

Effective Downregulation of HLA-A*2 and HLA-B*57 by Primary Human Immunodeficiency Virus Type 1 Isolates Cultured from Elite Suppressors[∇]

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Elite controllers or suppressors (ES) are human immunodeficiency virus type 1 (HIV-1)-infected patients who control viral replication to <50 copies/ml without antiretroviral therapy. Downregulation of HLA class I molecules is an important mechanism used by HIV-1 to evade the immune system. In this study, we showed that primary isolates from ES are as effective as isolates obtained from patients with progressive HIV-1 disease at downregulating HLA-A*2 and HLA-B*57 molecules on primary CD4⁺ T cells. Thus, a diminished ability of viral isolates from ES to evade HIV-specific immune responses probably does not contribute to the control of viral replication in these patients.

Long-term nonprogressors (LTNP) are human immunodeficiency virus type 1 (HIV-1)-infected individuals who maintain normal CD4⁺ T-cell counts and remain asymptomatic for longer than 10 years without therapy (7). Although many LTNP have detectable levels of HIV-1 RNA in their plasma, patients known as elite suppressors (ES) have viral loads of <50 RNA copies/ml. Understanding the factors involved in the maintenance of LTNP and ES statuses may be critical for the development of effective vaccines and immunotherapeutic treatments. One such factor under investigation is the role of cytotoxic-T-lymphocyte (CTL) responses. Several studies have shown that the HLA-B*27 and -B*57 alleles are overrepresented in cohorts of ES (13, 16, 19, 28, 29, 34). These findings suggest important roles for major histocompatibility complex class I (MHC-I) restriction and CD8⁺ T-cell responses in the control of viremia. Indeed, multiple studies have documented qualitatively superior CD8⁺ T cell function in ES compared to that in chronic HIV progressors (CP) (2, 5, 12, 27, 28, 37, 47).

Other studies suggest that some ES and LTNP are infected with attenuated viruses. One illustrative example comes from studies done on the Sydney Blood Bank Cohort, in which an LTNP donor transmitted an HIV-1 isolate with a large deletion in *nef* and the U3 region of the long terminal repeat to multiple recipients, all of whom became LTNP (11, 21). As in the Sydney Blood Bank Cohort studies, several other investigators have detected viruses with defective *nef* genes in LTNP and ES (1, 8, 18, 23, 25, 35, 36, 38, 43). In contrast, other studies showed that CD4⁺ T cells from ES could produce Gag when they were stimulated in vitro (20, 26), and full-length sequence analyses of plasma and proviral genomes revealed no evidence of significant deletions (30). Recent studies have suggested that plasma isolates (31) and replication-competent viruses (32) from HLA-B*57/B58*01 ES and LTNP, respec-

tively, are less fit than isolates from B*57/B*5801 CP, but the difference in fitness observed is unlikely to fully explain the control of viral replication in these patients. Furthermore, we recently performed detailed genotypic and phenotypic analyses of replication-competent viruses isolated from ES and showed that these viruses were fully replication competent (6). Although *nef* is not required for viral replication in vitro, it has been strongly associated with pathogenesis in vivo (reviewed in reference 14). It is thus possible that some ES isolates are replication competent but have mutations in *nef* that result in diminished pathogenesis.

nef has been shown to be involved in the downregulation of both CD4 (15) and MHC-I (41). Several studies have shown that *nef*-induced MHC-I downregulation has a major impact on CTL function. In a seminal study, a dramatic reduction in HLA-A*2 expression by CD4⁺ T cells infected with wild-type virus but not by those infected with a virus carrying a defective *nef* gene was demonstrated. This downregulation resulted in diminished killing of HIV-1-infected cells by CTL clones specific for an HLA-A*2-restricted HIV-1 Gag epitope (10). Similarly, *nef*-mediated MHC-I downregulation was shown to impair the ability of HIV-1-specific CTL clones to suppress viral replication (42, 44). While these findings strongly suggest that HIV-1 partially evades the immune response by inducing MHC-I downregulation, other studies have demonstrated that primary CD8⁺ T cells from some ES and CP could effectively respond to autologous viral replication in autologous CD4⁺ T cells (26).

