



HIV-1-infected patients with full suppression of viral replication but impaired recovery of CD4 cells display immune activation with decreased CD28 expression and IL-10 production

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INTRODUCTION

Successful HAART therapy with full suppression of viral replication as assessed by undetectable circulating levels of HIV-RNA most often results in a CD4 cell count increase. However, in a few patients the CD4 cell count remains low even though there is no evidence of viral failure. The reason for this lack of CD4 cell count increase is not known. Even though these patients often feel well clinically, there is concern about their long term prognosis.

While the CD4 T cell loss during acute infection may be due to direct killing of cells by HIV, this cannot account for the loss during chronic infection as less than 0.1% of CD4 cells are infected with HIV [1]. However, the immune activation also drives CD4 apoptosis and the grade of immune activation of T cells has repeatedly been suggested to be a main feature of HIV pathogenesis [2-3].

This reason for this chronic immune activation is not fully determined. But immune activation may be due to the lack of increase in CD4 cell count among some patients with undetectable HIV-RNA.

The importance of chronic immune activation in the development of AIDS is indicated in a study with persistent activation of CD4 cells in untreated mice constitutively expressing CD70 [4]. The activation lead to depletion of naive CD4 cells and the mice died of Pneumocystis carinii infection. SIV-infected sooty mangabeys is another example. In contrast to SIV-infected rhesus macaques and HIV-infected humans the sooty mangabeys preserve their CD4 subsets and remain free of AIDS despite heavy viral replication but with low levels of immune activation [5].

We hypothesized that immune activation would be increased in these discordant responders.

OBJECTIVE

To assess differences in the level of immune activation among patients with lack of an increase in CD4 cell count despite undetectable HIV-RNA levels.

METHODS

Design

In the Danish HIV Cohort Study we identified patients who conformed to the following criteria:

- 1) They had initiated HAART with a CD4 cell count <100 cells/μL.
- 2) They had undetectable HIV-RNA (<50 copies/mL) for at least three years prior to the inclusion in this study.
- 3) Twenty-one male patients met the inclusion criteria, had a CD4 count <200 cells/μL after the 3 years of viral suppression, and were associated with the clinics at the departments of infectious diseases at Rigshospitalet or Hvidovre Hospital, Copenhagen, Denmark. These patients were identified as possible cases. Forty-two patients, routinely followed at the same departments, with a CD4 count >200 cells/μL were randomly selected as controls. Subsequently, patients were included at routine visits in the case group. 18 patients were recruited, while 33 patients were included in the control group.

Flow cytometry

Whole blood samples were stained according to the manufacturer's recommendations. The monoclonal antibodies used were conjugated to fluorescein-isothiocyanate (FITC) phycoerythrin (PE), peridinin chlorophyll protein (PerCP), PE-Cy7, allophycocyanin (APC), or APC-Cy7. These antibodies were run for each sample: 1: CD14, CD16, CD45, HLA-DR, IL-2, IL-10, CD38, CD45RA, CD28, CD28 CR2, 2: CD4, CD8, CD45RA, CD45RO, HLA-DR, CD38. The stained samples were subjected to six-parameter flow cytometry (FACSscan, BD Biosciences, Franklin Lakes, NJ, USA). The subsequent computer analyses were performed with BD FACSdiva v4.1.2. Samples from 7 case patients were lost due to technical issues.

Whole blood stimulations

Heparinized whole blood was mixed with RPMI 640 and stimulated with endotoxin (LPS), phytohemagglutinin (PHA), or control medium (unstimulated) and incubated at 37°C, 5% CO₂. Supernatants were harvested after 24 hours and kept at -80°C until analysis.

Plasma separation

- Blood was drawn into EDTA-coated tubes and immediately placed on ice until separation.
- Plasma was stored at -80°C until analysis.

Cytokine measurements

- Plasma TNF-α was measured by the enzyme-linked immunosorbent assay (R&D Systems Minneapolis, MN, USA). Supernatant cytokine levels of TNF-α, IL-10, IFN-γ, IL-8, and IL-6 as well as plasma IL-10 were measured by a multiplexed assay (BioRad, MxP Multiplex kits, Human cytokine panel A, R&D Systems) on the Luminex platform (Luminex Corporation, Austin, TX, USA).

Statistics

- Parameters were compared between case and control groups by T test or the Mann-Whitney U test. Univariate tests were supplemented with analysis of covariance (ANCOVA) allowing for adjustment for age. Parameters were log₁₀ transformed when appropriate. Normality was checked graphically. Results are reported as means with 95% confidence intervals (CI). A P-value of 0.05 was considered significant.

RESULTS

At inclusion, the mean CD4 count was 214 (CI: 161-253) and 637 (177-252) cells/μL in the case and control groups, respectively. Patients in the case group had a median age of 54 years (interquartile range: 46-60) and were older than patients in the control group who had a median age of 44 years (42-50 years), P for difference between groups: <0.01.

- See Figures and tables for results.

