Rhizoctonia bataticola lectin (RBL) induces phenotypic and functional characteristics of macrophages in THP-1 cells and human monocytes

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We have previously reported that a fungal lectin, Rhizoctonia bataticola lectin (RBL), stimulates proliferation and secretion of Th1/Th2 cytokines in human peripheral blood mononuclear cells (PBMC). In the present study, we evaluated the ability of RBL to differentiate human monocytes to macrophages. RBL induced morphological changes indicative of differentiation in primary monocytes and THP-1 cells. Stimulation with RBL resulted in significant up-regulation of differentiation markers – CD54, HLA-DR, CD11b and CD11c and secretion of proinflammatory cytokines – IL-1β, TNF-α and IL-6. Functionally, RBL profoundly increased phagocytic activity in monocytes. In THP-1 cells, RBL-induced phagocytosis was higher compared to the effect induced by combination of phorbol-12-myristate-13-acetate (PMA) and lipopolysaccharide (LPS). RBL induced a significant increase in matrix metalloproteinase-9 (MMP-9) activity in comparison with a combined treatment of PMA + LPS. Mechanistic studies revealed the involvement of the NF-κB pathway in RBL-induced differentiation of monocytes. The data suggest that RBL mimics the combined action of PMA and LPS to induce morphological and functional differentiation in human monocytes and monocytic cell line – THP-1 to macrophages. Human monocytes differentiated to macrophages with RBL have the potential as an in vitro model to study macrophage biology.

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1. Introduction

The mononuclear phagocyte system consists of monocytes, macrophages and dendritic cells (DCs). While the central role of the system is maintaining homeostasis, it is also involved in regulating inflammation, autoimmunity and infection [1]. The monocytes are produced in the bone marrow and undergo differentiation to macrophages after entering the tissues to become an important component of the innate immunity [2]. Macrophages exhibit morphological characteristics distinct from monocytes [3]. The tissue macrophages display important functions such as phagocytosis, antigen presentation and secretion of cytokines. There is considerable plasticity of macrophage phenotype and functions that depends on the external stimuli [4].

Monocytic cell lines have been widely used to study macrophage function since primary tissue macrophages cannot be readily obtained and expanded in vitro. Studies have shown that monocytic cell lines – U937, HL-60 or THP-1 can be differentiated into macrophages with stimuli such as phorbol-12-myristate-13-acetate (PMA) or 1,25-dihydroxyvitamin D3 (VD3) [5–7]. PMA induces differentiation in THP-1 cells with increased adherence and expression of surface markers associated with macrophage differentiation [8]. On stimulation with PMA, THP-1 cells mimic native monocyte-derived macrophages and respond to a second activation signal by LPS that result in secretion of cytokines [9,10]. We have earlier reported that RBL, a lectin isolated from phytopathogenic fungus Rhizoctonia bataticola, with exclusive specificity for complex high mannose type N-linked glycans demonstrated mitogenic activity toward human PBMC [11] and stimulated the production of Th1/Th2 cytokines [12]. We also demonstrated that the biological activity of RBL is by binding to CD45, a receptor-like protein tyrosine phosphatase, on the surface of normal PBMC and is mediated by activation of p38 MAPK and STAT-5 signaling pathways [13]. Differentiation of monocyte toward macrophage forms an important component in regulating immune responses. The goal of the present study was...
to examine the potential of RBL to differentiate human peripheral blood monocytes and monocytic cell line THP-1 to macrophages.

2. Materials and methods

2.1. Isolation and purification of RBL

Cation exchange chromatography on CM-cellulose was used to concentrate the lectin in the crude extract. The lectin was eluted from this column with 500 mM NaCl, resulting in a recovery of 94% of the hemagglutinating activity loaded and a 17-fold concentration and a six-fold purification of the lectin. The lectin was further purified to homogeneity on asialofetuin-Sepharose 4B affinity column, from which it was eluted in 100 mM glycine–HCl buffer, pH 2.0 containing 500 mM NaCl. Preparation of the fluorescein isothiocyanate (FITC)-labeled lectin was according to the methods described previously [11].