We tested the hypothesis that ES are infected with HIV-1 isolates that are less capable of downregulating MHC-I molecules. This could potentially cause the isolates to be more susceptible to CD8⁺ T-cell suppression of replication and may explain the superior CD8⁺ T-cell responses reported in prior ES studies (2, 5, 12, 27, 28, 37, 47). To date, fully characterized replication-competent isolates have been reported from just six ES subjects (1, 3, 6). We compared the MHC-I downregulation capacity of isolates from five of these ES to that of isolates obtained from resting CD4⁺ T cells of eight patients with progressive disease (viral load, >10,000 copies/ml). In order to

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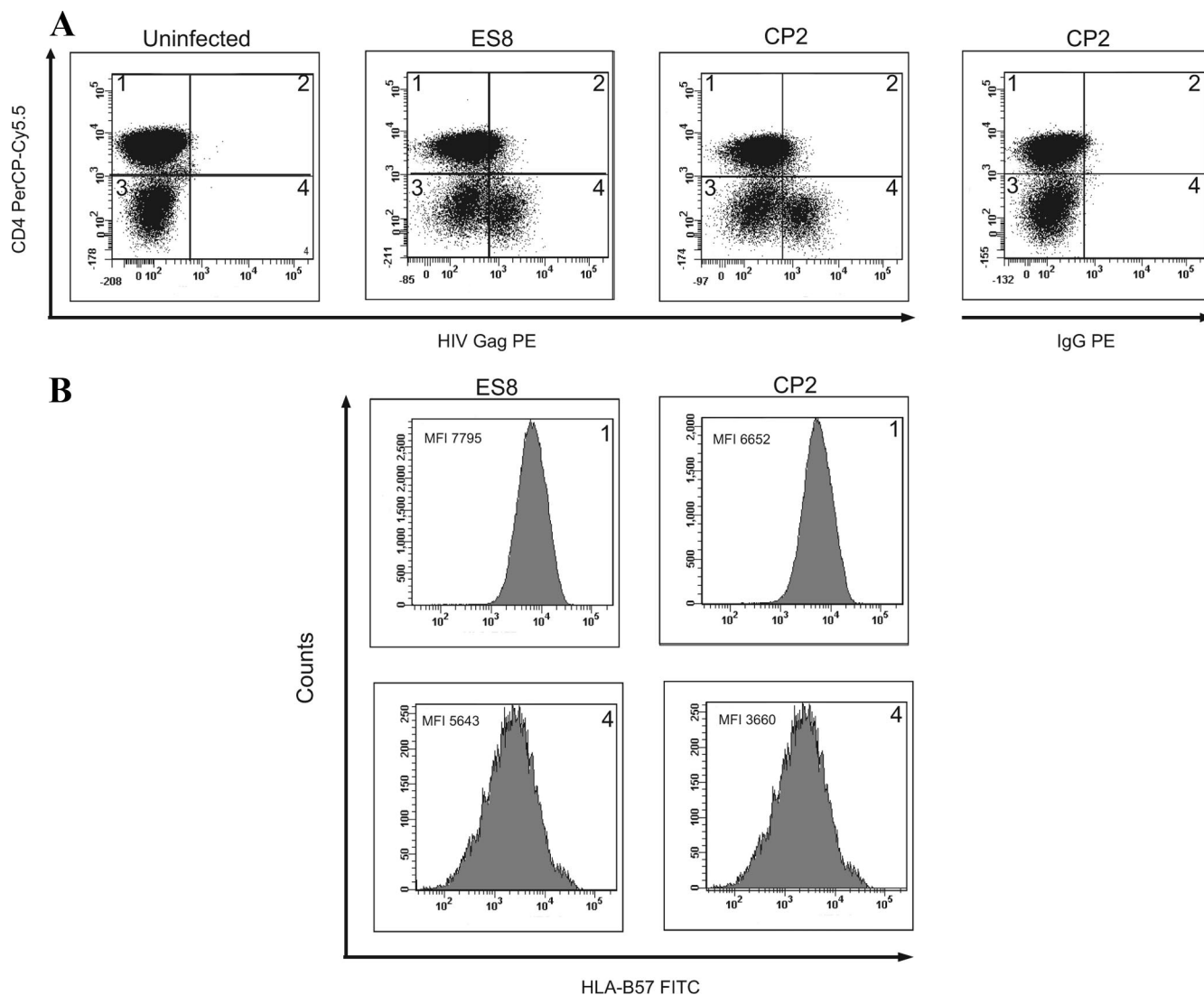


