Advanced Pharmaceutical Bulletin, **2013**, 3(2), 329-332
doi: http://dx.doi.org/10.5681/apb.2013.053
http://apb.tbzmed.ac.ir/

Induction of CD14 Expression and Differentiation to Monocytes or Mature Macrophages in Promyelocytic Cell Lines: New Approach

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ARTICLEINFO

Article Type:
Research Article

Article History:
Received: 22 February 2013
Revised: 18 March 2013
Accepted: 6 April 2013
ePublished: 20 August 2013

Keywords:
CD14
1, 25-D3
LPS
DMSO
Monocyte

ABSTRACT

Purpose: CD14, one of the main differentiation markers on the surface of myeloid lineage cells, acts as a key role in activation of LPS-induced monocytes. LPS (lipopolysaccharide) binds to LPS-binding protein in plasma and are delivered to the cell surface receptor CD14. In this study, Various stimuli [Dimethyl Sulfoxide (DMSO), active 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and LPS], either alone or in combination, have been recognized that have an effect on the level of CD14 expression in the human HL-60 and U937 promonocytic cell lines and therefore induce their terminal differentiation into monocytes or mature macrophages. ***Methods:*** U937 and HL-60 cells were cultured in RPMI 1640 supplemented with 10% FBS. For each cell line, 1×10^6 cells were seeded for 72 hours with DMSO, 14 days with LPS and 18 days with 1, 25-D3 in each well plate; then ELISA method was used to study their responses to the factors by means of anti-CD14. ***Results:*** ELISA assay demonstrated that U937 and HL-60 cells were induced by both [1,25(OH)2D3] and DMSO to obtain characteristics of adherent cells and express CD14 protein; moreover, LPS at a low dose increased CD14 expression on surface of this cells. ***Conclusion:*** According to the our results, it is speculated that CD14 gene expression may be induced in human U937 and HL-60 cell lines by different factors including 1,25-D3, DMSO and LPS.

Introduction

Cluster of differentiation 14 (CD14) was described as monocyte/ macrophage differentiation antigen on the surface of myeloid lineage, such as monocytes, macrophages and dendritic cells (DCs).[1] This protein plays a crucial role in the immune recognition and reactivation in microbial cell wall components from Gram-positive and Gram-negative bacteria.[2] Recently, CD14 role is known in phagocytic clearance of apoptotic cells.[3] CD14 isoforms, 52-55 kDa expressed on the surfaces of monocytes and neutrophils are attached to the cell surface by a glycosyl phosphatidylinositol (GPI) anchor, membrane protein (mCD14) and the serum soluble 48–56 kDa (sCD14, an acute phase protein).[4] Soluble CD14 found in human serum has been attributed to the shedding of mCD14 from monocytes, macrophages and PMN. Membrane CD14 as a receptor for lipopolysaccharide (LPS) on the membrane of the mononuclear phagocyte (MPS) binds to LPS-binding protein (LBP) in plasma and transfers to the cell surface receptor CD14.[5] It has been commonly used in normal tissue or blood and in leukemia as a marker for myeloid cells. LPS stimulates the human monocytes activation via several intracellular signaling pathways that involves the proinflammatory factors.[6]

On the cell surface, CD14 associates with Toll-like receptor 4 (TLR4). Binding of the LPS and LPS-binding protein complex to CD14 induces signal transduction through TLR4, which then triggers the synthesis and release of proinflammatory chemokine (IP10), and cytokines (TNF-α, IL-6 and IL-1).[7,8] CD14 Expression on myeloid cell line can be induced. Among several myeloid cell lines (HL60, THP-1, Mononomac- 1, and U937), U937 cells and HL-60 cells are the most frequently used as valid model for investigating monocytic differentiation and consequent biological functions of differentiated cells in vitro. U937 cells of histocytic lymphoma basis are arrested in a more advanced step of differentiation (promonocyte/monocyte).[9] HL-60 human leukemia cells can be induced to differentiation into monocyte, macrophage and granulocytes by inducing factors. Various stimuli [LPS (lipopolysaccharide), Dimethyl Sulfoxide (DMSO), 1, 25-dihydroxyvitamin D3 [1,25(OH)2D3], either alone or in combination, have been recognized that have an effect on the level of CD14 expression in the human HL-60 and human