2.2. Isolation of monocytes from peripheral blood

PBMC were first isolated by density centrifugation of heparinized blood of healthy donors using Histopaque 1077 (Sigma Chemicals). Cells collected from the interface were suspended in RPMI 1640 medium supplemented with heat inactivated fetal calf serum (10%), penicillin (100 U/ml), streptomycin (100 µg/ml) and allowed to stand overnight at 37 °C in 5% CO₂. Monocytes were separated from PBMC by plastic adherence and used for further experiments. To examine the potential of RBL to induce differentiation of monocytes to macrophages, monocytes were exposed to RBL (2.5 µg/ml) for 48 h and analyzed for morphological changes, phenotypic markers of macrophages and functional properties. Monocytes treated with LPS (1 µg/ml) extracted from Escherichia coli serotype O111:B4 (Sigma) were used as positive control.

The study was approved by the ethics committee of NCCS. Written informed consent was obtained from all volunteers.

2.3. Cell culture

Human mononuclear cell line, THP-1 was procured from American Type Culture Collection (ATCC Rockville, USA) and maintained in RPMI 1640 (Gibco) supplemented with 10% heat inactivated fetal calf serum (FCS), 1 mM glutamine, 100 µg/ml streptomycin and 100 U/ml penicillin at 37 °C in 5% CO₂ and 95% humidified air. THP-1 cells were exposed to RBL (2.5 µg/ml) for 48 h to study the effect on cell morphology, adherence, expression of specific cell surface markers and cytokine profiles. Cells differentiated to macrophage by treatment with PMA (2 ng/ml) for 24 h followed by activation with bacterial LPS (1 µg/ml) extracted from E. coli serotype O111:B4 (Sigma Chemicals) for 24 h were used as positive controls. Phase contrast microscopy images of treated and untreated cells were captured to record changes in cell morphology.

2.4. Analysis of lectin binding

PBMC were incubated with FITC-RBL (1 µg/100 µl) and phycoerythrin labeled CD14 antibody at 4 °C for 1 h. The binding of FITC-labeled RBL was determined in CD14 positive-gated population by flow cytometry analysis. Data was acquired for 10,000 events on BD FACS Calibur cytometer and analyzed using Cell Quest-Pro Software. Unstained cells and cells stained with FITC-gelatin processed similarly were used as negative control. Localization of RBL-binding sites on the surface of primary monocytes derived from peripheral blood and THP-1 cells was studied by confocal laser scanning microscope (510 Meta, Zeiss, USA). To determine the sugar specificity of RBL, FITC-labeled RBL (1 µg/100 µl) was incubated with 10 µg/100 µl of mucin, asialofetuin, and 200 mM of N-acetyl-d-galactosamine, β-d-glucose and sucrose (Sigma Chemicals) for 1 h at room temperature and the lectin–sugar complex was used for staining the cells at 4 °C for 1 h. Flow cytometry analysis was performed as described above.

2.5. Cell adhesion assay

THP-1 cells (1 x 104 cells/well) were plated in a 96-well tissue culture plate that was blocked with 0.5% BSA. The cells were treated with RBL for 6 and 12 h. The adherent cells were stained with crystal violet and phase contrast images were acquired on an inverted phase contrast microscope. The dye was eluted in a solution of 1% SDS and absorbance was measured at 595 nm. Cells treated with phorbol-12-myristate acetate (PMA) were used as positive control and untreated cells served as negative control.

2.6. Expression of cell surface molecules

Expression of cell surface molecules was quantified by flow cytometry analysis. THP-1 cells were stained with phycoerythrin tagged primary antibodies to CD11b, CD11c, CD54, CD14, HLA-DR and CD80 (BD, Bioscience) followed by data acquisition and analysis using Cell Quest-Pro Software.

2.7. Cytokine ELISA assay

Culture supernatants were collected from monocytes and THP-1 cells stimulated with RBL, PMA and PMA + LPS and analyzed for IL-1β, TNF-α, IL-6 and IL-10 levels by ELISA (BD Bioscience). Conditioned medium from unstimulated PBMC was used as control.

