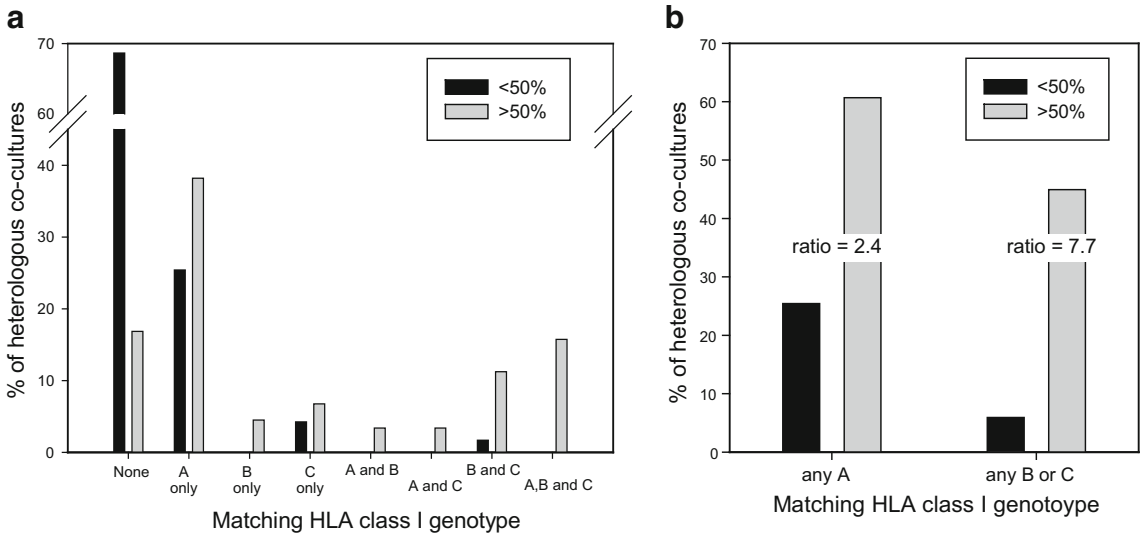

Fig. 4 Relationship between CD8$^+$ T cell anti-HIV activity and HLA class I loci. **a** Shown are the frequencies of heterologous co-cultures (*y-axis*) having no matching HLA class I alleles or matching alleles at specific loci. **b** Shown are the frequencies of heterologous co-cultures having any matching alleles at the HLA-A locus and at the HLA-B and/or HLA-C loci. For both *panels*, *black bars* and *gray bars* denote co-cultures exhibiting ≤50 and >50% suppression of HIV replication, respectively

