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Nevirapine Inhibits the Anti-HIV Activity of CD8+ Cells

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Abstract

Antiretroviral therapy (ART) significantly reduced the CD8+ cell non-cytotoxic anti-HIV response (CNAR) in twelve HIV-1-infected subjects ($p < 0.0001$). In separate experiments, CD8+ cells from long term survivors (LTS) were co-cultured with HIV-infected CD4+ cells using varying concentrations of anti-HIV drugs. The antiviral function of CD8+ cells from four of fourteen LTS was reduced with exposure to 10μM nevirapine ($p < 0.05$). The antiviral activity of CD8+ cells from two LTS was inhibited by 5μM zidovudine. These studies indicate that nevirapine and probably zidovudine can inhibit the anti-HIV activity of CD8+ cells and thus could influence the effectiveness of ART.

Keywords

Drug resistant HIV-1; CD8+ cell Non-cytotoxic Antiviral Response; Antiretroviral therapy; Nevirapine; Zidovudine

Introduction

Antiretroviral therapy has significantly prolonged the lifespan of HIV-infected people[1,2]. However, drug-resistant HIV-1 can arise during the course of ART. The prevalence of antiretroviral drug resistance is increasing among subjects newly infected with HIV-1[3–5]. The cause for the emergence of drug-resistant viruses can involve selection due to changes in the immune system. Some studies have shown that antiretroviral therapy is associated with a reduction in number of effector cytotoxic T lymphocytes[6], HIV-specific CD4+ T-cell activity[7], the CD8+ non-cytotoxic anti-HIV response[8], and anti-HIV antibody production[9,10]. The specific effects of individual antiretroviral drugs on distinct CD8+ cell anti-HIV responses have not been evaluated. We focused on the CD8+ non-cytotoxic anti-HIV response (CNAR) that correlates with control of HIV infection[11–13].

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There is no conflict of interest.

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Materials and Methods

Human Subjects

The subjects from an established cohort of HIV-infected individuals at the University of California, San Francisco (UCSF) were analyzed. The study subjects included 12 HIV-1-infected individuals who were receiving combination antiretroviral therapy and 14 long-term survivors (LTS). LTS are HIV-1-infected subjects, who have remained asymptomatic with normal CD4+ lymphocyte counts (> 500cells/μl) without therapy for more than 10 years of infection. The levels of total leukocytes, granulocytes, lymphocytes, monocytes, platelets, and T cell subsets were determined using a BD FACSort. Measurements of plasma HIV RNA levels were performed using a branched-DNA (bDNA) assay (Siemens Diagnostics, Emeryville, CA) or were self-reported. This study was approved by the UCSF Committee on Human Subjects.

Human primary cells

Buffy coats from HIV-seronegative subjects were obtained from the Blood Centers of the Pacific, San Francisco, CA. Blood from HIV-infected individuals was collected at UCSF. Peripheral blood mononuclear cells (PBMC) were prepared by Histopaque-1077 (Sigma-Aldrich) density gradient centrifugation. Primary CD8+ cells were purified from HIV-infected individuals by positive immunomagnetic (IM) isolation using Dynal beads (Cat. No. 113.33D, Invitrogen). Primary CD4+ cells from the buffy coats were purified by positive selection using Miltenyi IM beads (Mat. no. 120-000-440, Miltenyi Biotec). The purity of both T-cell populations was >95%. The primary T cells were stimulated for 3 days with PHA-L (3μg/ml) (Sigma).

CD8 + Cell HIV-1 Suppression Assay

The stimulated human CD4+ T cells were acutely infected with different HIV-1 isolates or clones: nevirapine (NVP) -resistant virus (NIH Cat # 1392), multidrug resistant virus (NIH Cat # 7391), efavirenz (EFV) -resistant virus clone v7205-2, nelfinavir (NFV) -resistant virus clone v16970-2, or the ritonavir (RTV) -resistant virus clone v40539-1. For measurement of CNAR, CD8+ cells at different CD8+ cell: CD4+ cell input cell ratios (0.5:1, 1:1, 2:1) were co-cultured with HIV acutely infected CD4+ cells in triplicate wells in a 96-well plate with varying concentrations of antiretroviral drugs[11]. To measure HIV replication levels in the cultures, 100μl aliquots of culture supernatants were collected from each well on days 4, 7 and 10 post-infection. Fluids were treated with 1% Triton, and the HIV p24 ELISA (NIH AIDS Research and Reference Reagent Program) was performed to evaluate the concentration of p24 antigen in the culture. Alternatively, the collected fluids were centrifuged at 12,000g for 1 h at 4°C, and the resulting viral pellets were assayed for reverse transcriptase (RT) activity as described[14]. Results obtained from both assays were comparable[15].