2.8. Phagocytosis assay

THP-1 cells were treated with RBL for 48 h and fluorescently labeled latex beads of 2.0 µm size (Sigma Chemicals) were added to the cells and incubated for 12 h in dark at 37 °C in 5% CO₂ in humidified air. The cells were dislodged and the phagocytic uptake of beads was quantified by flow cytometry. Cells incubated with latex beads at 4 °C served as controls, where in latex beads are accumulated on the cell surface. Phagocytosis was also assessed by fluorescence microscopy.

2.9. p65 localization

THP-1 cells stimulated with PMA, PMA + LPS and RBL were fixed with 3.7% PFA, permeabilized with 0.2% Triton X100 and blocked with 5% BSA. The cells were then stained with p65 antibody (Santa Cruz biotechnologies) followed by species specific secondary antibody labeled with Cy3. DAPI was used for staining the nucleus. Untreated cells were used as control. The images were acquired by confocal laser scanning microscope (Ziess) under 60 × magnification.

2.10. Gelatin zymography

THP-1 cells were either left untreated or were stimulated with PMA, PMA + LPS and RBL for 4 h. The conditioned medium was collected and equal volumes was mixed with 5X Laemmli’s sample buffer without reducing agents and were subjected to polyacrylamide gel electrophoresis. Gelatin (0.1%) was incorporated during preparation of the Acrylamide gel. After electrophoresis the gel was washed twice with washing buffer (50 mM Tris–Cl, pH 7.5 and 2.5% Triton X-100) for 30 min and incubated overnight at 37 °C in renaturation buffer (50 mM Tris–Cl, pH 7.6, 10 mM CaCl₂, 150 mM NaCl, and 0.05% NaN₃). The gel was stained with 0.2% Coomassie Brilliant
Blue R-250 in 40% isopropanol and destained using 7% glacial acetic acid.

2.11. Inhibitor treatment

THP-1 cells were pretreated with a pharmacological inhibitor of NF-κB-BAY11-7082 (10 μM) for 1 h followed by RBL stimulation for 48 h. The involvement of the NF-κB pathway was confirmed by monitoring morphological changes and by gelatin zymography and phagocytosis assay.

2.12. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM) for various experimental groups. The statistical differences between groups were analyzed using an unpaired Student’s t-test. The graphs were plotted using graph pad software.

3. Results

3.1. Binding and carbohydrate specificity of RBL

Initial experiments were conducted to determine the binding of RBL to primary monocytes. PBMC were stained with FITC-labeled RBL and Phycoerythrin (PE)-tagged antibody to CD14, a monocyte specific marker, and analyzed by flow cytometry. Cells were gated for CD14 positivity and the population that stained for RBL was determined. As shown in Fig. 1A, CD14 positive -gated population showed 99% positivity for RBL staining with MFI of 176.36. RBL exhibited intense binding to THP-1 cells with 95% positivity and a MFI – of 201 (Fig. 1C). No significant staining was observed in cells incubated with FITC-gelatin (0.5% positivity and MFI of 35). The confocal laser scanning microscopy revealed uniform binding of RBL on the cell surface thereby indicating the presence of RBL binding sites on the cell membrane in monocytes and THP-1 cells (Fig. 1B and C).

To determine the sugar specificity of RBL, RBL-FITC (1 μg/100 μl) was preincubated with 10 μg/100 μl of mucin, asialofetuin, and 200 mM of N-acetyl-b-galactosamine, glucose and sucrose. The binding of RBL-FITC in the absence and presence of sugar complex was determined by flow cytometry analysis in THP-1 cells. As depicted in Fig. 1D, a significant reduction in the binding was observed in cells treated with a mixture of RBL with mucin (MFI of 9.4) or asialofetuin (MFI of 11.64) but not with simple sugars such as N-acetyl-galactosamine, glucose and sucrose suggesting that RBL binds to complex glycoproteins on the surface of THP-1 cells.