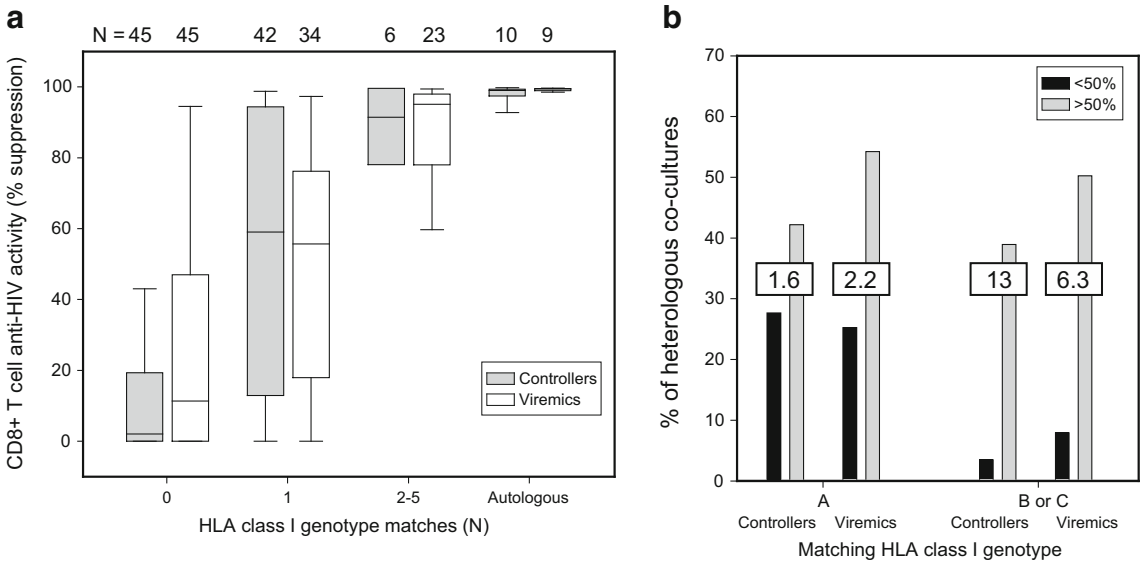

Fig. 5 Similar relationships between $CD8^+$ T cell anti-HIV activity and HLA class I matching among $CD8^+$ T cells from HIV controllers and viremic individuals. **a** *Box plots* show the distributions of the $CD8^+$ T cell anti-HIV activity data categorized by the number of HLA class I four-digit allelotype matches (0, 1, 2–5, and autologous) between the co-cultured $CD8^+$ T cells and the acutely HIV-infected $CD4^+$ T cells (1:1 cell input ratio). *Box plots* for HIV controllers and viremic individuals are shaded *gray* and *white*, respectively. The *lower*, *middle*, and *upper boundaries* of each *box* reveal the 25th, 50th, and 75th percentiles, respectively. *Whiskers* indicate the 10th and 90th percentiles. **b** Shown are the frequencies of heterologous co-cultures having any matching alleles at the HLA-A locus and at the HLA-B and/or HLA-C loci for HIV controllers and viremic individuals. *Black bars* and *gray bars* denote co-cultures exhibiting ≤50 and >50% suppression of HIV replication, respectively

presence of matching HLA-B/C alleles was more predictive of strong $CD8^+$ T cell anti-HIV activity than was the presence of matching HLA-A alleles (Fig. 5b). These results demonstrate that the described relationships between $CD8^+$ T cell anti-HIV activity and HLA class I are similar among asymptomatic HIV-1-infected individuals having disparate viral loads.

$CD8^+$ T cells can reduce HIV production in heterologous $CD4^+$ T cells having multiple compatible HLA class I supertypes To evaluate the import of HLA class I families that are able to present similarly recognizable peptides, supertype designations were assigned to the HLA class I genotypes and then analyzed with respect to the observed $CD8^+$ T antiviral activity in the co-cultures (Fig. 6a). A direct monotonic relationship was present between the number of HLA class I supertype matches and the magnitude of the $CD8^+$ T cell anti-HIV activity ($R_S = 0.516$, $p < 0.001$). Overall, 206 of the 209 (99%) heterologous co-cultures had at least one HLA class I supertype match at the -A, -B, or -C loci. However, notable supertype differences were present among the co-cultures having discordant antiviral activity. Each of the three co-cultures that lacked any HLA class I supertype matches exhibited less than 50% reduction in HIV production, suggesting that supertype matches are a necessary, but not a sufficient, condition for the $CD8^+$ T cell antiviral activity. All (15 of 15) of the heterologous co-cultures that exhibited greater than 50% anti-HIV activity without having any strict HLA class I four-digit genotype matches at the -A, -B, and -C loci featured HLA class I supertype matches; most had HLA-B matches (13 of 15 = 87%) and two or more distinct HLA class I supertype matches (11 of 15 = 73%). In comparison, fewer than half (40 of 81 = 49%) of the co-cultures that exhibited less than 50% anti-HIV activity without having any strict HLA class I four-digit genotype matches exhibited HLA-B supertype matches, but many (62 of 81 = 77%) exhibited two or more supertype matches at the HLA-A and HLA-C loci. The relationship between HLA class II supertype matches and $CD8^+$ T cell anti-HIV activity (Fig. 6b; $R_S = 0.317$, $p < 0.001$) was not as strong as the relationship with HLA class I supertype matches. These results indicate that $CD8^+$ T cells can reduce HIV replication in $CD4^+$ T cells having composite genotypes of closely related HLA class I alleles and provide further support for our observed associations with the number of HLA-matching alleles and matches at the HLA-B/C loci.