CONCLUSIONS

The proportions of naive cells among CD4 and CD8 lymphocyte subsets were lower among responders.

- CD28 is a co-stimulatory receptor that enhances T cell proliferation and cytokine secretion. It enhances long-term T cell survival and prevents energy induction. We previously reported that low expression of CD28 and impaired production of cytokines were linked to immune activation and poor prognosis among untreated patients [6]. HLA-DR expression on T lymphocytes is often used as a marker of T cell activation. Immune activation with decreased CD28 and increased HLA-DR expression on T cells suggests weak key findings among cases in this study.
- IL-10 is an anti-inflammatory cytokine. We have previously found that mortality was reduced among high-producers of IL-10 [7]. This study supports an impaired production of IL-10 as a cofactor in immune activation and attenuated CD4 recovery during HAART.

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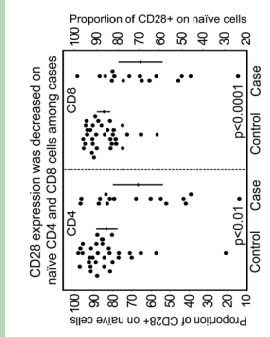


FIG. 1. A: The percentage of naive (CD45RA⁺ and CD62L⁺) cells in the CD4 or CD8 gated lymphocyte subsets. The percentage of naive cells was lower in the case group in unadjusted analysis (CD4, unadjusted age adjusted, p<0.01; CD8, p<0.01). Bars represent means with CIs.

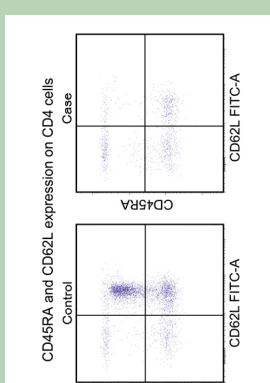


FIG. 1. B: The two panels show examples of scatter plots of CD45RA versus CD62L expression on CD4 gated lymphocytes from two patients from the control and case groups, respectively.

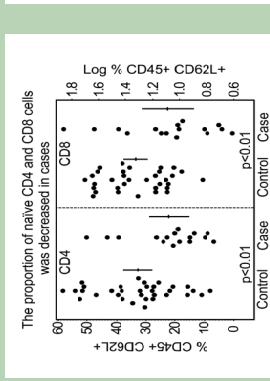


FIG. 2. A: The expression of the activation marker HLA-DR was higher in the case group on phenotypically naive (CD45RA⁺ CD45RO⁻) CD4 cells (unadjusted, p<0.001; age adjusted, p<0.01). CD8 cells (CD8⁺ CD45RO⁻) CD4 cells (p<0.01; age adjusted, p<0.05).

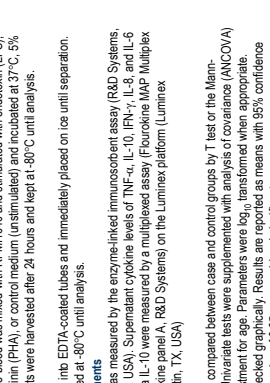


FIG. 2. B: The two panels show examples of histograms depicting CD28 expression from a control and case patient, respectively. The gate included CD4⁺ CD45RA⁺ CD62L⁺ lymphocytes.

	Control group	Case group	P-value				
L-10 to endotoxin	2.07	1.97	2.16	1.86	172	2.01	<0.05
L-10 to PHA	2.32	2.22	2.42	1.73	1.56	1.50	<0.001
TNF-α to endotoxin	0.39	0.69	0.99	0.38	0.72	0.00	ns
TNF-α to PHA	2.09	2.39	3.10	2.01	2.78	3.04	ns
IFN-γ to endotoxin	0.33	0.01	0.01	0.05	0.23	0.04	ns
IFN-γ to PHA	2.07	2.82	3.12	2.75	2.85	2.94	0.07
IL-6 to endotoxin	2.75	2.64	2.96	2.39	2.17	2.62	<0.01
IL-6 to PHA	1.50	1.35	1.65	1.28	1.01	1.58	0.15
IL-8 to endotoxin	3.76	3.64	4.08	3.86	3.79	4.17	ns
IL-8 to PHA	1.68	1.56	2.11	1.88	1.51	2.25	ns
IL-10 to endotoxin	0.33	0.33	0.33	0.33	0.33	0.33	ns
IL-10 to PHA	3.47	3.36	3.97	3.39	3.23	3.52	ns
IL-6 unstimulated	1.05	0.92	1.26	0.79	0.92	1.16	ns

TABLE 1: Cytokine production from whole blood stimulated with PHA, LPS, or unstimulated. Results are means with CIs in the case and control groups, respectively. The production of IL-10 to PHA, IL-10 to LPS, and IFN-γ to PHA was decreased in cases. Moreover, plasma IL-10 was lower in plasma (unadjusted, p<0.001; age adjusted, p<0.05) with no difference in plasma TNF-α.