Mass spectrometry

Intracellular drugs were measured using liquid chromatography tandem mass spectrometer (LC-MS/MS), consisting of two Shimadzu LC-20AD^{XR} pumps, a SIL-20AC^{XR} autosampler, and an AB Sciex API5000 mass spectrometer[16,17]. CD8+ cells pre-treated with 1 μM or 10 μM NVP, EFV or NFV were washed twice with 1xDPBS and pelleted. The cell pellets were resuspended in 400 μl of 60% methanol with 1% formic acid, vortexed for 1 min, and sonicated for 10 min. The lysed cells were centrifuged at 22,000 g for 7 min. A 10 μl aliquot of the supernatant was directly injected into the LC-MS/MS system. Ion pair 267/226 for NVP and 568/330 for NFV was used for MRM detection in ESI^+ mode. For EFV, ion pair 314/68 was used for detection in ESI^- mode. The lower limits of

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quantification for NVP, NFV and EFV were 0.038nM (S/N=15), 0.05nM (S/N=91), and 0.5nM (S/N=12.7), respectively.

Results

Effect of ART on the CD8+ cell anti-HIV response

CNAR activity was measured prior to therapy and during the course of ART for up to 5 years in 12 HIV-infected subjects. With treatment, the CD4+ T cell counts in these subjects declined before antiretroviral therapy and increased while on therapy (Fig. 1A). The HIV RNA levels in all these infected patients except subject 11 were undetectable after 1 year of antiretroviral therapy (Fig. 1B). For these studies, a conventional CNAR assay was conducted using the CXCR4-tropic, chemokine-resistant virus, HIV-1_{SF33}[11]. CNAR significantly declined in 10 of 12 HIV-infected subjects during antiretroviral therapy ($p <$ 0.001) (Fig. 1C). The data on Subjects 11 and 12 were not collected before therapy, but following therapy a similar decline was observed. The CNAR activity of CD8+ cells from 8 of the 12 subjects was less than 50% after 5 years of treatment (Fig. 1D). And, CNAR activity in 4 of the 12 subjects decreased more than 4-fold during the course of ART.

Effect of anti-HIV drugs on the anti-HIV response of CD8+ cells in culture

Five drug-resistant viruses confirmed to be CXCR4-tropic using the U373-MAGI CXCR4 cell line tropism assay[18] (data not shown) were used. The sensitivity of these viruses to the antiviral drugs was first assessed. Replication of the NVP-resistant virus and EFV-resistant virus were not affected by 10μM drugs, but were significantly reduced in the presence of 25μM of the drugs ($p < 0.01$). The replication of a mutidrug-resistant virus (NIH Cat # 7391) was not affected by 5μM abacavir (ABC), lamivudine (3TC) or zidovudine (ZDV) (data not shown). The replication of the NFV-resistant virus and the RTV-resistant virus was not affected by 2μM NFV or RTV. The plasma drug concentrations in subjects receiving these drugs as ART are equal to or greater than the amount used for these studies[19,20].

To determine the effect of antiretroviral medications on CNAR, we isolated CD8+ cells from 14 HIV-infected drug-naive LTS and co-cultured them with CD4+ cells infected by drug-resistant HIV. Co-cultures were performed with or without the presence of varying concentrations of the antiretroviral drugs. The concentrations used did not substantially reduce the replication of the drug-resistant viruses. The extent of HIV suppression was calculated by comparing the p24 levels or RT value in the supernatants of the co-culture to the average amount of HIV-1 virus replication in CD4+ cells infected with the same virus and exposed to the same concentration of the anti-HIV drug. CD8+ cells from 4 of the 14 LTS were sensitive to 10μM NVP. When exposed to this drug, their antiviral activity measured by CNAR, was significantly reduced ($p < 0.05$) (Table 1). No association was observed between the HLA genotype and the sensitivity of the CD8+ T cell anti-HIV activity to NVP (data not shown). CD8+ cells from 2 of 14 LTS were also sensitive to 5μM zidovudine and showed a reduction in CNAR. However, the difference was not significant ($p > 0.05$) (Table 1). All other antiretroviral medications did not affect CNAR in the 14 subjects (Table 1).

Each drug did not inhibit the proliferation of T lymphocytes in culture (data not shown). And, varying concentrations of the drugs used in this study (e.g. 0.5 μM – 10μM) did not show cytotoxicity to CD8+ and CD4+ T cells. The expression of activation markers, such as HLA-DR, CD25, CD69, on both CD8+ and CD4+ T cells in the co-culture were also not affected by 10μM NVP (data not shown).

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Residual presence of NVP, EFV, and NFV in primary CD8+ cells

The mass spectrometry data showed that 0.33 – 0.83 % of NVP remained in the CD8+ cells after 3 days. The percentages of residual CD8+ cell intracellular concentrations of EFV and NFV were 0.23–2.24% and 1.45–11.6%, respectively (Supplemental Figure 1). The reduction of CD8+ cell anti-HIV activity by only NVP suggests this drug has a specific effect on CD8+ cell function.