Anti-HLA-class I and anti-CD3 antibodies can block the $CD8^+$ T cell-mediated anti-HIV activity To further investigate the observed association between $CD8^+$ T cell anti-HIV activity and HLA class I, anti-HLA class I antibodies were added to the co-cultures (Fig. 7). Notably, the very short half-life (≤1 h) of HLA class I molecules at the cell surface (Su and Miller 2001) necessitates the frequent addition of relatively large quantities of anti-HLA class I antibody. When anti-HLA class I antibody (50 μg/ml) was added daily, the anti-HIV activity of $CD8^+$ T cells otherwise excellent was reduced (Fig. 7a). The anti-IgG antibody of the appropriate

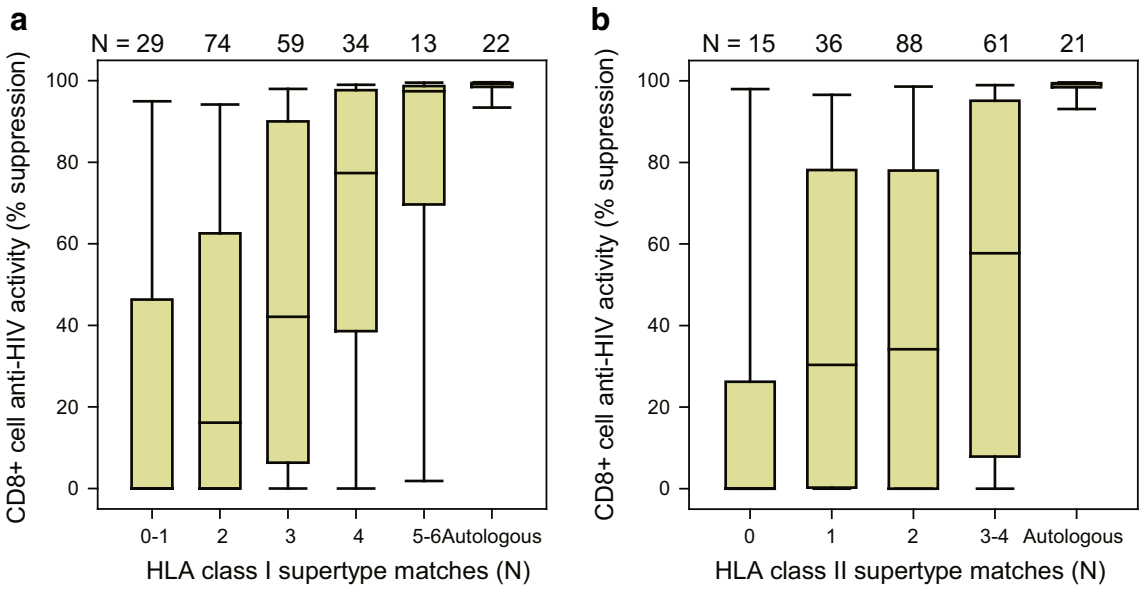

Fig. 6 HLA class I and class II supertype matches in relation to $CD8^+$ T cell anti-HIV activity. *Box plots* show the distributions of the $CD8^+$ T cell anti-HIV activity data categorized by **a** the number of HLA class I supertype matches and **b** the number of HLA class II supertype matches between the co-cultured $CD8^+$ T cells and the acutely HIV-infected $CD4^+$ T cells (1:1 cell input ratio). The *lower*, *middle*, and *upper boundaries* of each *box* reveal the 25th, 50th, and 75th percentiles, respectively. *Whiskers* indicate the 10th and 90th percentiles

isotype had no appreciable effect on HIV replication. Substantial reductions in $CD8^+$ T cell anti-HIV activity were noted in four of four experiments at both the 0.5:1 ($p < 0.001$) and 1:1 ($p = 0.013$) $CD8^+$ T cell to $CD4^+$ T cell input ratios (Fig. 7b). Additional experiments were performed to evaluate the potential requirement for T cell receptor (TCR) binding (Fig. 7c, d). In two of two experiments, the addition of anti-CD3 antibody to the co-cultures completely abrogated the ability of $CD8^+$ T cells to reduce HIV production. These results demonstrate that maximal $CD8^+$ T cell anti-HIV activity requires HLA class I and TCR interactions.