3.2. Differentiation of THP-1 cells

Further studies were directed toward examining the potential of RBL to induce differentiation of monocytes to macrophages using THP-1 cell line as a model system. Increased adherence is a functional indicator of macrophage differentiation [14]. The effect of RBL on adhesion was studied at two time points – 6 h and 12 h. As shown in Fig. 2A and B, exposure of THP-1 cells to RBL for 6 h led to adhesion of few cells while the PMA-treated cells exhibited a
significant increase (2-fold) in cell adhesion compared to controls. Interestingly, at a later time point (12 h) RBL induced a marked increase (2.5-fold) in adherence that was comparable to the effect induced by PMA. These observations clearly suggested that RBL enhances adhesion, an event, important in monocyte-macrophage differentiation.

The differentiation of monocyte to macrophage is accompanied by a reduction in the nucleocytoplasmic ratio due to increase in the cytoplasmic volume and granularity [5]. We observed that untreated THP-1 cells remained round and were present in suspension while cells exposed to RBL for 72 h exhibited macrophage-like flattened morphology with protruding pseudopodia. The appearance was similar to THP-1 cells treated with PMA followed by stimulation with LPS. Preincubation of RBL with mucin significantly blocked the ability of RBL to induce morphological changes associated with the differentiation of monocytes (Fig. 2C). RBL induced remarkable changes in morphology with extended filopodia-like extensions in primary monocytes isolated from PBMC (Fig. 2D). These results suggested that RBL induced differentiation of THP-1 cell line and primary monocytes to macrophage.

### 3.3. Expression of differentiation markers

CD54 or ICAM-1 is a marker for macrophage activation [15]. THP-1 cells showed constitutive expression of CD54 (45% positivity with low MFI of 24.71). Treatment with PMA increased the positive population to 82.39% (MFI of 77.51) and addition of LPS increased the positivity to 99% with MFI of 631.89. Interestingly, exposure to RBL resulted in a phenomenal increase in the proportion of CD54 positive cells (99%) with MFI 756.21 (Fig. 3A) in THP-1 cells. Analysis of other cell adhesion molecules – CD11b and CD11c revealed a marginal increase in CD11c expression on stimulation with combined treatment of LPS and PMA in comparison with RBL. There was no significant difference in the expression of CD14 and CD11b between the two treatment groups (Fig. 3B–D). There was a significant increase in the expression of HLA-DR (Fig. 3E) in cells stimulated with RBL (86.26% positivity and MFI of 23.35) that was higher than that observed in cells stimulated with PMA and LPS (66.38% positivity and MFI of 19.92). Analysis of CD80 expression revealed no significant increase upon RBL exposure (Supplementary Fig. 1). These data suggest that RBL induced expression of macrophage differentiation markers that was comparable in cells stimulated with PMA and LPS.

Supplementary Fig. 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.imlet.2014.12.005.

### 3.4. Effect of RBL on cytokine profiles of THP-1 and primary monocytes

To further study the impact of RBL on macrophage differentiation, cytokine profiles of RBL-treated THP-1 cells were compared with cells activated with PMA and PMA + LPS. Cell culture supernatants were collected after exposure to RBL for 48 h and the levels of IL-1β, TNF-α, IL-6 and IL-10 were estimated by ELISA. RBL-treated cells secreted high level of IL-1β (10-fold increase) that was comparable to cells stimulated with PMA + LPS. Stimulation with RBL did not affect IL-10 levels, while LPS activated cells showed very high secretion of IL-10 (Fig. 4). There was no significant difference in TNF-α and IL-6 levels between cells treated with RBL and PMA while activation with LPS induced very high levels of these cytokines.

PMA treatment differentiates THP-1 cells into adherent macrophage-like cells that resemble primary monocyte-derived macrophage obtained by adhesion to tissue culture plastic. LPS stimulation in PMA treated cells results in activation of the macrophages. Primary monocytes stimulated with RBL or LPS for 48 h showed profiles distinct from those in THP-1 cells. RBL induced a marked increase in IL-1β level (27-fold increase compared to 3-fold increase with LPS stimulation) and TNF-α (8-fold compared to 3-fold increase with LPS). On similar lines, IL-10 secretion was significantly higher with RBL (62-fold) compared to LPS stimulation (38-fold increase). There was no significant difference in the level of IL-6 induced by RBL and LPS (Fig. 5). These results suggest that RBL effectively stimulates the secretion of cytokines in monocytes, an
Fig. 3. Expression of surface markers in RBL-treated THP-1 cells. THP-1 cells were treated with PMA (2 ng/ml), PMA (2 ng/ml) + LPS (1 μg/ml) and RBL (2.5 μg/ml) for 48 h and the expression of CD54 (A), CD11b (B), CD11c (C) CD14 (D) and HLA-DR (E) was estimated by flow cytometry. The histograms depict fluorescence intensity on Y-axis and cell count on the Y-axis. The marker M1 was set at 1% positivity for unstained cells. The % positive cells and mean fluorescence intensity (MFI) values are incorporated in the figure.