Discussion

Antiretroviral medications can have adverse clinical effects. NVP can cause liver damage, skin reactions, and allergic reactions[21–23]. In some cases, hepatic injury progresses despite discontinuation of treatment. ZDV is associated with hematologic toxicity including neutropenia and anemia, particularly in patients with advanced HIV-1 disease. In addition, pure red cell aplasia occurs with ZDV after 6 weeks to 4 years of therapy[24–26].

Our data show that the immune system can also be affected by antiretroviral therapy. CNAR was significantly reduced during the 5 years clinical course of ART ($p < 0.0001$) (Fig. 1C). The anti-HIV response slowly declined as CD4+ cell counts recovered and viral loads became undetectable (Fig. 1A, B, D).

While these results could reflect the lack of viral antigen stimulation of CD8+ cell responses, a toxicity to the immune system could be involved. To investigate this possibility, CD8+ cells from HIV-infected long term survivors were isolated and co-cultured with CD4+ cells infected with drug-resistant HIV at different concentrations of the anti-HIV medications. The data showed that the CD8+ cells from 4 of these subjects were very sensitive to 10μM NVP (Table 1). The relevant concentration of 10 μM NVP is 2663 ng/ml. That amount is equal to the lowest plasma concentration of NVP (4,500±1,900ng/ml) in individuals on the drug[19]. The anti-HIV activity of CD8+ cells from these subjects was markedly inhibited by NVP. This effect did not involve a block in cell proliferation nor activation of CD8+ cells and CD4+ cells. Two subjects were also sensitive to 5μM ZDV (Table 1). The relevant concentration of 5μM ZDV is 1336 ng/ml. That amount is in the range of plasma concentrations of ZDV (1,220±210ng/ml) in subjects on the drug[20].

Due to the measurement limitation of intracellular concentration of drugs, residual NVP, EFV and NFV in primary CD8+ cells were evaluated by mass spectrometry. All three drugs were found in CD8+ cells. The reduction of CD8+ cell anti-HIV activity by only NVP suggests this drug has a specific detrimental effect on CD8+ cell function. Our data suggest that the effect of NVP and perhaps other ART on CD8+ cell anti-HIV responses should be considered when administering these drugs to HIV-infected patients.

In summary, NVP and to some extent ZDV can inhibit the anti-HIV activity of CD8+ cells and could influence the beneficial anti-HIV effects of ART. Drug-resistant viruses still occur especially when patients are treated with NVP alone over time despite the high anti-HIV efficacy of NVP[27]. If the CD8+ T cells from patients on ART still have anti-HIV responses, these immune responses could help prevent HIV replication and the emergence of drug-resistant viruses.

Supplementary Material

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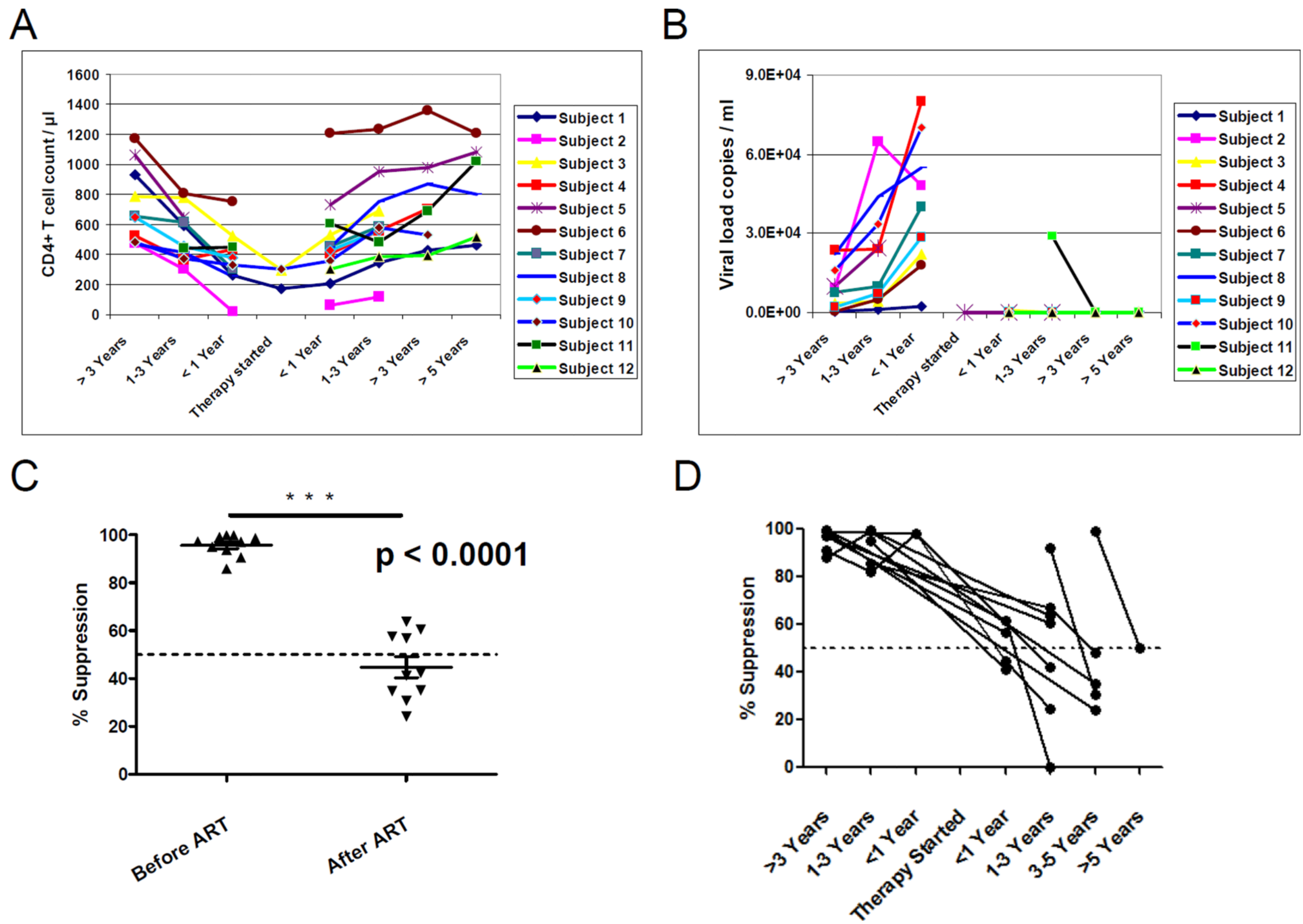