$CD8^+$ T cells that are stimulated in vitro exhibit enhanced and unrestricted anti-HIV activity in co-culture assays To determine their relative anti-HIV activities, unstimulated and PHA-stimulated $CD8^+$ T cells were evaluated for their abilities to reduce HIV replication levels in acutely HIV-1-infected autologous and heterologous $CD4^+$ T cells (Fig. 8). In parallel co-cultures with unstimulated $CD8^+$ T cells, the $CD8^+$ T cells that were stimulated in vitro with PHA exhibited significantly increased anti-HIV activity (Fig. 8a; Wilcoxon signed-rank test, $p < 0.001$). The anti-HIV activity increased with stimulation of the CD8+ T cells in nearly all (24/26) experiments. In 10 of 14 heterologous co-cultures where the unstimulated $CD8^+$ T cells exhibited very low anti-HIV activity, the stimulated $CD8^+$ cells reduced HIV production by greater than 50% (Fig. 8b). Moreover, in most (3/5) heterologous co-cultures lacking any HLA class I genotype matches, those containing in vitro stimulated $CD8^+$ T cells exhibited greater than 50% reduction in HIV production, while the co-cultures containing unstimulated $CD8^+$ T cells exhibited very little or no anti-HIV activity. The already high level anti-HIV activity observed in all (7/7) of the autologous co-cultures was further increased with stimulation of the $CD8^+$ T cells (Wilcoxon signed-rank test, $p = 0.016$). We also observed that stimulated, but not unstimulated, $CD8^+$ T cells from an HIV-2-infected individual exhibited appreciable anti-HIV activity (64% reduction; see s99 in Fig. 8b) in co-cultures with acutely HIV-1-infected heterologous $CD4^+$ cells, indicating that the anti-HIV activity of stimulated $CD8^+$ T cells is not virus specific. These results demonstrate that stimulated $CD8^+$ cells, unlike unstimulated $CD8^+$ T cells, are able to reduce virus production levels in acutely HIV-1-infected $CD4^+$ T cells without a requirement for HLA class I compatibility.

Discussion

$CD8^+$ T lymphocytes from HIV-1-infected individuals can potently reduce HIV-1 replication in $CD4^+$ T cells in vitro, and this antiviral activity is directly associated with a positive clinical prognosis (Castelli et al. 2002; Ferbas et al. 1995; Gomez et al. 1994; Streeck et al. 2014; Streeck and Nixon 2010). A previous study reported that the measurement of the $CD8^+$ T cell non-cytotoxic antiviral activity in vitro can vary with the source of the $CD4^+$ T cells (Mackewicz et al. 1998), but a conclusive association with HLA class I was not demonstrated. Several studies have indicated that the $CD8^+$ cell cytotoxic responses require HLA compatibility (Yang and Walker 1997). In this present large study, our data do not