important step in modulating immune responses and also demonstrates the differential response of THP-1 cell line and monocytes to RBL.

3.5. RBL promotes phagocytic activity of monocytes

The phagocytic activity is a functional property of mature macrophages. To study the effect of RBL on phagocytic activity, THP-1 cells were incubated with latex beads tagged with a red fluorescent dye at 37°C and flow cytometry analysis was performed. Cells stimulated with PMA+LPS were used as controls. To differentiate true phagocytosis from adherence to the cell surface, control experiments were performed in cells incubated with beads at 4°C. As shown in Fig. 6, RBL-stimulated cells were more efficient in phagocytosing latex beads (47.26%) compared to cells treated with PMA+LPS (36.36%). Blocking of RBL by mucin significantly reduced the phagocytic uptake of beads by THP-1 cells. As expected, very low phagocytic activity was observed in untreated cells and in cells maintained at 4°C (+11%). Analysis by fluorescence microscopy revealed accumulation of red fluorescent beads near the nuclei.

In monocytes derived from PBMC, stimulation with RBL resulted in enhanced phagocytic activity (22.51%) compared to untreated control cells (11.93%) and LPS-stimulated cells (17.5%) (Fig. 7). Collectively, these results indicated that RBL has potential to induce functional activity of monocyte derived macrophages and is more effective compared to the conventional protocol using PMA and LPS for in vitro studies.

3.6. RBL induces THP-1 cells to secrete MMP-9

Activated macrophages secrete several enzymes particularly the MMPs that cause degradation of the extracellular matrix thereby facilitating migration [16,17]. Macrophages have been known to produce MMP-9 in response to prolonged exposure to an inflammatory stimulus. We next determined the effect of RBL on the secretion of MMPs. RBL-stimulated THP-1 cells secrete enzymatically active MMP-9 and was higher than that secreted by the combined PMA and LPS stimulation (Fig. 8A).
3.7. Involvement of the NF-κB pathway in RBL-induced differentiation

The NF-κB pathway plays a pivotal role in differentiation of monocytes to macrophages [18–20]. RBL treatment resulted in increased p65 expression as well as nuclear localization. Similar results were observed in cells stimulated with PMA and PMA + LPS (Fig. 8B). In the presence of a specific pharmacological inhibitor of NF-κB—BAY11-7082, THP-1 cells did not undergo characteristic morphological changes induced on stimulation with RBL. In the

Fig. 4. Effect of RBL on cytokine secretion in THP-1 cells. THP-1 cells were stimulated with PMA (2 ng/ml), PMA (2 ng/ml) + LPS (1 μg/ml) and RBL (2.5 μg/ml) for 48 h and secretion of IL-1β, IL-6, TNF-α, and IL-10 in supernatants was measured by ELISA. All values are mean ± SE of three independent experiments done in triplicates. *p < 0.05, significant difference compared to untreated cells.

Fig. 5. Effect of RBL on cytokine secretion in primary monocytes. Primary monocytes were stimulated with LPS (1 μg/ml) and RBL (2.5 μg/ml) for 48 h and secretion of IL-1β, IL-6, TNF-α and IL-10 in supernatants was measured by ELISA. All values are mean ± SE of three independent experiments done in triplicates. *p < 0.05, significant difference compared to untreated cells.
Fig. 6. Effect of RBL on phagocytosis. THP-1 cells were treated with PMA (2 ng/ml) + LPS (1 μg/ml), RBL (2.5 μg/ml) and RBL (2.5 μg/ml) + mucin (100 μg/ml) for 48 h and were incubated with latex beads at 4°C and 37°C and phagocytic uptake was measured by flow cytometry and visualized by fluorescence microscopy. DAPI was used as nuclear stain and images were acquired at 60× magnification. The figure is representative of three independent experiments.

presence of BAY11-8082, a significant reduction in RBL-induced phagocytosis and MMP-9 activity was observed confirming the involvement of NF-κB pathway in RBL-induced differentiation and activation of monocytes (Fig. 9A–C).

