

In vivo measures of T-cell responsiveness are impaired even in early HIV-1 infection

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Abstract

Introduction

Delayed type hypersensitivity (DTH) reaction is impaired in advanced HIV-1 infection. We investigated cell mediated immunity by DTH-reactions in patients with HIV-1 infection and relatively preserved numbers of circulating CD4+ T-cell counts.

Methods

At 48 –72 h following the intradermal injection of candida, mumps, tuberculin and tetanus antigens or normal saline as a control, sites of injection/induration were sampled from HIV-1-seropositive (pos.) and –seronegative (neg.) individuals with a 4 mm punch cylinder and were snap frozen in ice-cold isopentane. 8 µm histological sections were performed on frozen tissue and monoclonal antibodies (mABs) against CD4, CD8, HLADR, Granulysin, CD1a and FoxP3 were used to label cells for immunohistochemical characterization by digital imaging analysis.

Results

Five HIV-pos. patients and 4 HIV-neg. controls were recruited. All HIV+ patients were antiretroviral therapy naïve. In patients, median CD4+ cell counts were 650/µL (range: 170-830) and median HIV-RNA levels were 3650 copies/mL (range: 157 –750000). In HIV-neg. persons the staining for CD4 (p=0.049), CD8 p=(0.007), Granulysin (p=0.002), CD1a (p=0.049), and FoxP3 (p=0.024) was significantly greater at sites of antigen injection than at the site of saline injection, whereas among the HIV-pos. patients, only staining for CD4 (p=0.048), and HLA-DR (p=0.008) at antigen sites exceeded that at control sites. At these sites, CD4 staining was highly correlated with staining for CD1a (p<0.001, ρ=0.87), HLADR (p=0.003, ρ=0.76), FOXP3 (p=0.004, ρ=0.65), and Granulysin (p<0.001, ρ=0.77) but none of these *in situ* indices was predicted by circulating CD4+ T-cell counts.

Conclusion

In vivo measures of adaptive cellular immune responses are impaired even in early HIV- infection and are not well reflected by numbers of circulating CD4+T-cells.

Introduction

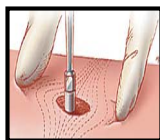
Cell mediated immunity as reflected in delayed type hypersensitivity (DTH) following the intracutaneous application of antigen is severely impaired in patients with advanced HIV-1 infection. In contrast DTH-reactions, e.g. the Mendel-Mantoux-test for the diagnosis of latent infection with *Mycobacterium tuberculosis*, may be preserved in early HIV-1 infection.

To gain a better understanding about cell mediated immune-defects in early HIV-infection, we analyzed skin biopsies from the sites of DTH-reactions (left forearm) and a control site (right forearm) from patients with early HIV-infection and ongoing viral replication and from HIV-seronegative controls.

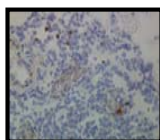
Methods



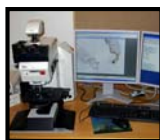
Skin testing for DTH-reactions in response to intradermal application of candida, mumps, tuberculin and tetanus antigens or normal saline as a control were performed on the volar sides of forearms



48 – 72 h following injection of the antigens 4 mm punch biopsies were performed at the sites of the antigen application/induration. Biopsies were snap frozen in ice-cold isopentane



8 µm histological sections were performed on frozen tissue and monoclonal antibodies (mABs) against CD4, CD8, HLADR, Granulysin, CD1a and FoxP3 were used to label the cells



Immunohistochemical characterization of the stained histological sections was performed by digital imaging analysis. Results were expressed as percentage of stained area in the cell area or as numbers of positive cells per mm²

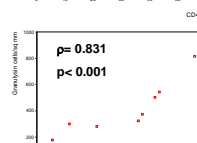
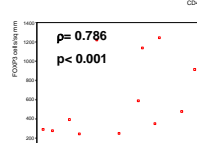
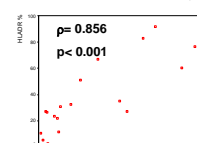
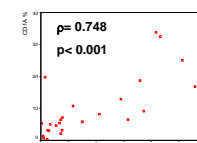
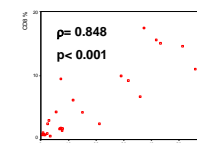
Results

	HIV+	HIV-
n	5	4
age	37	38
CD4+	650	nd
ARV	naive	na
VL	3650	na

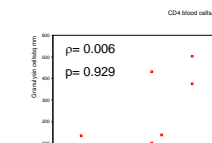
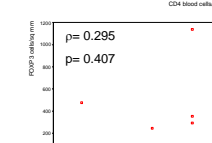
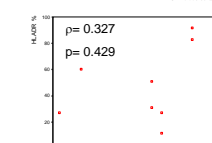
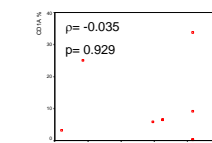
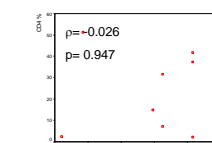
Median values, nd = not done, na = not applicable

The influx of immunocompetent cells into the skin correlates well with numbers of CD4+ T-cells in the skin, but not with numbers of circulating CD4+ T-cells

CD4+ T-cells in the skin



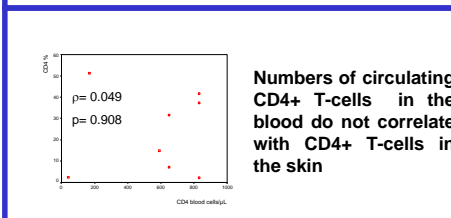
CD4+ T-cells in the blood



After antigen application there is less influx of immunocompetent cells into the skin in patients with early HIV-infection vs. HIV-negative controls

HIV +			
	antigen panel	normal saline	p
CD4 %	23.3	2.2	0.048*
CD8 %	4.2	0.7	0.106
CD1a %	6.4	4.0	0.604
HLADR %	41.1	3.9	0.008*
FOXP3 c/mm ²	160	26.5	0.106
Granulysin c/mm ²	119	11.6	0.077

HIV -			
	antigen panel	normal saline	p
CD4 %	16.4	0.8	0.049*
CD8 %	6.4	1.0	0.007*
CD1a %	9.4	3.1	0.049*
HLADR %	33.8	20.5	0.286
FOXP3 c/mm ²	337	0	0.024*
Granulysin c/mm ²	231	10.5	0.002*



Numbers of circulating CD4+ T-cells in the blood do not correlate with CD4+ T-cells in the skin

Conclusion

In vivo measures of adaptive cellular immune responses are impaired even in early HIV- infection and are not well reflected by numbers of circulating CD4+T-cells