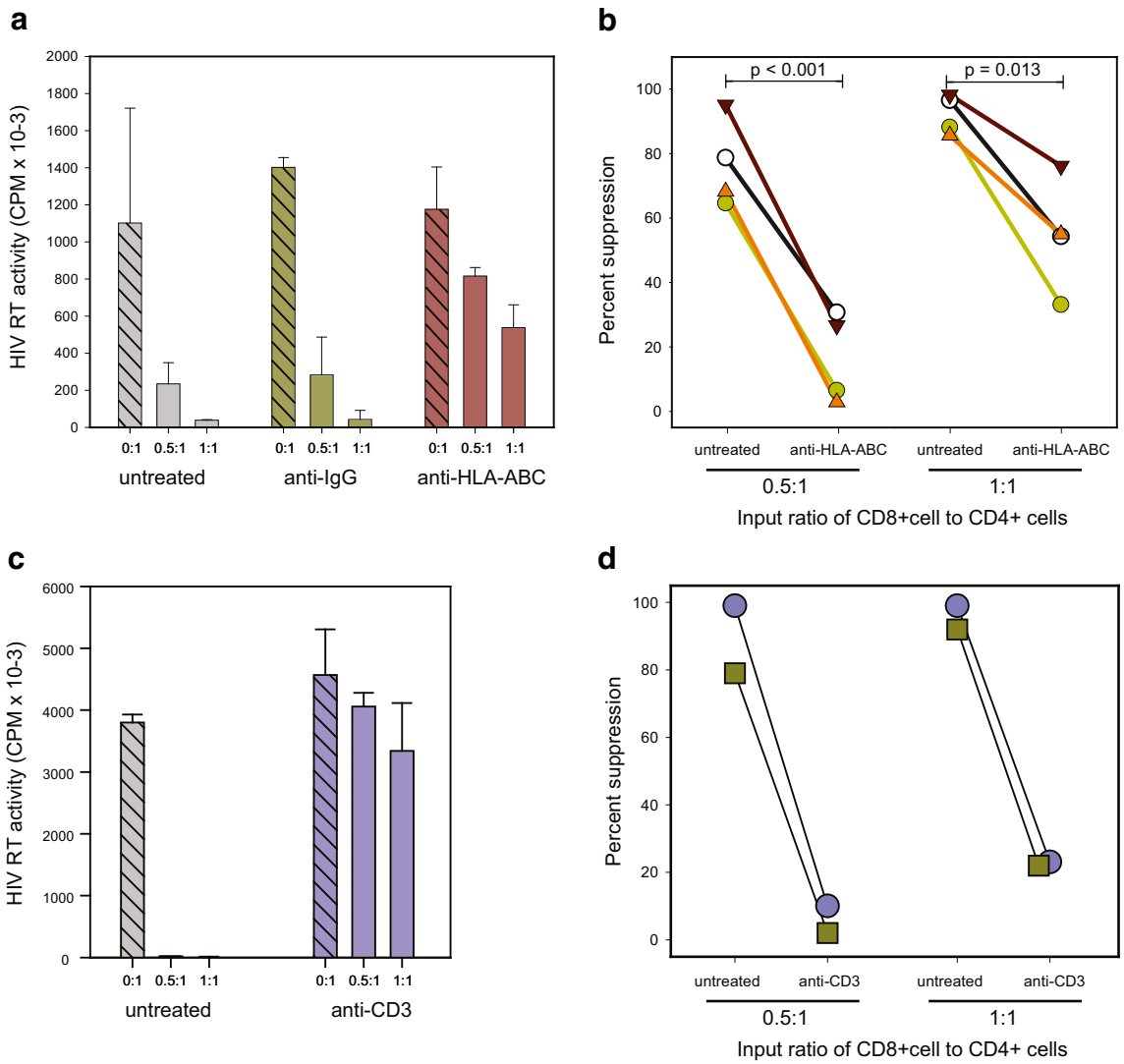

Fig. 7 Anti-HLA-class I antibody and anti-CD3 antibody block $CD8^+$ T cell anti-HIV activity. **a** Shown is a representative example of the levels of HIV replication in co-cultures containing medium alone, mouse anti-human IgG (50 μg/ml), or anti-HLA class I antibody (50 μg/ml). For each culture condition, 100,000 HIV-infected $CD4^+$ T cells were cultured alone (0:1) or with 50,000 (0.5:1) or 100,000 (1:1) $CD8^+$ T cells. **b** The effects of anti-HLA class I antibody on $CD8^+$ T cell antiviral activity are shown for the four experiments that were performed with the daily addition of antibody (50 μg/ml). **c** Shown is a representative example of the levels of HIV replication in co-cultures containing medium alone or anti-CD3 antibody. For each culture condition, 100,000 HIV-infected $CD4^+$ T cells were cultured alone (0:1) or with 50,000 (0.5:1) or 100,000 (1:1) $CD8^+$ T cells. **d** The effects of anti-CD3 antibody on $CD8^+$ T cell antiviral activity are shown for the two experiments that were performed. **a**–**d** Results from autologous co-cultures

directly address whether the requirement for HLA class I compatibility is associated with a cytotoxic or non-cytotoxic mechanism. However, we have demonstrated that bulk $CD8^+$ cells taken from HIV-infected subjects and not stimulated in vitro can reduce HIV production by $CD4^+$ cells in a manner that is directly proportional to the number of HLA class I matching alleles.

Analysis of the HIV replication levels in 231 (22 autologous and 209 heterologous) co-cultures with respect to the degree of HLA class I relatedness between the co-cultured $CD8^+$ T and $CD4^+$ T cells revealed that the anti-HIV activity of unstimulated $CD8^+$ T cells is strongly associated with concordance in the HLA class I genotype (Fig. 3 and Table 2). The diminished $CD8^+$ T cell anti-HIV activity in the presence of anti-CD3 and anti-HLA class I antibodies (Fig. 7) supports the conclusion that an interaction between the T cell receptor (TCR) complex and HLA class I is associated with the mechanism by which unstimulated $CD8^+$ T cells reduce HIV production by acutely HIV-infected $CD4^+$ T cells. We also observed that the HLA class I-restricted anti-HIV activity observed with unstimulated $CD8^+$ T cells can be overcome following the in vitro stimulation of the $CD8^+$ T cells (Fig. 8), reasonably due a mechanism that mimics the natural TCR-peptide-HLA class I interaction (Valentine et al. 1985).

