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### **CYTARABINE TREATMENT OF HUMAN T-LYMPHOID CELLS INDUCES DECREASED HIV-1 RECEPTOR EXPRESSION AND REDUCED HIV-1 SUSCEPTIBILITY**

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## **CYTARABINE TREATMENT OF HUMAN T-LYMPHOID CELLS INDUCES DECREASED HIV-1 RECEPTOR EXPRESSION AND REDUCED HIV-1 SUSCEPTIBILITY**

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### **ABSTRACT**

Continuous cultivation of T-lymphoid C8166 cells in the presence of pharmacological relevant concentration of cytarabine (Ara-C) results in significantly decreased expression of CD4 and CXCR4 molecules, the major cellular receptor and co-receptor of T-lymphotropic HIV-1 isolates. This change in receptor expression leads to decreased susceptibility of Ara-C resistant cells to HIV-1 infection demonstrated by reduced binding and penetration of HI-virus.

Human immunodeficiency virus (HIV-1) entries target cells by interaction of the viral envelope protein complex gp120-gp41 to the cellular surface protein CD4, followed by interaction with one of several co-receptors (1). Macrophage-tropic HIV-1 uses CCR5 as a co-receptor, while T cell line-tropic HIV-1 uses CXCR4 (2). Dual-tropic HIV-1 that can infect T cell lines and macrophages uses both CCR5 and CXCR4 and occasionally other chemokine receptors such as CCR2b and CCR3 (3). In the present study we investigated the effects of continuous Ara-C treatment of human T-lymphoid cells on the expression rate of cellular surface proteins, serving as HIV-1 receptors, such as CD4, CXCR4 and CCR5, as well as leukocyte functional antigen (LFA-1, CD11) and intercellular adhesion molecule

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1(ICAM-1, CD54), which contribute to the interaction or fusion of HIV-1 with target cells (4,5). FACS analysis and RT-PCR were used to compare protein expression and mRNA levels in parental and Ara-C resistant cells. Furthermore, HIV-1 binding and penetration studies showed whether Ara-C resistant cells are susceptible to HIV-1 infection.

**Chemotherapeutic agents:** Ara-C was obtained from Sigma (Deisenhofen, Germany), dFdC was kindly provided by Lilly (Indianapolis, USA). Drugs were dissolved in dimethylsulfoxide at a concentration of 10 mM. **Selection of Ara-C resistant cell line:** Ara-C resistant cell line was established by the continuous cultivation of C8166 cells in IMDM + 10% FCS containing increasing concentrations of Ara-C. The cell subline resistant against 5  $\mu$ M Ara-C, designated C8166<sup>r</sup>Ara-C<sup>5</sup>, was used in these experiments. **Determination of cytotoxicity:** Cytotoxic effects of AraC and dFdC were determined by MTT assay as described previously (6). **Flow cytometric analysis:** Indirect immunofluorescent detection of cell surface proteins was carried out using specific mouse monoclonal antibodies and fluorescein isothiocyanate conjugated goat anti-mouse IgG as secondary antibodies (Becton Dickinson). Antibodies to IgG were used as control. Fluorescence was determined using Becton Dickinson FACScan and CellQuest software (Becton Dickinson) (7). **Determination of CD4 gene expression by RT-PCR:** RT-PCR was performed as described previously (4). For the amplification of a region out of the CD4 mRNA, following primers were used: CD4-1: 5'-CTG CCA TTT CTG TGG GCT CA-3', CD4-2: 5'-CCA TCT GGA GCT TAG GGT CCT-3' (PCR product: 885 bp). **Virus:** Virus stock of T-lymphotropic HIV-1 laboratory strain HTLV-III<sub>RF</sub> was obtained from MRC AIDS Reagent Project (Hertfordshire, UK). **Virus titration:** Infective dose (TCID<sub>50</sub>) of virus in C8166 parental and Ara-C resistant cells was quantified by endpoint dilution (8). **Virus entry studies:** C8166 parental and Ara-C resistant cells were infected with HIV-1 at MOI 0.1 and incubated by 37°C. After washing the cells with PBS, cells were disturbed by freezing and thawing and intracellular amount of virus particles was measured as HIV-1 RNA copies/ml by quantitative RT-PCR (Roche, Amplicor).

Human T leukemic C8166<sup>r</sup>Ara-C<sup>5</sup> cells, continuously exposed to 5  $\mu$ M Ara-C, showed 800- and 650-fold lower sensitivity to cytotoxic effects of Ara-C and dFdC, respectively, when compared to parental cells (Table 1). Cell surface

**Table 1.** Cytotoxic Effects of Ara-C and dFdC in C8166 and C8166<sup>r</sup>Ara-C<sup>5</sup> Cells Measured by the MTT Assay

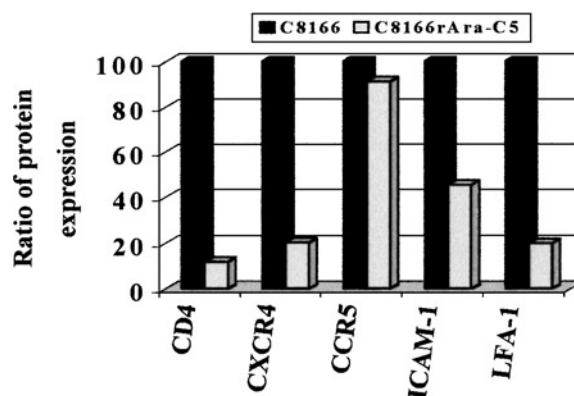
Drug	CC <sub>50</sub> [ $\mu$ M] <sup>a</sup>		RI <sup>b</sup>
	C8166	C8166 <sup>r</sup> Ara-C <sup>5</sup>	
Ara-C	0.04 $\pm$ 0.001 <sup>c</sup>	32.0 $\pm$ 1.9	800
dFdC	0.008 $\pm$ 0.0001	5.2 $\pm$ 0.2	650

<sup>a</sup>Concentration of drug, which inhibits cell growth by 50%.