FIG. 1. Analysis of HLA-B*57 downregulation on HIV-1-infected cells. (A) CD8⁺ T-cell-depleted PBMC were stained with anti-HLA-B*57 and anti-CD4 monoclonal antibodies 3 days after infection with primary isolates from an ES (ES8) or a CP (CP2). Cells in quadrant 1 are uninfected CD4⁺ T cells, and cells in quadrant 4 (Gag-positive, low levels of CD4) are infected cells that have downregulated CD4. (PE, phycoerythrin; IgG, immunoglobulin G.) (B) The MFI of HLA-B*57 were compared for uninfected (quadrant 1) and infected (quadrant 4) cells from each sample.

develop a physiological model for HIV-1-induced MHC-I downregulation, we enriched primary CD4⁺ T cells from peripheral blood mononuclear cells (PBMC) from donors who were HLA-A*2 and/or HLA-B*57 positive by CD8⁺ T cell depletion with magnetic beads (Dyna), followed by activation in vitro with phytohemagglutinin for 3 days. For evaluation of HLA-A*2 downregulation, CD4⁺ T cells were obtained from HIV-seronegative donors. CD4⁺ T cells from ES were used for the evaluation of HLA-B*57 downregulation. This allele was as effectively downregulated in these ES as it was in multiple HLA-B*57 CP (data not shown). Following activation, the cells were infected with primary HIV-1 isolates from ES or CP by spinoculation (33). The primary isolates were obtained as previously described from latently infected CD4⁺ T cells (9). The median peak viral load and CD4⁺ T-cell nadir of the CP from whom viral isolates were obtained was 81,000 copies/ml

and 279 cells/ μ l, respectively, and thus these isolates should be effective at HLA downregulation (22).

At different time points, the cells were harvested and stained with either fluorescein isothiocyanate (FITC)-conjugated anti-HLA-A*2 (Becton Dickinson) and tricolor-conjugated anti-CD4 antibodies (Caltag) or biotinylated anti-HLA-B*57 antibody (One Lambda) followed by FITC-conjugated streptavidin, peridinin chlorophyll protein-Cy5.5-conjugated anti-CD4 antibody (Becton Dickinson), and allophycocyanin-conjugated anti-CD3 antibody. The cells were fixed and permeabilized with Cytotfix/Cytoperm solution (Becton Dickinson). Intracellular staining was then performed with the phycoerythrin-conjugated Gag-specific monoclonal antibody Kc57 or an immunoglobulin G1 mouse isotype control (Beckman Coulter). A total of 100,000 to 500,000 events were analyzed for each sample. HLA typing of ES was performed as

