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Increased expression of negative regulators of cytokine signaling during chronic HIV disease cause functionally exhausted state of dendritic cells

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ABSTRACT

Mechanisms of functional impairment of dendritic cells (DCs) during chronic HIV-1 infection are not well understood. In order to understand this phenomenon, we aimed to study the expression of negative regulators of cytokine signaling and correlate with DC exhaustion during chronic HIV-1 disease. Monocyte-derived DCs (mo-DCs) from 27 HIV-1 infected patients (CD4+ T-cell counts: 429 ± 44 cells/μL, plasma viral load: Log_{10}3.9 ± 1.0 copies/ml) and 19 healthy controls (HCs) were stimulated *ex vivo* with TLR4 agonist, lipopolysaccharide (LPS) for 2 days to evaluate their functional fitness. The expression of a set of genes associated with cytokine signaling was evaluated in a custom designed PCR array by Real-Time PCR. The mo-DCs from HIV-1 infected patients depicted functional exhaustion as evident by decreased allo-stimulation index (mean ± SD: 10 ± 6 vs. 24 ± 16) ($p < 0.05$), decreased cytokine production (pg/ml) (IL-12: 4.6 ± 16 vs. 25 ± 85; TNF-α: 128 ± 279 vs. 286 ± 544; IL-10: 6 ± 12 vs. 13 ± 20; IL-8: 10,688 ± 11,748 vs. 17,470 ± 125,049) and retained endocytosis (1.1 ± 0.3 vs. 1.0 ± 0.29) ($p < 0.05$) even after LPS-stimulation, as compared to HCs. Significantly upregulated expression of SOCS-1 (mean ± SD fold change: 2.2 ± 2vs.0.8 ± 0.6), SOCS-3 (6.3 ± 7.4vs.1.4 ± 0.4), PIAS-1 (1.6 ± 0.1vs.1.0 ± 0.3) and SHP-1 (0.8 ± 0.4vs.0.4 ± 0.2) correlated positively with PD-L1 expression in these DCs (Spearman's coefficient, SOCS-1: 0.63, SOCS-3: 1.0 and PIAS-1: 0.7) ($p < 0.05$). The expression of these molecules trended positively with plasma viral load and negatively with CD4+ T-cell counts. These findings suggest that the upregulation of negative regulatory factors during chronic HIV disease have profound down-modulatory effects on DC functions and establishment of an overall exhausted state. Understanding mechanisms causing upregulation of these factors may lead to the design of new generation therapeutics based on silencing of their gene expression.

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1. Introduction

Dendritic cells (DCs) are the most potent professional antigen presenting cells (APCs), that after initial processing, present the antigen to naïve-T lymphocytes to mount an appropriate immune response [1]. Infection with HIV adversely affects all components of the immune system but the dysfunctional status of DCs may have far-reaching consequences, as the aberrant signals emanating from these defective APCs could be considered a major factor responsible for immune disarmament. Among the strategies adopted by HIV for its survival and dissemination, down-modulation of DC functions represents a key aspect of viral pathogenesis. Both, the host as well as viral factors, are known to be involved in facilitating DC impairment [2–5], although a clear understanding of the mechanisms is still lacking.

A role of the programmed death pathway in DCs has been suggested in causing exhaustion of immune cells wherein their ability to produce cytokines is severely impaired, but how these receptors mediate inhibitory signals is not yet clear [6]. An increased expression of programmed death (PD) receptors correlates with the viral load in the HIV infected individuals [7]. An upregulation of programmed death ligand (PD-L)-1 can be induced by virus-derived ligands via MyD88 pathway on DCs, suggesting another mechanism contributing to functional exhaustion of the immune cells [8].

The cytokine secretion pattern from DCs decides the fate and differentiation of naive-T cells and is under the control of certain positive and negative regulators [9,10]. A family of eight members, called the suppressor of cytokine signaling (SOCS) protein family includes factors that play a major regulatory function in macrophages and DCs [11,12]. Besides SOCS, the role of other negative

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regulators such as protein tyrosine phosphatases (SHP)-1 and the protein inhibitors of activated STATs (PIAS) is also becoming increasingly clear [13]. In this study, we have investigated the relationship of DC exhaustion with negative regulators of cytokine signaling to determine the intrinsic mechanism of DC dysfunction during chronic HIV-1 infection.

2. Materials and methods

2.1. Study groups

A total of 27 HIV-1 infected patients (mean age: 33 years) in the chronic stage of disease (mean ± SD of CD4+ T-cell counts: 429 ± 44 cells/μL, mean ± SD of plasma viral load: Log_{10}3.9 ± 1.0 copies/ml) visiting the Integrated Counselling and Testing Center (ICTC), Department of Immunopathology and the ART clinic, Department of Internal Medicine, at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India were recruited. The study was approved by the Institutional Ethics Committee (IEC) of PGIMER, Chandigarh, India. Fifteen ml peripheral blood was drawn from each enrolled subject after obtaining an informed consent. Patients with any other chronic infection like Tuberculosis, Hepatitis B or C were excluded. The HIV-1 infected patients were compared with a group of age and sex matched healthy controls (HCs). The absolute counts of human CD4+ T lymphocytes were determined by flow cytometry using BD Tritest™ CD3 FITC/CD4 PE/CD45 PerCP reagent with BD Trucount™ tubes (BD Bioscience, San Jose, CA, USA) in erythrocyte-lysed whole blood. HIV-1 plasma viral load was quantitated by COBAS Taqman HIV-1 test (Roche, Branchburg, USA) as per the manufacturer's guidelines. Table 1 depicts the demographic/clinical details of the study participants.

