



# EXPRESSION OF INTERLEUKIN-23 FROM DENDRITIC CELL USING BCG VACCINE *Bacilli*

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**Abstract**— Dendritic cells are antigen presenting cells which activates T cells and maintain immune memory. However the macrophages become site of *Mycobacterium tuberculosis* growth and therefore, it evades immune response. The present study investigated the effect of BCG vaccine *Bacilli* on dendritic cell to secrete Interleukin-23 (IL-23) an important cytokine for the memory T cells survival. Peripheral blood mononuclear cells (PBMC) were isolated from blood and the dendritic cells were derived from the PBMC. Dendritic cells were treated with BCG vaccine *Bacilli* and cultured for three days. Total mRNA was isolated from the dendritic cells and analysed for the presence of IL-23 RNA using Reverse Transcriptase-PCR (RT-PCR). Results have shown that the dendritic cells exposed to BCG vaccine *Bacilli* produced IL-23 RNA. This study will provide some clue on BCG induced memory T-cell response.

**Keywords** - Dendritic cells, BCG vaccine *Bacilli*, Interleukin-23, Lipo-polysaccharide, mRNA, cDNA, RT-PCR.

## I. INTRODUCTION

Dendritic cells are antigen presenting cells which induces primary immune response. Dendritic cells were first discovered by Paul Langerhans and the term “Dendritic cell” was coined by Ralph Steinman [13]. The dendritic cells are named so because of the dendrite like projections. The functions of dendritic cell include antigen processing and presentation, activation of T cells and maintenance of immune memory. Dendritic cells can take up whole cells, including necrotic and apoptotic cells. They can also acquire antigens from live cells for presentation to cytolytic T cells [10]. Matured dendritic cell stimulate T helper and T cytotoxic cells via interactions between their antigen loaded MHC class II/I complex and T cell receptors present on the surface of naive T cells [1].

Interleukin -23 supports the T helper 17 cells that are part of the development of memory T cells [5]. Interleukin-23, a member of the Interleukin-12 (IL-12) cytokine family, it is a heterodimer with a 19,000 molecular weight fourfold helical core a subunit (IL-23p19), disulphide linked to an additional 40,000 molecular weight distinct b subunit (IL-12p40) [8]. The formation of biologically active Interleukin-23 requires the synthesis of both subunits p40 and p19 within the same cell [14] [9]. The balanced expression of Interleukin-12 and Interleukin-23 are crucial to maintaining an effective cell-mediated response [11]. The dendritic cells and macrophages are obtained from blood monocytes.

In case of tuberculosis patient’s blood macrophages harbored, contains *Mycobacterium tuberculosis*. Therefore, understanding the role of *Mycobacterium* infected dendritic cell in memory T cell stimulation was under intense study [6]. A review demonstrated that the BCG vaccine reduced infection and progression to active Tuberculosis [12]. A previous study from the investigators lab has shown that the treatment of BCG vaccine *Bacilli* and purified protein derivative on dendritic cells modulated the expression of CD80, antigen up-take and lymphocyte stimulation property of dendritic cell [1]. It also suggests that *Mycobacteria* efficiently modulate the functionality of dendritic cell [1].

## II. MATERIALS AND METHODS

### A. Isolation of peripheral blood mononuclear cells [2]

Buffy coat sample byproduct of platelet cephesis were collected from VHS blood bank Tharamani, Chennai. The collected buffy coat was diluted equally with 1X phosphate buffered saline (PBS) (Himedia, India) and layered on the Ficoll-Hypaque lymphocyte separation medium (Himedia, India) kept in a tissue culture centrifuge tube. The tube was then centrifuged at 400g for 30 minutes at room temperature in a Swinging bucket centrifuge (Plasto crafts, India). The interface layer between plasma and Ficoll Hypaque were collected in a new tube and then diluted twice with PBS. The cell pellet obtained was mixed with RPMI-1640 incomplete medium (Himedia, India) and placed in a T-75 tissue culture flask in 5% Carbon dioxide (CO<sub>2</sub>) incubator (Sanyo, Japan).