<sup>b</sup>Resistance index (Ratio of CC<sub>50</sub> C8166<sup>r</sup>Ara-C<sup>5</sup> and CC<sub>50</sub> C8166).

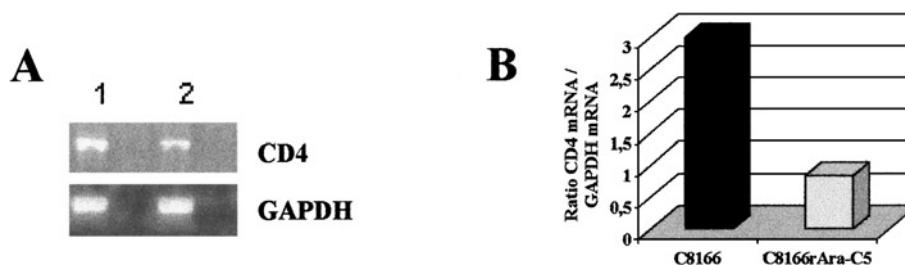
<sup>c</sup>Results represent mean value  $\pm$  SD of three different experiments.





**Figure 1.** Protein expression of CD4, CXCR4, CCR5, ICAM-1 and LFA-1 in C8166 and C8166<sup>r</sup>Ara-C<sup>5</sup> cells determined by FACS analysis.

protein expression of cellular HIV-1 receptor CD4 and co-receptor CXCR4 was strongly decreased in C8166<sup>r</sup>Ara-C<sup>5</sup> cells compared to parental cells, while expression of CCR5 co-receptor was not influenced (Fig. 1). In addition ICAM-1 and LFA-1 were remarkably down regulated in Ara-C resistant cells (Fig. 1). RT-PCR analysis showed a 3.6 fold reduction in CD4 mRNA levels between C8166 parental and Ara-C resistant cells (Fig. 2A and B). The strong decrease in HIV-1 receptor expression on the cell membrane of C8166<sup>r</sup>Ara-C<sup>5</sup> resistant cells arises the question of susceptibility of these cells to infection with a T-lymphotropic HIV-1 strain. Virus titer (TCID<sub>50</sub>/ml) of HTLV-III<sub>RF</sub> was  $3.1 \times 10^4$ /ml in C8166 cells, whereas no infectious virus production was detected in Ara-C resistant cells. Virus binding and entry were interrupted in C8166<sup>r</sup>Ara-C<sup>5</sup> cells, seen by the strong difference in intracellular HIV-1 RNA copy number for more than five orders of magnitude in C8166 parental and Ara-C resistant cells infected with HIV-1 (Table 2). After inhibition of HIV-1 binding and entry by incubation of cells with 10  $\mu$ g/ml dextran sulfate, which was used as a reference substance for virus entry inhibition (9), amount of HIV-1 RNA copies were comparable in C8166 parental and Ara-C resistant cells (Table 2).



**Figure 2.** Specific PCR products from cDNA of CD4 (885 bp) and GAPDH mRNA (126 bp) separated by agarose gel electrophoresis (lane 1: C8166, lane 2: C8166<sup>r</sup>Ara-C<sup>5</sup>) (A) and ratio of CD4 and GAPDH mRNA levels (B).

**Table 2.** Virus Entry and Binding of HTLV-III<sub>RF</sub> Strain in C8166 and C8166<sup>r</sup> Ara-C<sup>5</sup> Cells Measured as Intracellular Amount of HIV-1 RNA Copies/ml by RT-PCR

Treatment	HIV-1 RNA copies/ml $\times 10^4$	
	C8166 <sup>a</sup>	C8166 <sup>r</sup> Ara-C <sup>5</sup>
Without	150.0 $\pm$ 95 <sup>b</sup>	4.5 $\pm$ 0.4
With 10 $\mu$ g/ml dextran sulfate	8.0 $\pm$ 0.4	5.0 $\pm$ 0.2

<sup>a</sup>Cells were HIV-1 infected with MOI 0.1 and incubated with and without 10  $\mu$ g/ml dextran sulfate.

<sup>b</sup>Results represent mean value  $\pm$  SD of two different experiments.

The data of the present study demonstrates for the first time, that treatment of T-lymphoid cells with pharmacological relevant concentrations of Ara-C is associated with resistance to HIV-1 infection. This effect is accompanied by decreased mRNA level and protein expression of HIV-1 receptors and co-receptors on the surface of Ara-C resistant cells. Current treatment strategies of HIV-1 infection including reverse transcriptase and protease inhibitors often fail after a period of time due to the rapid development of resistant virus strains against these therapeutics (10). Therefore, the need for more therapeutic options is growing. Inhibition of HIV-1 entry, as the first step in replication cycle of HI-virus has reemerged as an attractive target of suppression of HIV-1 infection. Substances which decrease the expression rate of HIV-1 receptors at the surface of target cells without modifying the physiological function of cells might represent a promising therapeutic strategy to prevent HIV-1 infection in host cells. Although, the use of Ara-C for the treatment of patients may be limited by cytotoxic side effects such as myelosuppression, neutropenia and gastrointestinal epithelial injury (11), the present results encourage further studies to show whether continuous treatment with pharmacological relevant concentrations of Ara-C may influence sensitivity of normal T lymphocytes to HIV-1 infection.

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