2.2. Differentiation of monocytes to monocyte-derived dendritic cells (mo-DCs) for functional assays

Monocyte-derived dendritic cells (mo-DC) were obtained from monocytes isolated from peripheral blood mononuclear cells (PBMCs) of study subjects using method described earlier [14]. The functional assays were performed on these mo-DCs on day 8.

2.3. Analysis of inflammatory cytokines

The levels of various cytokines like IL-12, IL-10, TNFα, IL-1β, IL-6 and IL-8 were measured in culture supernatants of the LPS stimulated mo-DCs using Cytometric Bead Array (CBA Human Inflammation Kit, BD Biosciences, USA) as per manufacturer's instructions.

2.4. Mixed Lymphocyte Reaction (MLR) and endocytosis assay

The MLR using carboxyfluorescein succinimidyl (CFSE Invitrogen, CA, USA), stained lymphocytes from a healthy donor and LPS stimulated mo-DC from study subjects was performed to calculate the stimulation index. Endocytosing capacity of LPS treated mo-DC of study subjects was assayed using FITC-dextran (Sigma-Aldrich, USA). The detailed methods have earlier been described elsewhere [14].

Table 1
Clinical characteristics of study participants.

	HIV-1 infected patients	Healthy controls
Number	27	19
Mean age	33 years	32 years
Gender (males/females)	20/7	13/6
Mean CD4+ T-cell counts (cells/μL)	429 ± 44 cells/μL	NA
Mean plasma viral load (Log_{10} copies/ml)	3.9 ± 1.0	NA
Time since infection (years)	2 ± 2	NA

NA, not applicable.

2.5. Gene expression profile

For delineation of the mechanism causing DC impairment among HIV-infected patients, we analyzed the expression profiles of some selected genes by quantitative real-time RT-PCR in a custom designed PCR-array (Qiagen, Hilden, Germany). The array contained selected genes that influence the cytokine signaling in DCs. These included SOCS-1, SOCS-3, PIAS1, SHP-1 and PD-L1. The real-time PCR (Light cycler, Roche Diagnostics, Indianapolis, USA) was performed on cDNA obtained form the extracted RNA of mo-DCs in presence of SYBR Green (Roche, CA, USA) and data analyzed by comparing the Ct values after normalizing with the house keeping genes, GAPDH and β-actin as described earlier [15].

2.6. Statistical analysis

Statistical analysis was done using GraphPad Prism software (version 5.0, GraphPad Software Inc., La Jolla, CA, USA). Data were expressed as mean ± standard deviation (mean ± SD). For comparison between HIV-1 infected patients and healthy control groups, Mann-Whitney test was done. Correlation coefficients were calculated using the Spearman rank sum test. Linear regression analysis was also done and the residuals were calculated. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Impairment of DC functions during chronic HIV-1 infection

3.1.1. Reduced cytokine production

The level of secreted cytokines (IL-12, TNFα, IL-10, IL-8, IL-6 and IL-1β) measured in the culture supernatants of mo-DCs post LPS-stimulation revealed IL-12 (mean ± SD: 4.6 ± 16 vs. 25 ± 85 pg/ml), TNFα (128 ± 279 vs. 285 ± 544 pg/ml), IL-10 (6 ± 12 vs. 13 ± 20 pg/ml) and IL-8 (10,688 ± 11,748 vs. 17,470 ± 12,504 pg/ml) were significantly lower ($p < 0.05$) in HIV-1 infected patients as compared to the HC group (Fig. 1a–d). We did not observe any significant difference for IL-6 and IL-1β between the patients and HC group (data not shown).

3.1.2. mo-DC from HIV patients showed weak allo-stimulatory capacity

The SI of mo-DCs derived from HIV infected subjects was found to be significantly lower (mean ± SD: 10 ± 5.6) in the MLR assay with allogenic PBMC of a healthy donor, as compared to the HC group (22 ± 15) ($p < 0.05$) indicating a diminished antigen presenting ability of mo-DC of patients with chronic HIV disease (Fig. 1e).

3.1.3. The mo-DC of HIV infected individuals etained endocytosis capacity post LPS stimulation

The mo-DCs from HIV-infected individuals demonstrated significantly higher uptake of dextran even after LPS stimulation as compared to mo-DCs from HCs (mean ± SD: 1.1 ± 0.3 vs. 1 ± 0.29) ($p < 0.05$) (Fig. 1f). This retained endocytosing capacity post-LPS stimulation indicates an immature state of mo-DCs from HIV-1 infected individuals and may be related to defective maturation capacity of DCs during HIV-1 infection.

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