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Disparity in circulating peripheral blood dendritic cell subsets and cytokine profile of pulmonary tuberculosis patients compared with healthy family contacts

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ABSTRACT

Dendritic cell (DC) subsets, myeloid DCs (mDCs), and plasmacytoid DCs (pDCs) play a fundamental role in immune response to *Mycobacterium tuberculosis* (*M. tuberculosis*). Flow-cytometric estimation of DC subsets showed differences in the ratio of these subsets in untreated, smear-positive pulmonary tuberculosis patients compared with healthy family contacts (HFC, $p < 0.05$). The percentage of pDCs (0.14 ± 0.01) was higher than mDCs (0.12 ± 0.01) in patients, whereas in HFC, mDCs (0.15 ± 0.01) was higher than pDCs (0.1 ± 0.01). The percentage of mDCs (0.15 ± 0.01) and pDCs (0.11 ± 0.01) was restored in treated patients. Alteration in the DC subsets before and after chemotherapy was confirmed in the follow-up of acid-fast bacilli (AFB)–positive patients. This reversal in the percentage of mDC vs pDCs implicates the influence of active disease on circulating DC subsets. The cytokine bead array revealed an inverse relationship in the circulating levels of IL-12 and IFN-γ. High IL-12 (37.9 ± 15.2) and low IFN-γ (11.09 ± 3.6) was seen in HFCs derived serum samples compared with that of patients ($p < 0.05$). The higher percentage of mDCs and elevated IL-12 levels was found to be associated with high risk HFCs investigated. Furthermore CpG/LPS–stimulated whole-blood culture of untreated patients expressed high IFN-α in pDCs and less IL-12 in mDCs compared with those of treated patients.

1. Introduction

Dendritic cells (DCs) play a central role in the genesis of an immune response. The initial interaction between naive T cells and DCs and the ensuing cytokine environment generated critically influence the quality of immune response [1,2]. DCs have been subdivided into myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) [3,4]. pDCs originate from lymphoid precursors, express interleukin (IL)–$3^{hi.}$ receptor-α chain (CD123), secrete interferon (IFN)–α, and induce T-helper 2(T_h2) immune responses [5]. mDCs originate from myeloid precursors, express β_2 integrin CD11c, CD13, and CD33, secrete IL-12, and induce T-helper 1(T_h1) responses [6].

This study was designed to enumerate the percentage of circulating DC subsets in untreated, smear-positive pulmonary tuberculosis (PTB) patients and their healthy family contacts (HFC). Our data showed that untreated acid-fast bacilli (AFB)–positive patients had a higher percentage of pDCs and a lower percentage of mDCs compared with HFCs. The ratio of these subsets was re-established in treated AFB-negative patients to that seen in HFCs. Serum IFN-γ levels in untreated smear positive PTB patients were found to be higher compared with HFCs. IL-12, the principal cytokine associated with interferon (IFN)–γ production, was found to be low in the patients. Furthermore, *in vitro*–stimulated whole-blood cultures derived from untreated patients showed a lower percentage of mDCs expressing IL-12 compared with treated patients.

2. Subjects and methods

2.1. Study population

The institutional ethical committee approved the study. Informed consent was obtained from all included in the study. A total of 95 PTB patients (69 male and 36 female, age range 15–75 years) registered in the OPD (Out Patient Department) of LRS (Lala Ram Sarup) Institute of Tuberculosis and Respiratory Diseases (New Delhi, India) and 31 HFC who were related to and closely associated with the patients, were included in the study (20 male and 11 female, age range 14–62 years). All patients underwent clinical examination, chest X-ray, sputum microscopy for AFB, and routine laboratory tests. Diagnosis of tuberculosis was made on the basis of clinical and radiological assessment and detection of AFB in sputum. At the time of enrollment, the 95 PTB patients were untreated and were sputum AFB positive. Scrutinizing of patient clinical histories, physical examination, and laboratory investigations ruled out the occurrence of concomitant intracellular infections in the enrolled individuals. All patients were human immunodeficiency virus negative. After Directly Observed Treatment Short-Course (DOTS) treatment, the patients were sputum AFB negative and

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showed radiologic changes commensurate with clinical improvement. HFCs were screened for clinical signs of tuberculosis and were radiologically examined; when warranted on the basis of symptoms, additional tests such as sputum examination for AFB and erythrocyte sedimentation rate were undertaken. The 31 high-risk HFC included in the study were clinically free of overt signs of tuberculosis.

2.2. Reagents

For identification of DCs and its subsets, the monoclonal antibodies (mAbs) used were a mixture of FITC–anti-lin1 mAbs (anti-CD3, CD14, CD16, CD19, CD20, and CD56); PE-Cy7-HLA-DR, PE-CD11c, and PerCP-CD123 were obtained from BD Biosciences (San Diego, CA). Heparin (Gibco, Grand Island, NY), paraformaldehyde (SD. Fine-Chem, Mumbai, India), azide (CDH, New Delhi, India); a FlowCytomix Multiplex kit was obtained from Bender Medsystems (Vienna, Austria). Cytokines were detected by PE-IL-12 (eBioscience), PE-IFN-α; GolgiStop, Cytofix-Cytoperm, FACS lysing solution and recombinant human IFN-γ (BD Biosciences), lipopolysaccharide (LPS), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (St. Louis, MO) and CpG-A oligodeoxynucleotides from Hycult Biotechnology (Uden, The Netherlands).

