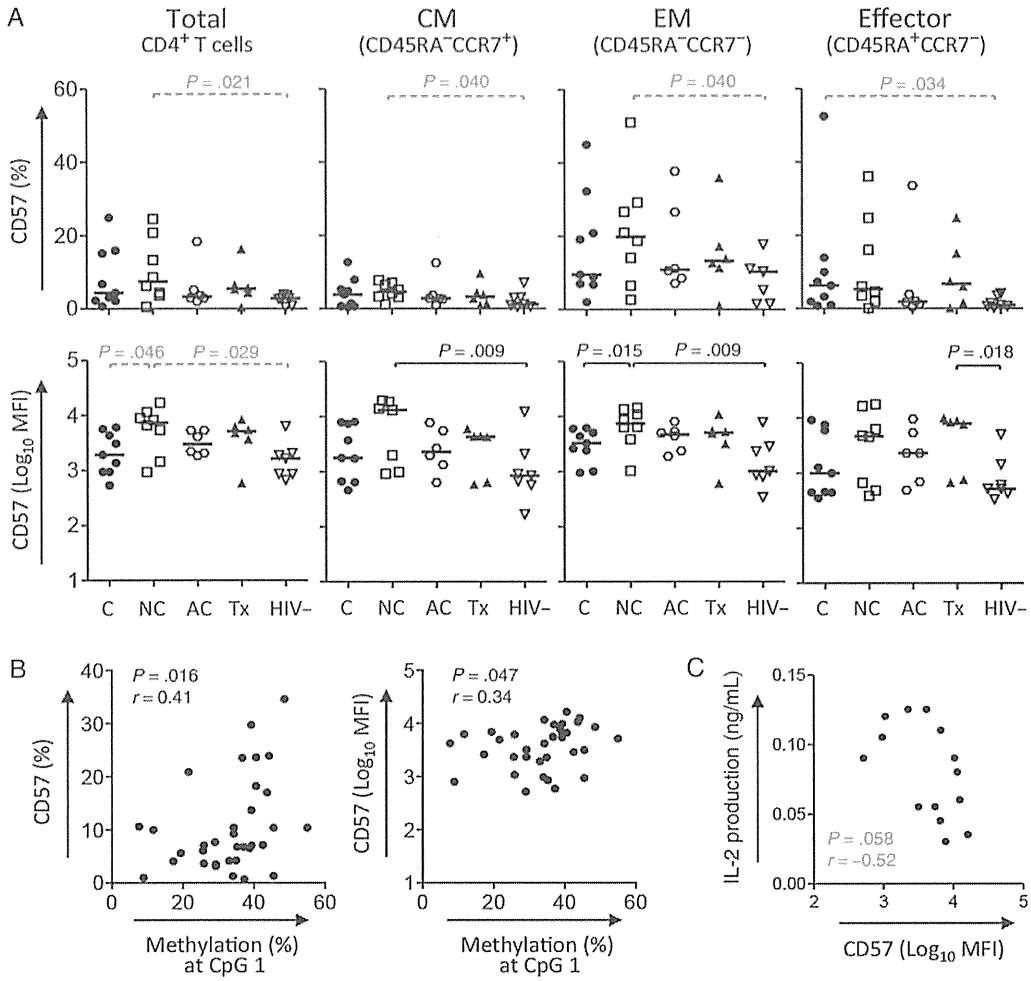


**Figure 6.** CD57 expression and methylation analysis of CpG site 1 in the *IL2* locus of CD4 memory T-cell subsets from individuals with human immunodeficiency virus type 1 (HIV-1) infection. *A*, Summary graph of CD57 expression level in each subset of $CD4^+$ T cells classified by CD45RA and CCR7. The Mann–Whitney *U* test was used for statistical analysis. *B*, Correlation plot of methylation at CpG site 1 vs CD57 expression in memory $CD4^+$ T cells. *C*, Correlation plot of methylation at CpG site 1 in $CD4^+$ T-cell vs IL-2 production in PHA-stimulated PBMCs. Correlation coefficient and *P* values in Spearman's rank correlation test are shown. *P* values less than .05 with $q > 0.2$ show in gray. Abbreviations: C, controller; NC, noncontroller; Ac, acute HIV-1 infection; Tx, treated with cART; HIV-, HIV-1-uninfected; CM, central memory; EM, effector memory.

We next measured the level of CD57 expression on individual $CD4^+$ T-cell subsets in all study groups (Figure 6*A*). Our data reveal that the frequency of $CD57^+$ cells was higher in noncontrollers than other groups and that the mean fluorescence intensity of CD57 expression on the total pool of memory $CD4^+$ T cells was significantly higher in noncontrollers.

Finally, we performed a correlation analysis between CD57 expression, DNA methylation of the *IL2* promoter, and IL-2 expression. A positive correlation was observed between CD57 expression and DNA methylation at CpG site 1 in the *IL2* promoter (Figure 6*B*). We also found a negative correlation between CD57 expression and IL-2 production in HIV-1–infected individuals (Figure 6*C*). Together, these data support a model whereby prolonged exposure of memory $CD4^+$ T cells to the chronic inflammatory environment in HIV-1 noncontrollers results in upregulation in CD57 expression and hypermethylation of the *IL2* promoter, ultimately resulting in senescence of polyclonal memory $CD4^+$ T cells.