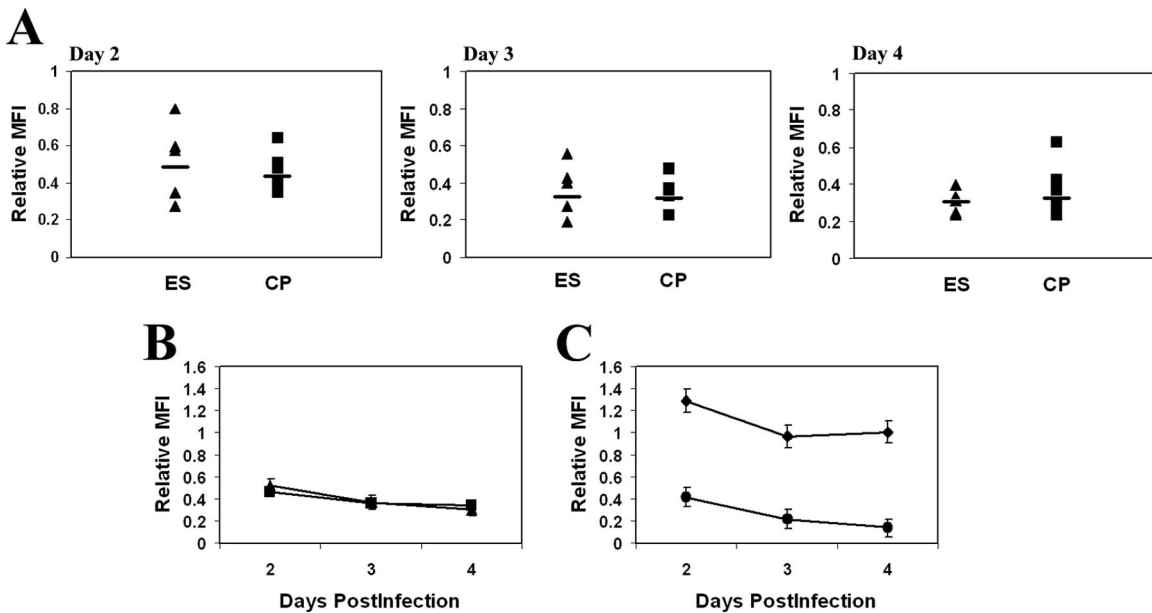


FIG. 2. (A) Relative MFIs of HLA-A*02 on cells infected with isolates from five ES (triangles) and eight CP (squares) on days 2 to 4 postinfection. The relative MFI is defined as the MFI of the infected cells divided by the MFI of the uninfected CD4⁺ T cells in each sample. The horizontal bars represent the median for each group. (B) Average relative MFI of HLA-A*02 for cells infected with isolates from ES and CP on each day. (C) Average relative MFI of HLA-A*02 for cells infected with the wild-type NL4-3-green fluorescent protein virus (diamonds) or the Nef⁻ Vpr⁻ mutant virus (circles).

previously described (4). The HLA-specific antibodies were tested on cells from a panel of ES with known HLA types to confirm specificity.

MHC-I downregulation was measured by comparing the mean fluorescence intensities (MFI) of HLA-A*2 and HLA-B*57 on HIV-1-infected versus noninfected CD4⁺ T cells. Infected cells were defined as cells that stained positive for intracellular Gag and had downregulated CD4 (Fig. 1). Uninfected CD4⁺ T cells were defined as cells that expressed high levels of CD4 and were negative for intracellular Gag protein. In order to standardize values, we determined relative MFI by dividing the MFI of the infected population by that of the CD4-positive, uninfected population. The Wilcoxon Mann-Whitney test was used to analyze the data.

To determine if there was a difference in the ability of HIV-1 isolates cultured from ES versus CP to downregulate HLA-

A*2, we measured the MFI of this molecule on infected CD4⁺ T cells that had downregulated CD4. On average, primary CD4⁺ T cells infected by ES viruses had levels of MHC-I downregulation of about two- to threefold, with relative MFI of 0.51, 0.37, and 0.30 on days 2, 3, and 4, respectively. Similarly, cells infected by isolates cultured from CP had relative MFI of 0.46, 0.36, and 0.33 on days 2, 3, and 4, respectively (Fig. 2B). These differences were not significantly different at any time point.

In order to rule out nonspecific downregulation of MHC-I on infected cells, we determined the MFI of HLA-DR and CD45 RO on cells infected with isolates from two subjects. The average relative MFI of the two proteins were 1.28 and 1.48, respectively, indicating that the MHC-I was in fact specifically downregulated. Since mutations in Nef have been shown to abrogate HLA downregulation, we also compared HLA-A2