The mononuclear cell suspension 50 $\mu$ l was mixed with equal volume of 0.4% trypan blue (Himedia, India). From this mixture, 20 $\mu$ l was taken using a micropipette (Tarsons, India) and placed in the hemocytometer (HBG, Germany). The total number of cells per ml was calculated using the following formula,

$$\text{Cell density (cells/ml)} = [\text{No. of cells counted} * 2 (\text{dilution factor})] / 0.0001$$

#### B. Treatment of immature dendritic cells with BCG vaccine Bacilli

The flasks containing mononuclear cells were incubated for 2 hours at 37 $^{\circ}$ C in 5% CO<sub>2</sub> incubator. Suspension cells were removed from the flask. Additionally two washes were done with Rosewell Park Memorial Institute-1640 (RPMI-1640) incomplete medium to remove the floating cells. The medium was replaced with 30ml of fresh RPMI-1640 complete medium containing 50ng/ml recombinant Human Granulocyte Macrophage-Colony Stimulating Factor (rHu GM-CSF) (Himedia, India) and 100ng/ml recombinant Human Interleukin-4 (rHu IL-4) (Himedia, India). The flasks were then incubated at 37 $^{\circ}$ C in a 5% CO<sub>2</sub> incubator for 3 days. Then 10 $\mu$ l of BCG vaccine (0.1 ml contains 0.2 - 0.8 million colony forming units) (Green chemicals, India) and Lipopolysaccharide (1mg/ml) was added separately and incubated at 37 $^{\circ}$ C in 5% CO<sub>2</sub> incubator.

#### C. Isolation of mRNA [15]

The growth media was removed from the tissue culture flask and 1ml of Trizol (Invitrogen, India) was added to the culture flask. The cells were lysed directly in the tissue culture flask by pipetting the cells up and down several times. The sample was kept at room temperature for 5minutes for complete homogenization. 0.2ml of chloroform (Himedia, India) was added for and shaken vigorously for 15 seconds. The sample was then centrifuged at 300g for 15minutes at 4 $^{\circ}$ C and the upper aqueous phase containing the RNA was transferred to another tube. 0.5ml of 100% isopropanol (Himedia, India) was added to the aqueous phase and incubated at room temperature for 10minutes. This mixture was then centrifuged at 3000g for 10minutes at 4 $^{\circ}$ C. The pellet obtained was washed with 75% ethanol (Himedia, India) by centrifuging at 1875g for 5minutes at 4 $^{\circ}$ C. The pellet was resuspended in 10 $\mu$ l RNase free water (Progen, India) and kept in water bath at 55-60 $^{\circ}$ C for 10-15minutes.

#### D. PCR amplification

5 $\mu$ l of RNA sample was added to a sterile RNase-free tube. The volume was made upto 10 $\mu$ l by adding 9 $\mu$ l of sterile water and 1 $\mu$ l of oligo(dT) primer (0.5 $\mu$ g/ $\mu$ l). The vial was placed at 65 $^{\circ}$ C for 10 minutes. Then following reagents were added: 1 $\mu$ l RNase inhibitor (20U/ $\mu$ l), 2 $\mu$ l 0.1M DTT, 4 $\mu$ l RT buffer (5x), 2 $\mu$ l of 30mM dNTP mix, 1 $\mu$ l of 25mM MgCl<sub>2</sub>, 0.5 $\mu$ l M-MuLV reverse transcriptase (50U/ $\mu$ l), 1 $\mu$ l sterile water for the first stand synthesis. The solution was mixed well and incubated at 37 $^{\circ}$ C for 1 hour, followed by incubation at 95 $^{\circ}$ C for 2 minutes to denature RNA-cDNA hybrids.

Detection of RNA for Interleukin-23 was done using the Sequence specific primers was added and the final mixture was kept for amplification in PCR reaction. The primers used are as follows,

Forward primer- AGGCAAAAAGATGCTGGGGA

Reverse primer- GCTGCCTTTAGGGACTCAGG

50ml of 1% agarose gel was prepared and 2 $\mu$ l of ethidium bromide (10 mg/ml) (Himedia, India) was added. The gel solution is poured in the gel tray. The cDNA product obtained from the RT-PCR was loaded in the wells and electrophoresed at 100-120 volts. The gel was viewed in a UV transilluminator.

