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Research paper

Profiling dendritic cell subsets in the patients with active pulmonary tuberculosis

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ABSTRACT

Dendritic cell (DC) plays an important role in the immune response against pulmonary tuberculosis. However, the phenotypic profile of DC subsets in peripheral blood in individuals with active pulmonary tuberculosis (APT) is still inconclusive. Here, we demonstrated that the absolute numbers of total DC (tDC), myeloid DC (mDC) and plasmacytoid DC (pDC) in individuals with APT were decreased compared to healthy controls (HCs). The decreased number of DCs, especially of pDC, seems to be a useful diagnostic marker of APT. Meanwhile, the number of DCs was associated with the prolonged/complicated TB, ATD treatment effect and lymphocyte immune reactions, as manifested that relapsed APT patients with a higher number of tDC and lower number of pDC compared to newly diagnosed patients. Interestingly, mDC from APT patients displayed high expressions of CD83 and CCR7, but pDC displayed low expressions of CD83 and CCR7. Moreover, DCs from APT patients expressed lower levels of HLA-DR and CD80, but expressed a higher level of CD86 than those from HCs. However, the antigen uptake capacity of DC subsets was not different between APT and HCs, despite the antigen uptake capacity of pDC was much lower than that of mDC in both APT patients and HCs. Our data represent a systematic profile of DC subsets in the blood of APT patients, and would represent a useful biomarker for APT.

1. Introduction

Tuberculosis (TB) remains a major health problem, with millions of deaths and 9 million new cases annually (WHO, 2014). At the same time, one third of the world's total population is predicted to be infected with *Mycobacterium tuberculosis* (*Mtb*), although fewer than 10% of the infected individuals will eventually develop disease. The persistence of *Mtb* in discrete lesions in healthy individuals indicates that although the immune system is highly effective in constraining the pathogen, it fails to eradicate *Mtb* (Barry et al., 2009; Urdahl, 2014). The chronic nature of this infection implies that the bacilli have developed strategies to avoid both the innate and adaptive immune responses (Orme and Basaraba, 2014; Yamashiro et al., 2014). Dendritic cells (DCs) are professional antigen-presenting cells and have a primary role in the activation and coordination of primary immune responses. DCs play pivotal roles in anti-tuberculosis immunity. They express different surface markers and home to lung tissues, with some re-circulating back to the bloodstream (Banchereau et al., 2000; Boltjes and van Wijk, 2014; Crosignani et al., 2016; Merad et al., 2013). DCs sense pathogen-associated molecular patterns (PAMPs) of tuberculosis bacilli with innate receptors such as TLRs and RLRs (Boltjes and van Wijk, 2014; Merad et al., 2013). Immature DCs are primarily sampling the immunological milieu of the tissue where they reside. However, upon activation immature DCs undergo a transformation process that includes up-regulation of class I and class II MHC molecules and co-stimulatory molecules (such as CD80 and CD86), production of

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Table 1
The clinical data of studied subjects.

Groups	APT(n = 65)	HC(n = 25)
Age (years)	(14–69)	(15–60)
Age (Mean ± SEM)	38.62 ± 1.66	36.12 ± 2.42
Gender (Female/Male)	23/42	9/16
Newly/Relasped	54/11	-/-
Sputum smear (+/−)	19/46	-/-

Note: Newly:Newly diagnosedpatients; Relasped: Relasped patients. There were no signification differences among age and gender groups ($P > 0.05$).

interferons, pro-inflammatory cytokines (IL-12, IL-15, IL-18, IL-10), and radical changes in their chemokine receptor and adhesion molecule profile (Banchereau et al., 2000; Collin et al., 2013; Crosignani et al., 2016; Merad et al., 2013). Activated mature DCs migrate to the lymphoid organs, where they interact with and activate both naïve and experienced T cells (Collin et al., 2013; Crosignani et al., 2016). However, the overall profiling of DC subsets in peripheral blood in the patients with active pulmonary tuberculosis (APT) remains elusive.

In the present study, we demonstrate that dendritic cell subsets in peripheral blood of APT patients were changed compared to healthy controls (HCs), with lower expressions of HLA-DR and CD80.

2. Materials and methods

2.1. Subjects and ethics statement

Sixty-five patients with active pulmonary tuberculosis, from 14 to 69 years old, and 25 healthy individuals, from 15 to 60 years old, were enrolled in this study (Table 1 and Table S1). APT subjects were inpatients in the Department of Respiration of Dongguan 6th Hospital (Dongguan, China). All the patients were confirmed based on typical clinical symptoms, chest X-radiography, acid fast bacilli staining of sputum smears, positive bacterial culture, bronchoalveolar lavage or biopsy direct examination and culture. Subject exclusion criteria include HIV^+ test results, diabetes, cancer, autoimmune diseases, immune suppressive treatment (Zeng et al., 2015). Samples were collected before or within ~1 week after the patients received anti-tubercular drugs (ATD) of individualized isoniazid, rifampicin, pyrazinamide, and ethambutol. Some newly diagnosed patients were followed-up for 1 month. All the healthy control (HC) volunteers had no bacteriological and clinical evidence of APT disease. No significant differences in terms of age and gender were noted between patients and HCs. The study was approved by the Internal Review and the Ethics Boards of Guangdong Medical University and Dongguang 6th Hospital, and informed consent was obtained from all study subjects. All the participants provided their written informed consent to participate in this study and the IRBs approved our consent procedure.