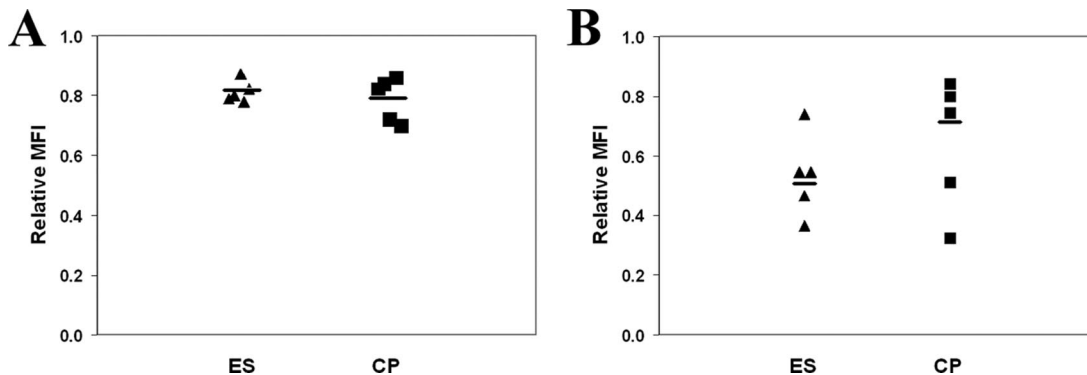


FIG. 3. (A) Relative MFI of CD3 on cells infected with isolates from five ES (triangles) and five CP (squares) on day 3 postinfection. The horizontal bars represent the median for each group. (B) Relative MFI of HLA-B*57 on cells infected with isolates from ES and CP.