### III. RESULTS AND DISCUSSION

The Ficoll hypaque separation medium contains a higher density 1.077gm/ml when compared to that of the buffy coat sample hence the mononuclear cells were formed in the layer above the Ficoll layer. After the development of immature dendritic cells, the cells were treated with Lipo polysacrides (LPS) and BCG vaccine *Bacilli*. The dendritic cells are critical for priming of T cell maturation and cytokine production in response to infection with bacteria [5]. This hypothesis supports the use of BCG vaccine *Bacilli* as a model organism to stimulate the dendritic cells. The LPS treated cells were used as positive control [16] while untreated cells were used as negative control. The cells from the tissue culture flasks were collected (Figure 1a, 1b and 1c) and the RNA was isolated using the Trizol method. Gene specific primers were used for cDNA synthesis using the RT-PCR.

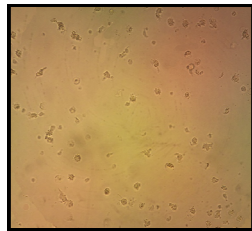


Figure 1a: Day 7 image of untreated dendritic cells in an inverted microscope at 40X magnification.

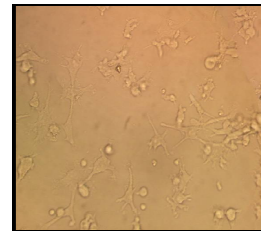


Figure 1b: Day 7 image of dendritic cells treated with LPS in an inverted microscope at 40X magnification.

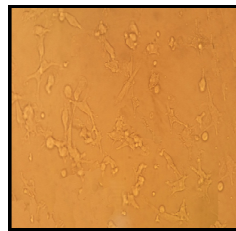


Figure 1c: Day 7 image of dendritic cells treated with BCG *Bacilli* vaccine in an inverted microscope at 40X magnification.

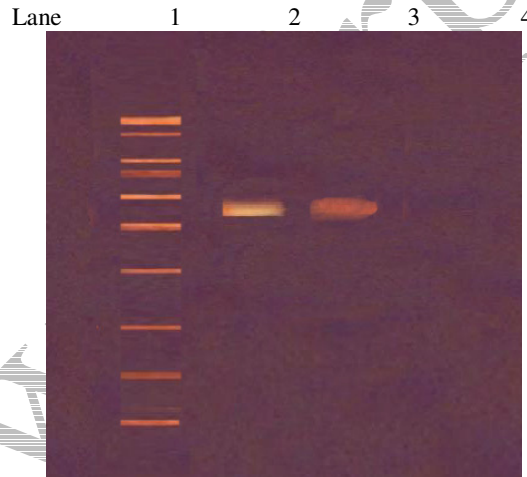


Figure 2: An image of cDNA product run on a 1% agarose gel as viewed in UV trans-illuminator

Lane 1 contains 100bp ladder, Lane 2 contains cDNA product obtained from the LPS treated sample and Lane 3 contains cDNA product obtained from the BCG treated sample and Lane 4 contains cDNA product obtained from the untreated sample. The PCR products from Lane 2 and 3 show the presence of mRNA for interleukin-23. The expression of interleukin-23 in the dendritic cells treated with the BCG vaccine *Bacilli* showed a positive result which relates lymphocyte stimulation property of dendritic cells. This study will provide some clue on the TB induced memory T-cell response in the human population. Further studies are underway to confirm the above findings. Earlier studies have shown that Interleukin-23 is associated with the generation of the T helper 17 response when treated with *Mycobacterium tuberculosis* [10]. Another study using zymosan for activation of dendritic cells enhanced IL-23 production, resulting in activation of a Th17 response [16]. These studies commonly used the LPS as a positive control for the IL-23 gene expression in dendritic cells.

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