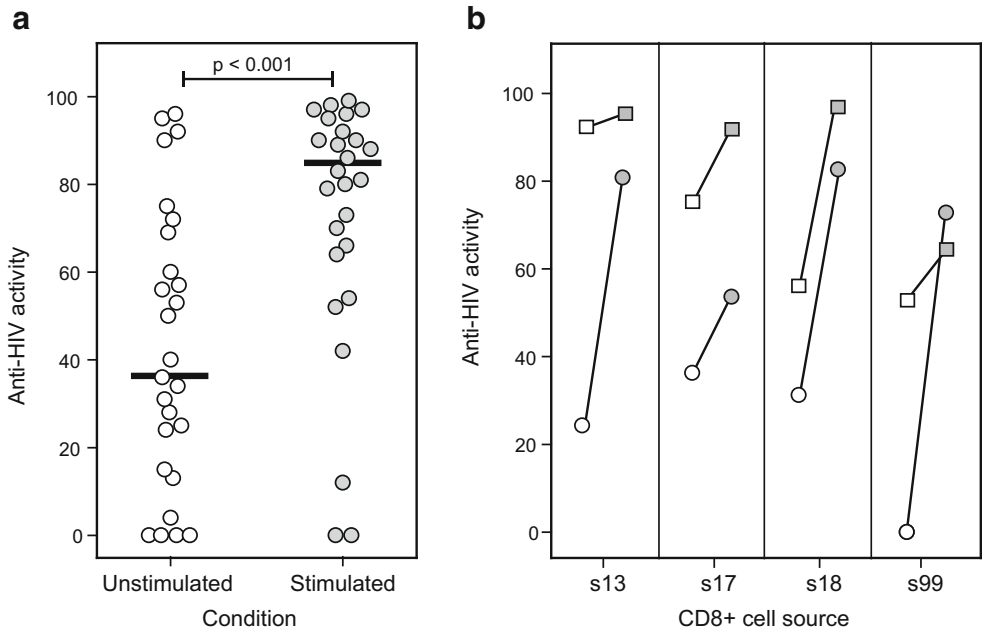

Fig. 8 $CD8^+$ T cells stimulated in vitro reduce HIV-1 production in HLA class I discordant $CD4^+$ T cells. **a** Parallel co-cultures were established with unstimulated and stimulated $CD8^+$ T cells. Shown are the anti-HIV activities of co-cultures containing 1:1 input ratios of $CD8^+$ T cells and acutely HIV-infected $CD4^+$ T cells. *Horizontal bars* indicate median values. **b** Representative examples are shown of the anti-HIV activities of unstimulated and stimulated $CD8^+$ T cells co-cultured with acutely HIV-infected autologous (*squares*) or heterologous (*circles*) $CD4^+$ T cells lacking any HLA class I genotype matches. *Symbols* are *shaded gray* to denote results with stimulated $CD8^+$ T cells

Associations between HLA class I and HIV-1 disease progression have been observed in numerous epidemiologic studies (Carrington et al. 1999; Pereyra et al. 2010; Saah et al. 1998; Trachtenberg et al. 2003). Given the well-characterized binding of CD8 to HLA class I (Cole and Gao 2004), these findings implicate a role for $CD8^+$ T cells. However, the biologic mechanism(s) underlying these associations remain unclear. One theory is that variable fitness costs are associated with mutations across the HIV genome and that protective HLA types are those that present relatively immutable viral epitopes (Carlson et al. 2015; Kloverpris et al. 2015). Consistent with this theory is the longstanding observation that heterozygosity of HLA class I alleles is associated with a protective advantage in terms of HIV-1 survival (Carrington et al. 1999). Based on instances of reduced HIV production in the presence of a single HLA class I match, as well as the direct association between the numbers of HLA class I matches and the $CD8^+$ T cell anti-HIV activity, our findings provide further evidence for a HLA class I heterozygosity advantage.