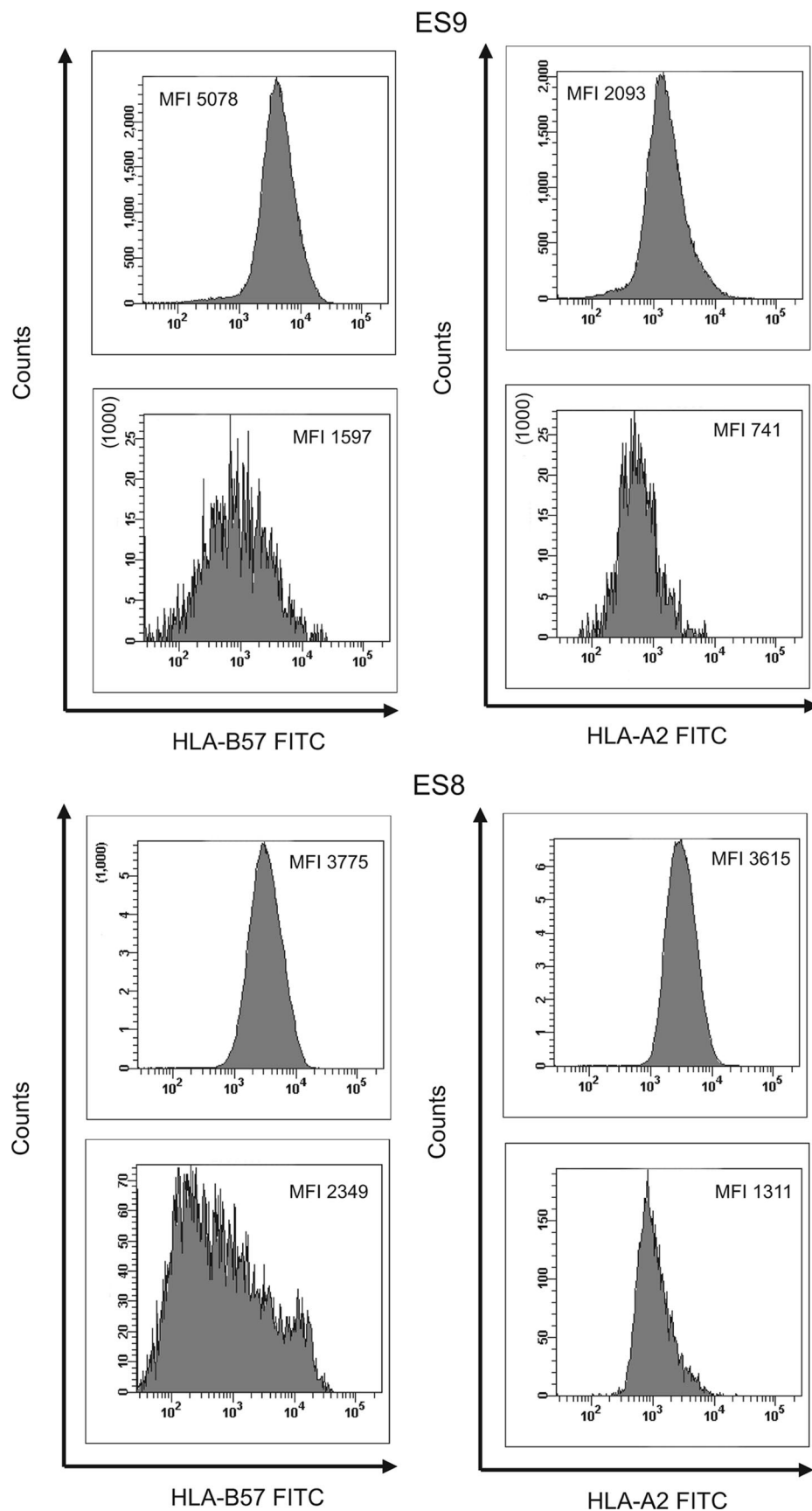


FIG. 4. Comparison of the relative MFI of HLA-A*02 and HLA-B*57 on CD8⁺ T-cell-depleted PBMC from ES8 and ES9 that were infected with autologous virus (ES8) or with the primary isolate from the CP who transmitted the virus to ES9. The MFI of HLA-A*2 or HLA-B*57 on uninfected CD4⁺ T cells (top panels) and infected cells that had downregulated CD4 (bottom panels) are shown.

downregulation by the HIV-1-based reporter construct NL4-3-green fluorescent protein and a Nef⁻ Vpr⁻ mutant vector (45, 46). As shown in Fig. 2C, no downregulation of HLA-A2 was seen at any point after infection with the Nef⁻ Vpr⁻ mutant virus, whereas infection with wild-type virus caused a degree of downregulation that was similar to that seen with primary isolates from ES and CP. Finally, we also looked at CD3 downregulation, as this molecule has been shown to be downregulated by Nef from HIV-2 and many simian immunodeficiency virus (SIV) isolates but not from HIV-1 (39). Furthermore, since SIVsmm *nef* isolated from sooty mangabeys with preserved CD4⁺ T-cell counts causes significantly more downregulation than SIVsmm *nef* from sooty mangabeys with CD4⁺ T-cell depletion (40), we determined whether isolates from ES also selectively downregulated this molecule. As shown in Fig. 3A, there was no significant downmodulation of CD3 after infection of cells with isolates from ES or CP.

Epidemiologic studies have suggested that HLA-B alleles play a larger role than HLA-A alleles in determining the outcome of infection (17). Furthermore, while HLA-B*57 is the most overrepresented allele seen in ES, there have not been any studies looking at downregulation of this MHC-I protein. Activated CD4⁺ T cells from an HLA-B*5703-positive ES were infected with isolates from five ES and five CP, and the degree of HLA-B*57 downregulation was measured on day 3. As shown in Fig. 3B, the average relative MFI of cells infected with isolates from five ES was 0.53, which was not significantly different from the average relative MFI of 0.64 that was seen in cells infected with isolates from five progressors.

While it appeared that there was generally more downregulation of HLA-A*2 than HLA-B*57, the studies were performed in cells from different donors, and this precluded a direct comparison of the MFI of the two MHC-I alleles. Two ES in our cohort were positive for both HLA alleles, and the degrees of downregulation of these proteins could thus be compared. CD4⁺ T cells from ES8 were infected with autologous virus (6), and cells from ES9 were infected with a primary isolate from the CP who transmitted virus to her (3). For patient ES8, HLA-A2 showed a greater degree of downregulation than HLA-B57 at day 3 (a relative MFI of 0.36 versus 0.62) (Fig. 4). In contrast, in ES9 the degrees of downregulation of the two proteins were nearly identical (a relative MFI of 0.35 for HLA-A2 versus 0.31 for HLA-B*57).

This is the first study to look at downregulation of MHC-I proteins on CD4⁺ T cells infected with HIV-1 isolates cultured from ES CD4⁺ T cells. We used a physiological model where primary CD4⁺ T cells were infected with primary HIV-1 isolates. One advantage of this approach is that it accounts for HLA downregulation mediated by viral proteins such as Tat (24), as well as Nef. Similar amounts of MHC-I downregulation were seen for cells infected with replication-competent isolates cultured from ES and progressors. These results demonstrate that most ES are not infected by HIV-1 virions that are deficient in downregulating MHC-I compared to those of CP. Thus, it is likely that other factors enable ES to control viremia. The identification of these factors will have implications for the design of HIV-1 vaccines.

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