Fig. 1. Effect of antiretroviral drugs on CD4+ T-cell count, viral load and CD8+ cell anti-HIV response

12 subjects were enrolled to study the CD8+ cell non-cytotoxic response (CNAR) prior to therapy and during the course of ART. The data on Subjects 11 and 12 were not collected until therapy had already been initiated. CNAR activity was measured by the reduction of HIV replication using the reverse transcriptase (RT) assay[14] or p24 ELISA. A. Patients' CD4+ T cells counts. B. Patients' viral loads. C. The extent of suppression of HIV-1_{SF33} virus production was significantly reduced during therapy ($p < 0.0001$, n=10). D. All but 4 subjects showed a notably decline in CNAR. Four subjects had a limited reduction in CNAR during the course of ART.

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Table 1

Effect of antiviral drugs on CD8+ cell anti-HIV response in culture

CNAR	Efavirenz		Nevirapine		Abacavir		Lamivudine		Zidovudine		Nelfinavir		Ritonavir	
Subject	**5μM**	**10μM**	**5μM**	**10μM**	**0.5μM**	**5μM**	**0.5μM**	**5μM**	**0.5μM**	**5μM**	**0.5μM**	**2μM**	**0.5μM**	**2μM**
S 01	n/d	n/d	–	–	n/d	n/d	n/d	n/d	–	–	–	–	n/d	n/d
S 02	n/d	n/d	–	**84%→21%***	n/d	n/d	n/d	n/d	–	–	–	–	n/d	n/d
S 03	n/d	n/d	–	**57%→ – 41%***	n/d	n/d	n/d	n/d	–	**63%→30%**	–	–	n/d	n/d
S 04	n/d	n/d	–	–	n/d	n/d	n/d	n/d	–	–	–	–	n/d	n/d
S 05	n/d	n/d	–	**88%→35%***	n/d	n/d	n/d	n/d	–	–	–	–	n/d	n/d
S 06	n/d	n/d	–	–	n/d	n/d	n/d	n/d	–	–	–	–	n/d	n/d
S 07	–	–	–	**83%→25%***	–	–	–	–	–	–	–	–	–	–
S 08	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S 09	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S 10	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S 11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S 12	–	–	–	–	–	–	–	–	–	**93%→63%**	–	–	–	–
S 13	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S 14	–	–	–	–	–	–	–	–	–	–	–	–	–	–

CD8+ cells isolated from long term survivors were cocultured with CD4+ cells acutely infected with drug-resistant HIV and exposed to varying concentrations of the medications. The extent of suppression was calculated by comparing the RT value or p24 concentration from the coculture fluid to the values of supernatants from HIV-infected CD4+ cells cultured alone with the same concentration of drug. CNAR, CD8+ cell non-cytotoxic antiviral response.

A – sign indicates < 30% change in CNAR activity.

* $p < 0.05$ as determined by t-test. n/d, not done.