

HIV-1-Specific CTL Recognizing a Scarce Epitope Are Not Suppressed by HIV-1 Protease Inhibitors

Otto O. Yang

Division of Infectious Diseases, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA and

Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA and
AIDS Institute, University of California, Los Angeles

Abstract: HIV-1-protease inhibitors have received growing attention as agents that can affect proteases in human proteasomes, with potentially deleterious effects for HIV-1-specific cellular immunity. Studying the antiviral activity of HIV-1-specific CTL that recognize an epitope that is presented at limiting concentrations on infected cells, we find that several HIV-1 protease inhibitors do not affect their function. These data indicate that these protease inhibitors may have minimal impact on the antiviral activity of CTL.

Key words: HIV, HLA class I antigens, protease inhibitor, antiviral immunity

INTRODUCTION

HIV-1 protease inhibitors (PIs) are commonly-utilized antiviral drugs for HIV-1 infection that exert antiviral effects by interfering with maturational processing of Gag. These agents are intended to be specific for viral and not host proteases, including the proteasome proteases that generate CD8⁺ cytotoxic T lymphocyte (CTL) epitopes^[1]. Given that HIV-1-specific CTL responses are important for immune containment of HIV-1 infection (reviewed in [2]), the impact of these PIs on human proteasome function has been an area of interest.

Multiple lines of evidence have been presented to demonstrate PI interference with human proteasome function and production of viral epitopes. Several *in vitro* studies have suggested that PIs can inhibit or alter proteasome function^[3-6] and an early study suggested *in vitro* interference with proteasome function in human cells and *in vivo* interference in LCMV-infected mice^[7]. More recently, Legrand *et al* presented data from PI-treated humans suggesting a greater boost of HIV-1-specific CTL responses when PI therapy is interrupted^[8]. These findings have raised concerns that PIs could interfere with antiviral immunity. Here we evaluate the impact of PIs on the antiviral function of HIV-1-specific CTLs, using a functional assay.

MATERIALS AND METHODS

Protease inhibitor-resistant HIV-1: A virus stock of NL4-3 containing multiple protease mutations (L10R, M46I, L63P, V82T) rendering the virus highly resistant against protease inhibitor drugs^[9] was the kind gift of Richard D'Aquila. The mutations in this strain allow replication in the presence of drug concentrations that would normally prevent HIV-1 replication and thus allowed evaluation of CTL antiviral efficiency in the presence of excess drug.

CTL: The HIV-1-specific CTL clone 68A62, recognizing the HLA A*0201-restricted RT epitope ILKEPVHGV, was derived and maintained as previously described^[10]. This clone was the generous gift of Bruce D. Walker.

Protease inhibitors: The protease inhibitor drugs (indinavir, nelfinavir, ritonavir and saquinavir) were the generous gift of Martin Hirsch. Test concentrations were chosen to exceed the 95% inhibitory concentration of wild type NL4-3 virus replicating in H9 cells^[9].

HIV-1 suppression assay: A modification of a previously described assay to assess CTL-mediated inhibition of HIV-1 in acutely infected cells was utilized^[10]. Briefly, T1 cells (HLA A*0201⁺), which had

Corresponding Author: Otto O. Yang, Division of Infectious Diseases, 37-121 CHS, UCLA Medical Center, 10833 LeConte Ave, Los Angeles, CA, 90095, USA, Tel: 1-310-794-9491, Fax: 1-310-825-3632

been pre-cultured for 7 days in the absence or presence of protease inhibitors at the indicated concentrations, were acutely infected with the virus at a multiplicity of 2×10^{-2} TCID₅₀/cell. These acutely infected cells then were cocultured with the CTL clone 68A62 at a ratio of 5×10^5 target cells and 1.25×10^5 CTL in a total volume of 2ml per well in 24 well plates, in the continued absence or presence of the protease inhibitor drugs. Viral replication was then assessed by quantitative HIV-1 p24 antigen ELISA (Dupont, Boston MA). Suppression of viral replication by the drugs was calculated by comparing cultures of T1 cells alone versus those with drugs. Suppression of viral replication by CTL in the absence or presence of drugs was calculated by comparing cultures of T1 cells (with or without drugs) to those of T1 cells (also with or without drugs) in the presence of CTL.

