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Infection with prevalent clinical strains of *Mycobacterium tuberculosis* leads to differential maturation of monocyte derived dendritic cells

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Abstract

The link between innate and adaptive host immune response to *Mycobacterium tuberculosis* (*M.tb*) is driven by dendritic cells (DC). In this study, we examined the ability of prevalent clinical strains from south India (S7, S10) and laboratory strain H37Rv (Rv) to induce maturation of monocyte derived dendritic cells (MoDC). The phenotypic and functional changes of DC upon infection with different strains of *M.tb* were evaluated. It was observed that S7 and Rv strains partially hampered the maturation of MoDC as reflected by the low expression of maturation markers and co-stimulatory markers when compared to LPS stimulated MoDC. In contrast, strain S10 infected DC showed a marked increase in the expression of these markers. The functional property was investigated by the ability of infected MoDC to induce T-cell proliferation and to stimulate secretion of IFN-γ by $CD4^+$T-cells. It was found that Rv and S7 infected MoDC were less efficient in inducing T-cell proliferation. The secretion of IL-12 by Rv and S7 infected MoDC was also found to be significantly lesser than LPS stimulated MoDC. On the other hand, S10 infected MoDC showed enhanced T-cell stimulation and cytokine secretion. Together these results indicate that there is a substantial variability in the capacity of *M.tb* clinical strains to induce maturation of DC which may be dependent upon their virulence.

Keywords: Dendritic cells; *Mycobacterium tuberculosis*; Maturation; Clinical strains; Lipopolysaccharide

1. Introduction

Dendritic cells (DC) play a key role in the host defense against *Mycobacterium tuberculosis* (*M.tb*) infections. Being part of the innate immune system, it triggers T-cells to polarize towards Th-1 or Th-2 type of immune response [1,2], depending upon their maturation status [3]. Progenitors of DC from different cell lineages exist in an immature state and are potential antigen capturing cells [4]. Hence, effective containment of *M.tb* infection requires optimal maturation of DC.

M. tuberculosis is a successful pathogen, known for its various evasive strategies to curb the immune responses [5–7]. It has been evidenced that monocyte derived dendritic cells (MoDC) infected with avirulent mycobacteria, namely BCG or *Mycobacterium avium* have efficiently induced its maturation and T-cell activation [8,9]. Contrary to this, Hanekom et al. have reported that virulent laboratory strain H37Rv (Rv) inhibits maturation of human MoDC [10]. But there are no studies regarding the effects of clinical strains on DC maturation. Of late, it is becoming evident that various clinical strains of *M.tb* differ in their transmission potential, have distinct interaction with the host and induce differential immune response [11–13]. So we speculated that distinct clinical strains of *M.tb* may modulate the maturation of DC for their advantage.

The above hypothesis was investigated using the most prevalent clinical strain of *M.tb*, isolated from the BCG trial area of Thiruvallur district, south India. Epidemiological studies have shown that, the difference in the transmissibility and wide spread nature of certain *M.tb* strain may pertain to virulence of the organism in a particular population. Our earlier studies based on restriction fragment length polymorphism (RFLP) showed that most (38–40%) of these clinical isolates of *M.tb* harbored IS6110 single copy strains. These strains were involved in the active transmission of the disease [14,15]. Further screening of these predominant strains with protein profiling and humoral

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immune responses revealed that two strains, namely S7 and S10 acted distinctly. Strain S7 was able to induce Th-2 response while strain S10 induced potent T-cell proliferation and IFN-γ secretion [16,17]. Both strains were found to contain single copy of IS6110 at the same locus and also showed similar clinical presentations. But, interestingly, by secondary spoligotyping they were found to be different [18]. Further it was found that these two strains adopted different mode of survival strategies and infection in macrophages. Though both strains exhibited low phagocytic index, S7 induced minimal apoptosis whereas S10 induced higher rate of apoptosis in macrophages [19]. Hence these two representative clinical strains (S7 and S10) were chosen to study their potential to induce in vitro maturation of human MoDCs.

2. Materials and methods

2.1. Study subjects and blood collection

The study followed the ethical guidelines of the institution (TRC-IEC No.: 2006007) and written informed consent was obtained from the blood donors. Experiments were performed in 10 healthy volunteers whose age ranged from 18 to 55. Blood was collected through venipuncture in a heparinised tube and processed for subsequent experiments.