2.2. Peripheral blood mononuclear cells preparation

Peripheral blood mononuclear cells (PBMCs) were prepared as previously described (Zeng et al., 2015). Approximately 5 ml blood was collected from each subject in EDTA-K2-containing blood collection tubes. PBMCs were freshly isolated from blood by standard Ficoll (GE healthcare) density gradient centrifugation. Cell viability were determined by typan blue exclusion (> 95% in all experiments). PBMCs were then aliquoted for the following experiments.

2.3. Antibodies and reagents

The following mouse anti-human Abs were used for flow cytometry: Lineage mAb Cocktail 1 (Lin1)-APC (UCHT1, HCD14, 3G8, HIB19, 2H7, HCD56, BioLegend), HLA-DR-PE-Cyanine7 (LN3, eBioscience), CD11c-APC-eFluor780 (BU15, eBioscience), CD123-PerCP- CyTM5.5 (7G3, BD Biosciences), CD80-FITC (2D10.4, eBioscience), CD86-FITC (2331(FUN-1), BD Biosciences), CD83-FITC (HB15e, BD Biosciences), CCR7-FITC (150503, BD Biosciences), and mouse IgG isotype controls (eBioscience).

2.4. Flow cytometry analysis

PBMCs were stained with anti-Lin 1-APC, anti–HLA-DR-PE-Cyanine7, anti-CD123-PerCP-CyTM5.5, and anti-CD11c-APC-eFluor780. The $Lin1^-HLA\text{-}DR^+$cells, representing total dendritic cell (tDC), $Lin1^-HLA\text{-}DR^+CD11c^+$ cells, representing myeloid dendritic cells (mDC), and $Lin1^-HLA\text{-}DR^+CD123^+$ cells, representing plasmacytoid dendritic cells (pDCs), were examined by flow cytometry (Kong et al., 2016). The absolute numbers of DC subsets were calculated by multiplying the frequency of DC subsets in PBMC with the absolute numbers of PBMC which are the sum of lymphocytes and monocytes in the white blood cells counts. In some experiments, the cells were stained with anti-CD80-FITC, anti-CD86-FITC, anti CD83-FITC, and anti-CCR7-FITC. Cells were incubated with mAb against surface antigens for 30 min at 4 °C and then washed twice in cold phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS). After washing, the stained cells were resuspended in 200 µl 2% FBS-PBS containing 2% paraformaldehyde and the samples were then acquired on a BD FACS Canto II flow cytometry and analyzed using FlowJo software (Tree Star) (Supplementary Fig. S1).

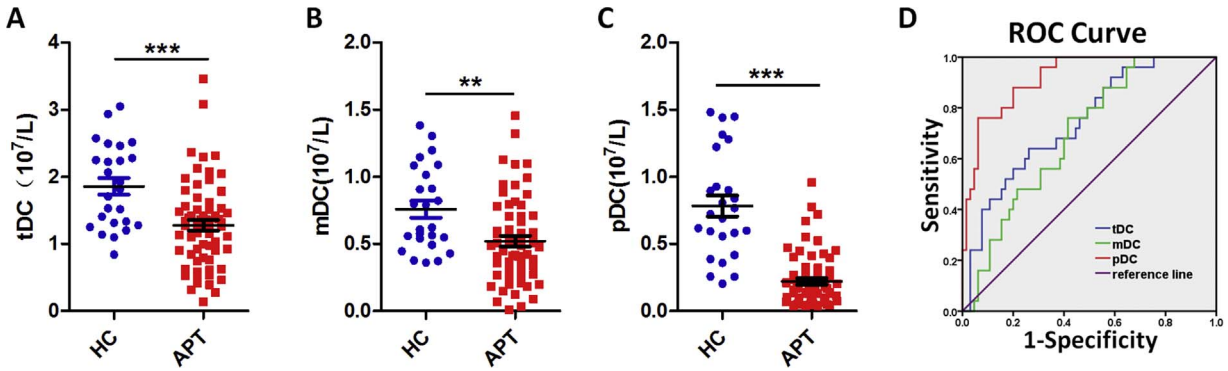

Fig. 1. Absolute number of DC subsets increased in APT patients displaying a diagnosis value.
Peripheral blood mononuclear cells (PBMCs) were prepared from the patients with APT and healthy controls. Cells were assessed for absolute number of DC subsets by flow cytometry as described in the materials and methods. Gating strategy was showed in Fig. S1. (A) Graph data showing the mean number of total dendritic cells (tDC),(B) graph data showing the mean number of myeloid(mDC) and (C) graph data showing the mean number of plasmacytoid dendritic cells (pDC) in patients with APT (n = 65) and HC (HC, n = 25), respectively. (D) Graph data showing the ROC of the tDC, mDC and pDC studied for diagnosis of APT. The *P* value is shown in each column. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.