RESULTS AND DISCUSSION

Therapeutic concentrations of protease inhibitor drugs did not interfere with the antiviral activity of HIV-1-specific CTL: The goal of PI treatment is to achieve an HIV-1-suppressive concentration. To examine the ability of CTLs to suppress HIV-1 replication under this condition, an HIV-1 clone with high-level PI resistance (L10R, M46I, L63P, V82T)^[9] was utilized, allowing assessment of CTL impact on virus replication in the presence of relevant drug concentrations. Indinavir, Ritonavir, Nelfinavir and Saquinavir were suppressive at one or both tested concentrations (Fig. 1A), indication therapeutic drug levels in this culture system.

Concurrently, the ability of CTL to suppress HIV-1 replication (in the presence or absence of PIs) was tested, using a CTL targeting an HIV-1 epitope that is presented on infected cells at limiting concentrations for CTL recognition^[10-12]. The degree of CTL-mediated viral suppression in the presence of each drug was consistently greater than in the absence of drug (Fig. 1B). This cellular antiviral activity was not related to the amount of drug-mediated inhibition (compare Fig. 1 A to B). Thus, the protease inhibitors at physiologically relevant intracellular concentrations did not apparently impair class I antigen processing and presentation of an epitope that is presented near the limit for CTL recognition.

Since an initial study by Andre *et al* proposed that the HIV-1 protease inhibitor Ritonavir impairs the chymotrypsin-like activity in murine and human proteasomes^[7], there has been interest regarding the implications for cellular immunity in HIV-1 infection. More recently, an interesting clinical study by Legrand *et al* suggested that a vigorous rebound in HIV-1-specific CTL activity after interruption of PI-containing

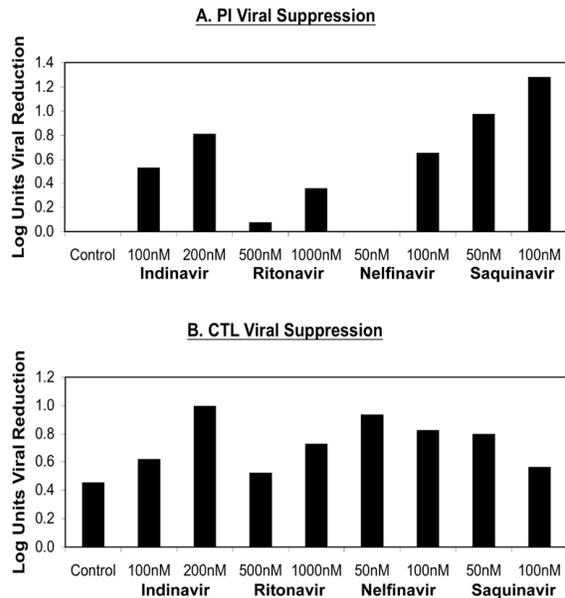


Fig. 1: HIV-1 protease inhibitors do not reduce the antiviral activity of CTL. T1 cells (pre-cultured for 7 days in the presence of the indicated concentrations of protease inhibitor drugs) were acutely infected with a protease inhibitor-resistant strain of HIV-1 NL4-3 and cultured in the continued presence of drug with and without an RT-specific CTL clone. Viral replication was assessed by p24 antigen ELISA; Day 10 data are plotted. A. The suppression of the virus by each PI is plotted (comparing viral replication in the presence and absence of PI, without CTL). B. The suppression of the virus by CTL in the presence of different PIs is plotted (comparing viral replication in the presence of each PI and the presence and absence of CTL)

treatment regimens supported a mechanism of PI interference with CTL responses^[8]. However, work from Kelleher *et al* demonstrated no evidence for PI effects on the presentation of HIV-1 epitopes^[13].

Our results agree with Kelleher *et al*, using a more physiologic experimental system. Their study examined HIV-1 epitopes presented by target cells infected with recombinant vaccinia viruses, which express proteins at high levels. Our data utilize target cells that express proteins and epitopes under conditions of actual HIV-1 infection. While Kelleher *et al* also examined CTL recognition of the ILKEPVHGV epitope expressed by vaccinia, HIV-1-infected cells process and present this epitope inefficiently, at an average of only about 14 copies per infected cell^[14]. Thus, despite experimental extremes of high concentrations of PIs (able to affect HIV-1 containing high level PI resistance mutations) and limiting levels of epitope, no effects of PIs were seen on the ability of CTL to clear HIV-1. Because this epitope is produced via the chymotrypsin activity of the proteasome^[15] that was proposed to be inhibited by PIs

(particularly ritonavir^[5,7]), our results suggest that the observed phenomena may not be functionally significant.

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