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SCIENCE DIRECT

Virology 332 (2005) 16–19

VIROLOGY

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Rapid Communication

# Decreased perforin and granzyme B expression in senescent HIV-1-specific cytotoxic T lymphocytes

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Received 8 April 2004; returned to author for revision 10 August 2004; accepted 17 November 2004
Available online 22 December 2004

**Abstract**

Cytotoxic T lymphocyte (CTL) senescence may be an important mechanism of immune failure in HIV-1 infection. We find that senescence of HIV-1-specific CTL clones causes loss of killing activity, preventable by transduction with telomerase. Furthermore, senescence is associated with reduced expression of the effector molecules granzyme and perforin, suggesting CTL "exhaustion" can result in hypofunction. These results agree with other studies showing that HIV-1-specific CTL exhibit abnormal phenotypes in vivo, and suggest the possibility that chronic turnover is an important mechanism of antiviral failure in HIV-1 infection.


*Keywords:* Perforin; Granzyme B; Cytotoxic T lymphocytes

## Introduction

The $CD8^+$ T lymphocyte compartment is markedly perturbed compared to $CD4^+$ T lymphocytes in HIV-1 infection (Effros et al., 1996; Wolthers et al., 1996), which is counterintuitive in light of the tropism of HIV-1 for $CD4^+$ cells. $CD8^+$ cytotoxic T lymphocytes (CTL) are believed to play a crucial role in suppressing the virus in vivo (Yang, 2003), and these cells likely proliferate constantly in the presence of ongoing viral replication during chronic infection. Clonal exhaustion, or replicative senescence, due to chronic proliferation therefore has been proposed as potentially important mechanism of immune failure. While the effects of senescence on cellular proliferative capacity, including lymphocytes (Hooijberg et al., 2000; Rufer et al., 2001), have been well established, the impact on cellular function remains less well understood. Here we evaluate a mechanism of dysfunction in HIV-1-specific CTL clones by preventing senescence by telomerase transduction.

## Results

### *Loss of cytolytic function due to senescence of HIV-1-specific CTL*

To evaluate the impact of senescence, HIV-1-specific CTL clones were transduced with hTERT, allowing constitutive expression of telomerase. Consistent with the known activity of telomerase to delay senescence and preserve replicative capacity of lymphocytes (Hooijberg et al., 2000; Rufer et al., 2001), the hTERT-transduced clones expanded more efficiently than the vector-transduced negative controls

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doi:10.1016/j.virol.2004.11.028

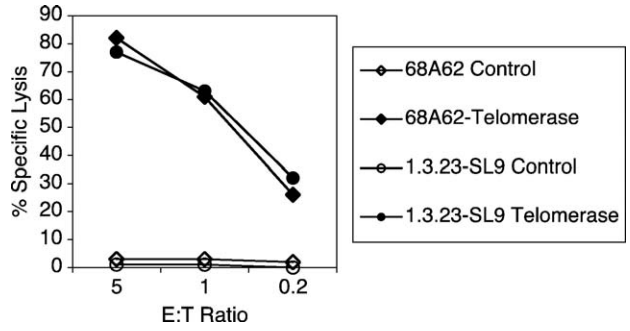


Fig. 1. Killing activity of hTERT transduced and nontransduced HIV-1-specific CTL. HIV-1-specific clones 68A62 and 1.3.23 were utilized in standard chromium release assays (effector to target ratio of 5:1) using the HLA A*0201-expressing cell line T1 pulsed with the appropriate synthetic peptide. Clone 68A62 had been passaged for 12 weekly passages after hTERT transduction, and 1.3.23-SL9 for 10 weekly passages.

after multiple passages [demonstrated for clone 68A62 in Dagarag et al. (2003) and data not shown]. When the passaged CTL clones were assessed for killing capacity against peptide-pulsed target cells in chromium release assays, the nontransduced CTL were found to have lost killing activity, while the hTERT-transduced CTL retained the ability to kill efficiently (Fig. 1). At effector/target cell (E/T) ratios of 5:1 or less, the nontransduced clones mediated specific lysis of 3% or less. In contrast, the same clones that had been transduced by hTERT retained efficient cytolytic capacity, mediating about 80% specific lysis at an E:T ratio of 5:1. Thus, there was a striking discrepancy in the ability of CTL to kill in the presence or absence of constitutive hTERT expression, in agreement with prior results with HIV-1-specific CTL (Dagarag et al., 2003).

*Reduced perforin and granzyme B expression in senescent HIV-1-specific CTL*

The perforin–granzyme pathway has been shown to be the major mechanism of killing by HIV-1-specific CTL (Shankar et al., 1999). The hTERT-transduced and non-transduced clones were therefore evaluated for expression of perforin and granzyme B, to evaluate whether altered levels might be responsible for differential killing activity (Fig. 2). Intracellular staining for perforin demonstrated nearly undetectable levels in the nontransduced cells, and weakly detectable levels in the hTERT-transduced cells. Staining for granzyme B revealed moderate staining of nontransduced cells, but clearly more intense staining of hTERT-transduced cells. The delay of senescence by hTERT transduction was