4. Discussion

Monocytes in the bloodstream migrate into tissues where they undergo differentiation into macrophages in the presence of certain

Fig. 7. Phagocytosis in primary monocytes. Primary monocytes derived from PBMC were treated with RBL (2.5 μg/ml) and LPS (1 μg/ml) for 48 h and phagocytosis was determined by the uptake of fluorescent latex beads by flow cytometry analysis. The flow cytometry histoplots depicts fluorescence intensity on X-axis and cell count on Y-axis. The figure is representative of three independent experiments.
growth factors and pro-inflammatory cytokines [22]. Macrophages play an important role in many inflammatory disease conditions including atherosclerosis and cancer [23]. Therefore, studying the macrophage biology is important in understanding the pathophysiology of diseases and development of novel therapies. Various approaches and strategies have been employed to develop experimental models to study macrophage differentiation. Recently, Fejer et al., reported a GM-CSF/STAT5 dependent macrophage model derived from mouse fetal liver, that reproduces the innate features of alveolar macrophages [24]. The most widely used system for monocyte-macrophage differentiation involves activation of undifferentiated cells with PMA and LPS [25–27]. While there are few studies describing activation of monocytes by plant and animal lectins, that include Korean mistletoe lectins [28] and Galectin 3 [29], little is known about the differentiation of monocytes to functional macrophages by fungal lectins. In the present study, we report that RBL, a fungal lectin isolated from Rhizoctonia bataticola induces differentiation of monocytes to activated macrophages as evidenced by morphological characteristics, adherence, expression of activation markers and functional activities. The study was performed with human mononcytic cell line, THP-1 and monocytes isolated from human PBMC. The inhibition of binding of wheat germ agglutinin (WGA), Solanum tuberosum lectin (STL), peanut agglutinin (PNA) and Sambucus nigra agglutinin (SNA) to THP-1 cells by complementary carbohydrates has been reported [30]. In this study, RBL exhibited very high binding (<90% positivity) to primary monocytes and THP-1 cells that was inhibited by complex glycoproteins–mucin and asialofetuin suggesting that RBL interacts with complex glycoproteins present on monocyte surface.

One of the initial events in the differentiation of monocytes to macrophage is increased adherence [14]. The kinetics of adhesion of THP-1 monocytes revealed that RBL-induced cell adhesion
was delayed compared to cells stimulated with PMA. The prerequisite of functional monocytes and macrophages is dynamic morphological changes such as formation of filopodial protrusions [31,32]. RBL treatment resulted in expansion of cytoplasm into filopodia-like projections in THP-1 cells and primary monocytes suggesting differentiation to macrophage lineage. CD54 (ICAM-1) is a macrophage activation marker [15] that functions as a ligand for leukocyte integrin complex to facilitate cell-cell interactions. CD54 expression is reported to be up regulated upon stimulation with macrophage stimulating factors such as GM-CSF, TLR3, TLR4 and TLR7 [14]. RBL upregulated CD54 expression in THP-1 cells comparable with the level induced by the combined treatment of PMA and LPS. HLA-DR is a MHC-class II cell surface receptor and is reported to be up-regulated during differentiation of monocytes to macrophages. RBL also enhanced the expression of other markers of macrophage differentiation – CD11b and CD11c [33]. CD14, a monocyte marker has been reported to be necessary for the phagocytic activity of macrophages [34-37]. However, other studies suggest that the expression of CD14 depends on the external stimuli. In this regard, while exposure to PMA did not increase CD14 expression in THP-1 and U937 cells, vitamin D3-treatment enhanced CD14 expression [5,38]. In the present study neither RBL nor a combination of PMA and LPS altered CD14 level in THP-1 cells.