2.3. Whole-blood staining and flow-cytometric analysis

A 200-μl aliquot of heparinized whole blood was incubated with a mixture of anti-lin1 mAbs, PE-Cy7-HLA-DR, PerCP-CD123 and PE-CD11c for 30 minutes in the dark at room temperature. The FACS lysis buffer was added, mixed, and incubated for $<$10 minutes in the dark at room temperature to lyse the RBCs. Cells were washed (1$\times$ phosphate-buffered saline [PBS], 0.1% azide). Stained cells were fixed in 1% paraformaldehyde and 500,000 cells were

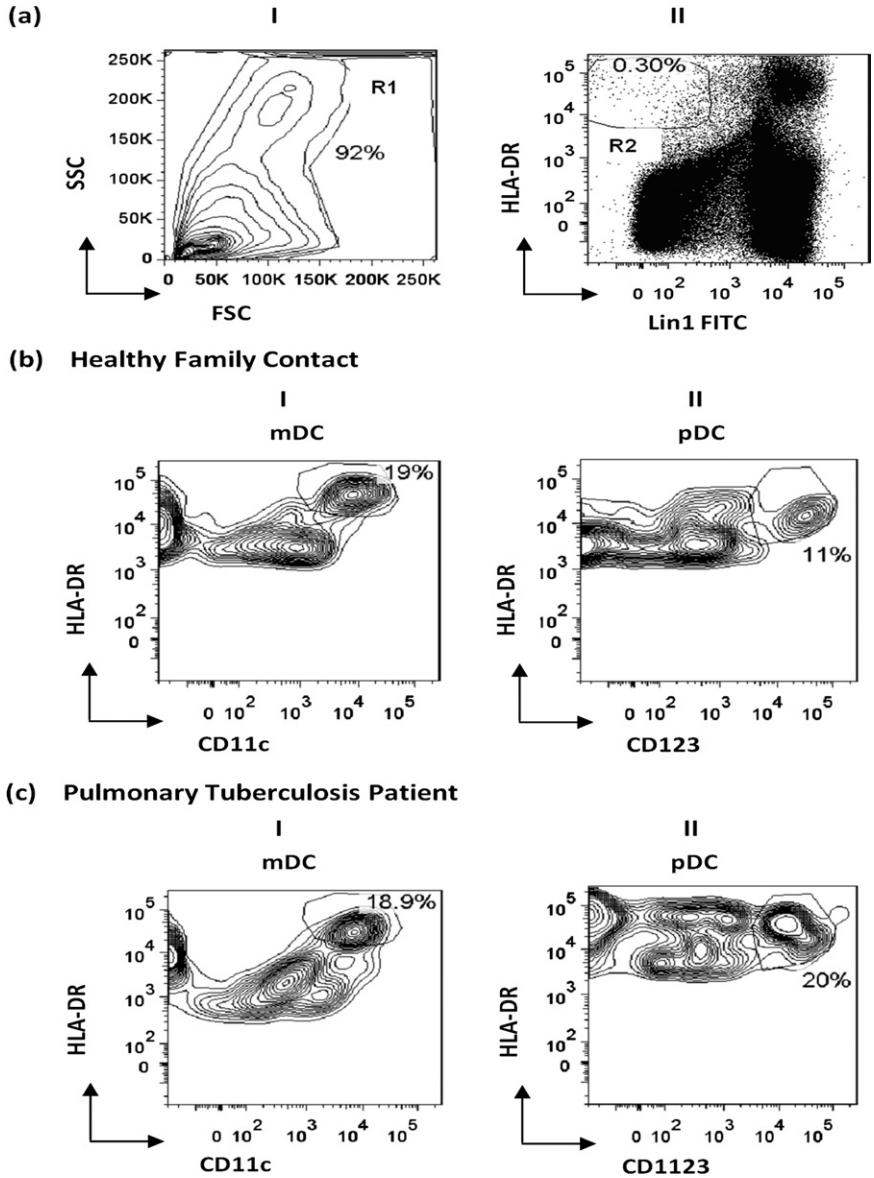

Fig. 1. Representative four-color flow-cytometric profiles of the myeloid (mDCs) and plasmacytoid (pDCs) DC subsets in peripheral blood of a household healthy contact (b) and an untreated tuberculosis patient (c). Whole-blood samples were stained with anti-HLA-DR (PECy7), anti-CD11c (PE), anti-CD123 (PerCP, anti-IL-3Rα), and FITC-conjugated lineage markers (lin1; anti-CD3, -CD14, -CD16, -CD19, -CD20, and -CD56). Erythrocytes were lysed and the cells were acquired and analyzed. Viable cells were selected on the basis of Forward Scatter (FSC) and Side Scatter (SSC) characteristics (R1 region). DCs were identified by negative lineage marker (lin1$^-$) and high positive expression of HLA-DR in the R2 region (a, II). The DCs were analyzed for the expression of CD123 and CD11c. pDCs were defined as lin1$^-$, HLA-DR$^+$, CD123$^+$ and mDCs as lin1$^-$, HLA-DR$^+$, CD11c$^+$ cells, respectively. Numbers in b and c indicate the percentage of double-positive cells for R2 gated cells.