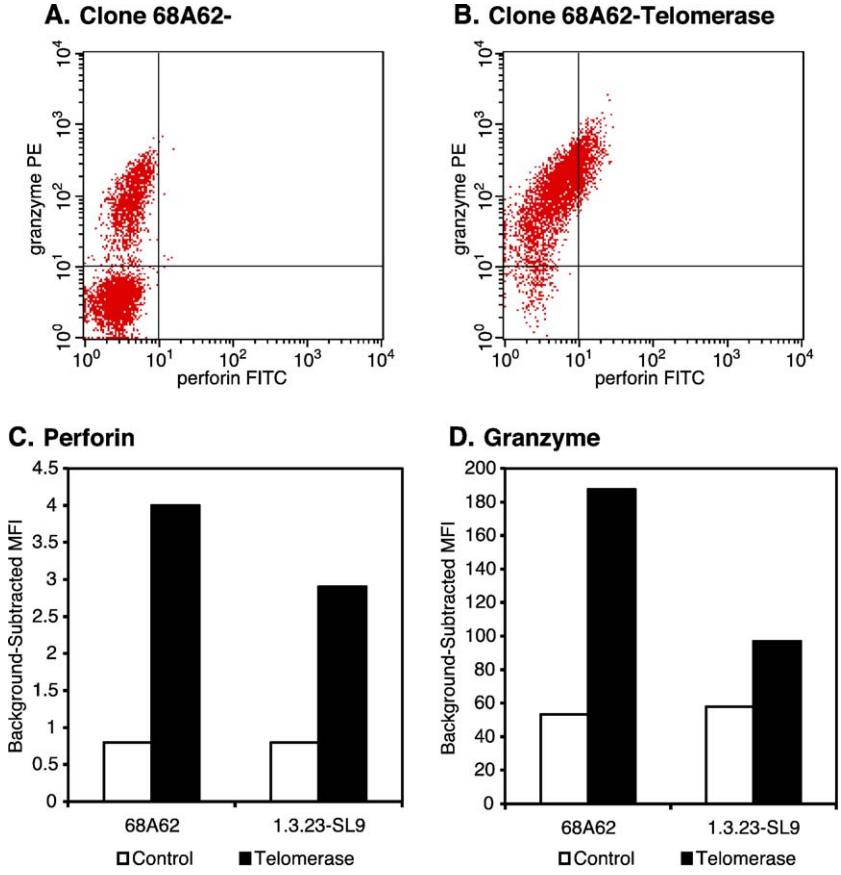


Fig. 2. Perforin and granzyme expression in hTERT transduced and nontransduced HIV-1-specific CTL. The same clones utilized in Fig. 1 were stained for intracellular perforin and granzyme B measurement by flow cytometric analysis. (A, B) Dot plots of clone 68A62 staining are shown. Mean fluorescence intensities of specific staining (above isotype control staining) are plotted for (C) perforin and (D) granzyme B.

therefore associated with reduced perforin and granzyme B expression, in addition to loss of cytolytic capacity.

## Discussion

These data suggest a mechanism for our prior observation that telomerase transduction prevents the selective loss of cytolytic function but not interferon-gamma production by senescent HIV-1-specific CTL clones (Dagarag et al., 2003). In the context of results showing extensive turnover and telomere shortening in the $CD8^+$ T lymphocyte compartment of HIV-1-infected individuals, these findings imply a functional derangement of $CD8^+$ effector T lymphocytes. Although the precise mechanisms whereby CTL suppress HIV-1 in vivo remain unknown, in vitro experiments have indicated that cytolytic function is a crucial component (Yang et al., 1997). Loss of CTL killing potential due to senescence therefore could contribute to ineffectiveness of cellular immunity in vivo. In support of this hypothesis, Migueles et al. (2002) have reported that perforin expression and proliferative capacity are associated with long-term nonprogression in HIV-1-infected persons.

CTL function is likely to be an important aspect when analyzing the response to HIV-1 infection (Yang, 2003). Global measurements of CTL frequency or breadth have indicated that the antiviral activity of CTL in vivo is not a simple quantitative function (Addo et al., 2003; Betts et al., 2001). It is probable that this interaction has multiple complex determinants, including the efficiency of infected cell recognition due to epitope targeting (Yang et al., 2003b), the ability of HIV-1 to escape through epitope mutation (Yang et al., 2003a), and the function of CTL after being triggered to recognize an infected cell. Addressing such factors may be crucial to the success of immune-based therapies for HIV-1 infection.

## Materials and methods

### *HIV-1-specific CTL clones*

HIV-1-specific CTL clones were obtained from the blood of infected persons through limiting dilution cloning, and maintained in tissue culture with periodic restimulations using anti-CD3 antibody and irradiated allogeneic feeder PBMC, as previously reported (Yang et al., 1996, 1997). Clones 68A62 and 1.3.23 recognized the HLA A*0201-restricted epitopes ILKEPGHGV (RT a.a. 309–317 numbered according to the HXB2 sequence) and SLYNTVATL (Gag p17 a.a. 77–85), respectively.

### *Transduction of CTL with telomerase*

The clones were transduced using a viral transduction vector generated from stably transfected PA317 packaging cells provided by Geron Corporation (Menlo Park, CA), as previous described (Dagarag et al., 2003).

### *Chromium release assays*

Transduced and nontransduced CTL clones were screened for lytic activity by standard chromium release assays using T1 cells (expressing A*0201) labeled with excess cognate peptides, as previously described (Yang et al., 1996).

### *Flow cytometric analysis of perforin and granzyme B expression*

Transduced and nontransduced CTL clones were stained for intracellular perforin (FITC conjugated antibody from Ancell Corporation, Bayport, MN) and granzyme B (PE conjugated antibody from Research Diagnostics, Flanders, NJ) after permeabilization (Becton Dickinson), and evaluated by flow cytometric analysis.

## Acknowledgments

This work was supported by NIAID grants AI043203 and AI054286. We thank Bruce D. Walker for the generous contribution of clone 68A62.

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