Production and secretion of cytokines from innate immune cells are crucial for the regulation of the immune response. Cytokine responses induced in macrophages vary depending on the stimulus. Studies have documented that THP-1 cells activated with LPS alone cannot secrete IL-1β and require prior exposure to PMA [9]. Interestingly, RBL induced IL-1β secretion in THP-1 cells that was comparable to combined stimulation of PMA + LPS suggesting that RBL might mimic the collective effect of PMA and LPS. The secretion of IL-1β in response to RBL-stimulation supports our finding that NF-kB pathway is involved in RBL-induced differentiation as IL-1 β is a target gene of NF-kB [21]. Furthermore, IL-1β release is also reported to be a result of pyroptosis, which is an inflammatory form of cell death in macrophages [39] and hence we also cannot rule out the possibility that RBL might induce death in a small fraction of cells. RBL stimulation also enhanced the levels of IL-6 and TNF-α but did not significantly induce IL-10 secretion in THP-1 cells suggesting that the differentiation of these cells is driven toward classically activated or M1 macrophages. The M1 macrophages produce pro-inflammatory cytokines which mediate killing of pathogens and more importantly exhibit anti-tumor immune responses. M2 macrophages, on the other hand are anti-inflammatory and play important role in tissue remodeling, angiogenesis and tumor progression [40,41]. Our study using monocyte -derived macrophages confirmed that RBL enhanced the secretion of pro-inflammatory cytokines - IL-1 β, TNF-α and IL-6. M1-type of macrophages has also been reported to exhibit increased expression of HLA-DR [42]. Our flow cytometry data showed enhanced HLA-DR expression upon RBL stimulation. The cytokine profile and the increased HLA-DR expression suggests that RBL stimulation might skew macrophage polarization toward M1 phenotype in contrast to THP-1 cells. RBL-stimulated primary monocytes secreted high IL-10 levels. However, it is not clear what causes the enhanced secretion of IL-10 in RBL-stimulated primary monocytes which depict the profile of M1-like phenotype. Furthermore, RBL significantly augmented the phagocytic activity in THP-1 cells and primary monocytes. Interestingly, the effect of RBL was more pronounced in monocyte-differentiated macrophage than in THP-1 cells. This may be possibly due to the differential status of THP-1 cells and primary monocytes [5].

Cells of the monocyte/macrophage lineage, including blood monocytes, dendritic cells, and tissue macrophages have been known to secrete MMPs. MMPs are required in the process of transmigration of monocytes/macrophages into tissues [43,44]. CD14+ monocytes from blood of healthy individual have been shown to express MMP-1, -3, -9, -10, -14 (MT1-MMP), -19, and -25 (MT6-MMP) [45]. A range of factors including cell adhesion to lymphocytes and ECM, stimulation by MCP-1, HIV-1 Tat, lectins, PMA, endotoxin, IL-1β, and prostaglandin E2 (PGE2) can induce MMP-9 production by monocytes [44,46,47]. We have shown that RBL-stimulation results in 2.8-fold increase in MMP-9 activity in THP-1 monocytes. The MMP-9 activity of RBL-stimulated cells was higher compared to the cells stimulated with PMA and LPS. MMP-9 is also the target gene of NF-κB pathway hence we next assessed the role of NF-κB pathway in RBL-induced differentiation of monocytes. RBL exposure increases the expression and nuclear translocation of p65 in THP-1 cells. Treatment with a inhibitor of NF-κB, BAY 11-7082, inhibited RBL-induced differentiation and activation of macrophages as evident from morphological changes, decreased phagocytosis and MMP-9 activity.

In conclusion, these findings indicate that RBL effectively induces differentiation of primary monocytes and monocyctic cell line, THP-1 depicting typical morphological changes, expression of specific markers, cytokine profile, phagocytic properties, MMP-9 activation that reflect the distinct features of macrophages. The study demonstrates that NF-κB pathway plays an important role in RBL-induced differentiation of monocytes into activated macrophages. The findings suggest that human monocytes differentiated to macrophages with RBL have the potential for use as an in vitro model to study macrophage biology.

Conflict of interest

The authors have no conflicts of interest to declare.

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