## DISCUSSION

Although many studies about T-cell immunopathogenesis during chronic HIV-1 infection have been reported, the molecular basis for T-cell dysfunction is not well understood. To better understand the mechanism for the broad decline in $CD4^+$ T-cell function during chronic HIV-1 infection, we performed

a combination of molecular and cellular assays that assessed the epigenetic profile of T cells relative to their ability to express cytokines in HIV-1 noncontrollers. Our results demonstrate that the CpG sites in the *IL2* promoter are highly methylated in $CD4^+$ T cells in noncontrollers and that the lower IL-2 expression is correlated with *IL2* promoter DNA methylation in $CD4^+$ T cells. Furthermore, DNA methylation was positively correlated with CD57 expression on memory $CD4^+$ T cells. To understand the relationship between $CD4^+$ T-cell quality and the *IL2* DNA methylation, we sorted $CD4^+$ T-cell subsets on the basis of differentiation and senescent markers and analyzed the methylation of CpG sites in the *IL2* locus. Naive $CD4^+$ T cells were fully methylated, and demethylation occurred at all CpG sites during T-cell differentiation, consistent with previous reports using mouse model systems [17, 19]. CpG sites were partially but significantly remethylated in terminally differentiated $CD4^+$ T cells in healthy individuals. We also found that $CD57^+$ cells, most of which possessed a terminally differentiated phenotype in HIV-uninfected individuals, were highly methylated at CpG site 1, compared with $CD57^-$ memory $CD4^+$ T cells. In contrast, the *IL2* promoter in memory $CD4^+$ T cells from HIV-1–infected noncontrollers was highly methylated. Further, the promoter was also methylated in less differentiated $CD57^+$ memory $CD4^+$ T cells. Taken together, our data suggest that loss of *IL2* expression in senescent $CD4^+$ T cells is regulated by DNA methylation during chronic HIV-1 infection.

Our results indicate that the genomic region proximal to CpG site 1 in the *IL2* promoter is important for transcriptional regulation of the gene. This regulatory region includes binding sites for critical transcription factors in the *IL2* promoter/enhancer region [18, 40, 41]. It has also been shown that the CpG site 1–specific methylation abrogates Oct-1/NFAT binding to the regulatory region and causes inhibition of *IL2* expression in Jurkat cells [18]. In the present study, we observed higher methylation in not only site 1 but all CpG sites in HIV-1 noncontrollers, suggesting that other CpG sites may be involved in *IL2* gene regulation by altering accessibility of chromatin and/or transcription factor binding.

IL-2 expression strongly depends on costimulatory signals through the CD28 superfamily of receptors during antigenic stimulation [40, 42]. It has been reported that the increase in histone acetylation and DNA demethylation at the *IL2* locus after T-cell receptor activation is impaired in the absence of CD28 costimulation [29, 30]. In this study, we observed increased levels of methylation of the *IL2* promoter only in terminally differentiated $CD4^+$ T cells without CD28 expression in HIV-1-uninfected individuals, supporting the idea that CD28 signaling plays an important role in DNA demethylation and/or remethylation of the *IL2* gene. However, we also observed DNA hypermethylation in early effector memory subsets with CD28 expression in HIV-1 noncontrollers, in which CD57 expression was abnormally elevated. In our experiments using HIV-1-uninfected subjects, CpG site 1 methylation was approximately 3-fold higher in $CD57^+$ versus $CD57^-$ memory $CD4^+$ T cells. Furthermore, we observed a positive correlation between CD57 expression in memory $CD4^+$ T cells and DNA methylation at CpG site 1 in the *IL2* promoter and also found a negative correlation between CD57 expression and IL-2 production in stimulated PBMCs. Taken together, our data indicate that CD57 engagement results in epigenetic modification of the *IL2* gene in a CD28-independent manner. The mechanism for gene regulation of CD28 and CD57 during the development of senescent T cells in the aged population remains unknown. Therefore, future studies should investigate molecular mechanisms underlying the relationship between the expression of IL-2 and these surface molecules. Of note, we observed no difference in *IL2* promoter DNA methylation in $CD8^+$ T cells. Meanwhile, it has been reported that IL-2 secretion in HIV-1-specific $CD8^+$ T cells was also impaired in progressors [7, 43]. Therefore, it is likely that mechanisms aside from DNA methylation regulate IL-2 expression. It will also be important for future studies to include analysis of epigenetic programs of effector molecules in HIV-1-specific T cells before and after cART initiation.

In the present study, we have identified epigenetic modification of the *IL2* promoter as a potential mechanism for the loss of IL-2 expression in $CD4^+$ T cells. This reprogramming of the *IL2* promoter may be coupled to the signaling events that also induce T-cell senescence during chronic HIV-1 infection. Individuals with chronic HIV-1 infection with a high viral load who maintained a certain $CD4^+$ T-cell count were recruited as noncontrollers, and individuals in late phase of HIV-1 infection and patients with AIDS were excluded in this study. Although there was no significant difference in $CD4^+$ T-cell count between controllers and noncontrollers in our cohort (by design), we observed DNA hypermethylation of the *IL2* locus in noncontrollers. In contrast, acutely infected individuals with a high viral load did not show the defect. These data suggest that $CD4^+$ T cells become senescent with loss of IL-2 expression before the decline in $CD4^+$ T-cell quantity if the immune system is persistently exposed persistently to high viral loads during chronic HIV-1 infection. Our data support the concept that early initiation of cART is a promising way to slow HIV-1 disease progression and immunosenescence.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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