2.2. Mycobacterium tuberculosis strains

M. tuberculosis strains S7 and S10 were obtained from clinical isolates of sputum culture positive patients from BCG trial area of Thiruvallur District and the laboratory strain Rv was included for comparison. The mycobacterial strains were grown as stationary cultures in minimal Sauton's liquid medium so as to obtain maximum gene expression in the bacilli until mid-log phase of 4–6 weeks. The bacterial cells were harvested, washed and bacterial clumps were dispersed by passing through 26-gauge needle 10 times. The cell suspension was centrifuged at low speed to remove the remaining clumps. Single cell suspension of bacilli in sterile phosphate-buffered saline (PBS) was stored in aliquots of 10×10^6 bacilli ml^{-1} at −80 °C until further use. The viability was checked before the start of the infection studies.

2.3. Generation of DC and its infection

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donors by density gradient centrifugation using Ficoll-hypaque (Amersham Biosciences, USA). Monocytes were positively separated by anti-CD14 magnetic beads according to the manufacturer's instructions (MACS Miltenyi Biotec, Germany). The cells were then resuspended in RPMI 1640 (Himedia, Mumbai, India) supplemented with 10% fetal calf serum (FCS, Gibco BRL) and cultured for 6 days in the presence of GM-CSF (20 ng/ml) and IL-4 (10 ng/ml, R&D Systems, MN, USA) to generate immature DC. On sixth day, these immature DC were either infected with different strains of *M.tb* at a multiplicity of infection (MOI) of 5 or stimulated with LPS (500 ng/ml) for 24 h. On day 7, the cells were harvested and the viability of infected cells was determined by trypan blue exclusion method.

2.4. Phenotype analysis of DC by FACS

The fluorescein isothiocyanate (FITC), phycoerythrin (PE) or phycoerythrin Cy5 (PECy5)-conjugated antibodies for anti-human leukocyte antigen class II (MHC-II) (clone L243), CD1a (clone HI149), CD14 (clone M5E2), CD80 (clone BB1), CD83 (clone HB15e) and CD86 (clone 2331 [FUN-1]) (Becton Dickinson Bioscience, Mountain View, CA) were used for direct immunofluorescence staining to characterize the phenotype of DC generated in different conditions. Briefly, the cells were washed twice in phosphate-buffered saline containing 1% bovine serum albumin (BSA) and 0.1% sodium azide and were stained with respective fluorescent labelled antibodies for 15 min at 4 °C. The cells were then washed, fixed and acquired using a FACSCalibur instrument (Becton Dickinson Bioscience). In each case, cells were stained with antibodies of the same isotype as the marker-specific monoclonal antibodies to evaluate non-specific binding. Analysis was performed on gated population that included CD1a positive cells and excluded dead cells. The geometric mean fluorescence intensity (MFI) for each marker on MoDC was determined by using CellQuest software (BD Biosciences) and FlowJo software version 7.1.1 (Tree Star).

2.5. Mixed lymphocyte reaction (MLR)

After 24 h of infection or stimulation, DC were added to 5×10^4 freshly isolated allogeneic PBMCs in 96-well U-bottom microplates (Corning-Costar) with different ratios of DC to T-cells. The proliferative response was measured after 5 days of culture with 18 h of prior incubation with [^{3}H] thymidine (0.5 μCi/well) (BARC, Mumbai, India) before harvesting. Cells were harvested in the cell harvester (PHD cell harvester, Cambridge Technology, UK) onto glass fibre disc and thymidine [^{3}H] incorporation was measured by the liquid scintillation counter (Wallac 1409, Finland).

2.6. Endocytosis assay by FITC dextran uptake

Mannose receptor (MR)-mediated endocytosis was measured as the cellular uptake of FITC-dextran. After 24 h of infection or stimulation, DC was incubated with 0.1 mg/ml of FITC-dextran (Sigma, St. Louis, USA) at 4 °C (internalization control) or at 37 °C for 1 h. Cells were then washed twice with cold PBS containing 5% FCS and acquired with FACSCalibur.

2.7. Cytokine assay

At the end of the incubation period, culture supernatants of DC were collected and stored at −80 °C. The levels of TNF-α and IL-12 were determined by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, MN, USA) according to the manufacturer's instructions. Supernatants of $CD4^+$T-cells co-cultured with DC at a ratio of 1:50 for 5 days were measured for