A fundamental question that has relevance to HIV vaccine design, and perhaps to the development of cell-based therapies, pertains to whether particular HLA genes are more important for the control of HIV. HLA-B types (e.g., B57, B58, B27, B51, and B81) are most frequently associated with improved prognosis (Carrington and O'Brien 2003; Pereyra et al. 2010). Possible mechanisms for the protective role of HLA-B include its relative resistance to downregulation by the HIV-1-negative regulatory factor (Nef) (Rajapaksa et al. 2012) and its rapid display of peptides on the cell surface (Neefjes et al. 2011). Our findings with unstimulated $CD8^+$ cells provide further support for a dominant role of HLA-B in HIV control by showing that matching HLA-B and/or C alleles are more predictive of strong $CD8^+$ T cell anti-HIV activity than matching HLA-A alleles (Fig. 4). This relationship was similarly observed among $CD8^+$ T cells from HIV controllers and viremic individuals and therefore appears to be independent of viral load (Fig. 5). Importantly, we show that bulk $CD8^+$ T cells from both HIV controllers and non-controllers require multiple matching HLA class I alleles, and presumably the recognition of multiple HIV epitopes, for the maximal suppression of HIV replication. Therefore, optimal vaccines may require an ability to elicit $CD8^+$ T cell responses to multiple HIV epitopes in each person vaccinated.

To our knowledge, this is the first study to evaluate the anti-HIV activity of $CD8^+$ T cells with respect to composite HLA class I supertypes. Nearly all of the heterologous co-cultures in this study had matching HLA class I supertype alleles, whereas fewer than half of the co-cultures exhibited greater than 50% reduction in HIV replication (Fig. 6 and Table 2). In comparison, the frequencies of co-cultures having matching HLA class I genotypes and exhibiting greater than 50% anti-HIV activity were more closely aligned (36 and 44%, respectively). Together, these observations strongly suggest that $CD8^+$ T cells are unable to functionally respond to many theoretically compatible HLA class I supertypes. Thus, the classification of HLA class I supertypes, as defined by others (Lund et al. 2004; Sidney et al. 2008), seems to have limited utility for predicting functional $CD8^+$ T cell responses.

A large number of studies of the $CD8^+$ T cell response to HIV-1 infection have been based on the use of synthetic peptides alone and/or in the context of HLA class I tetrameric complexes or antigens expressed by immortalized cell lines.

A vaccine conceptualized from those study results was ineffective (Sekaly 2008). The approach used in the present study can provide a more natural system for the examination of antigen-specific responses in terms of antigen processing, the amount of antigen present, and the interactions with co-stimulatory and inhibitory molecules. In this regard, our approach can potentially be used to reverse-engineer HIV peptides having the greatest capacity to induce $CD8^+$ T cell anti-HIV activity across discordant HLA class I types. This strategy would entail performing panels of heterologous co-cultures and determining the over-represented HLA types among co-cultures exhibiting strong $CD8^+$ T cell anti-HIV activity in the absence of matching alleles. The peptides presented by those HLA types could then be identified using bioinformatics (Larsen et al. 2007) and advanced mass spectrometry procedures (Mommen et al. 2014). The same approach can be used for validation and refinement of proposed HLA class I supertypes. Accordingly, co-cultured cells that exhibit anti-HIV activity in the absence of matching allotypes are hypothesized to share at least one HLA class I supertype. Alternatively, co-cultured cells that do not exhibit anti-HIV activity are likely void of any common biologically relevant HLA class I supertype. Reasonably, the approach can be adapted to investigations of other viral infections and cancer by performing panels of co-cultures with $CD8^+$ T cells from patients and variable heterologous target cells.

In summary, our studies show a direct relationship between $CD8^+$ T cell anti-HIV activity and the number of compatible (recognized) HLA class I alleles, as evidenced by the variable HIV replication levels in co-cultures containing autologous, partially matched, or completely mismatched $CD8^+$ T and $CD4^+$ T cells. Our experimental approach using unstimulated $CD8^+$ T cells and heterologous acutely HIV-infected $CD4^+$ T cells can be useful for vaccine development and the refinement of HLA class I supertype designations.

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Author contributions M.S.K. designed the studies, performed experiments and data analyses, and drafted the manuscript. F.T. performed experiments and data analyses. R.S. performed statistical analyses. All authors read and approved the final manuscript.

Compliance with ethical standards Each participant signed informed consent forms, and the study received approval from the Committee for Human Research at the University of California San Francisco (UCSF).

Competing interests The authors declare that they have no competing interests.

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