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U937 promonocytic cell lines and therefore induce their terminal differentiation into monocytes or mature macrophages.[10] Upon differentiation, U937 cells gain a large range of macrophage function through the concerted expression of several genes. Differentiated U937 cells can be further stimulated with LPS to mimic inflammatory response of activated macrophages.[11] In this study, U937 and HL-60 model system was employed to express monocyte-macrophage differentiation patterns. LPS 1, 25-Dihydroxyvitamin D3 [1,25(OH)2D3] and DMSO were used for inducing monocytic-macrophage differentiation of the U937 and HL-60 leukemic cell lines in a dose- and time dependent manner. It have been investigated that this maturation mimics the in vivo monocytic or myeloid differentiation.

Material and Methods

Cell Preparation and Seed

U937 and HL-60 cell lines were purchased from Pasteur Institute of Iran (cell Bank). The cells were grown and maintained in a humidified incubator at 37°C and in 5% CO2 atmosphere. RPMI-1640 medium (SIGMA) was supplemented with 15% heat inactivated Fetal Bovine Serum (FBS), 100 units/mL penicillin, 2.5 ml amphotericin B, 5× 10 M 2-mercaptoethanol and 2.5 mM L-glutamine, and 100 μg/mL streptomycin (all from Invitrogen Gibco) were used for cell cultures. Upon reaching appropriate confluence, the cells were passaged every 2-3 days and seeding was at in initial concentration of 1×10^6 cell/ml. LPS (Escherichia coli O111: B4) and 1,25-D3 were purchased from sigma chemical company. Five well of 12-well flat-bottom culture plates (Nunc, Denmark) were seeded per cell line. 1×10^6 cells per well was incubated and treated with DMSO + LPS for 14 days, 0.1 µmol 1,25-D3 , 1000 ng/ ml LPS for 18 days, and with LPS + Vitamin D3 for 18 day, respectively.

Microscopic Analysis

Morphological changes after 5-6 days incubation, maturation and morphology differentiation into macrophage-monocyte was detected by light microscopic analysis.

ELISA Assay

CD14 protein expression on cell membrane was analyzed by ELISA assay. Briefly, after incubation period, 1×10^6 cell was seeded in 12-well flat-bottom ELISA plate. Glotaraldehid 25% was used to fix the suspended cells into bottom of ELISA plate wells for 12-16 hour in 4°C. After the overnight incubation, the plates were washed 3 times with PBS containing 0.05% (v/v) Tween 20 (PBS-T). Each well was blocked with 200 µL of PBS containing 1% (w/v) BSA (Sigma) for 1 h at room temperature and washed 3 times with PBS-T. Subsequently 100 µl of first antibody (mouse anti-human CD14 /monoclonal antibodies: 1/1000 diluted in PBS buffer), was added and incubated for 2 hour in 37°C, and washed 3 times with PBS-T. Secondary antibody (rabbit anti-mouse IgG conjugated to horseradish peroxidase 1/3000 diluted in PBS buffer) was added to each well and incubated for 1 hour at 37 °C. 100 µL of 0.01% (w/v) 3, 3', 5, 5' tetramethylbenzidine (TMB) chromogenic/substrate solution (Sigma) was added to each well. The reaction was stopped by adding 50 µL of 2 N sulfuric acid, yielding a yellow color. The optical density (OD) colored solution was quantified at 450 nm wavelengths by using an enzyme linked immunoabsorbent assay reader (ELISA Reader, Bio-Rad).

Results

The morphological changes of cells were examined by light microscopy. After 5-6 days incubation, it was exposed that cells gained pseudopods and shift from a round shape to a macrophage-like morphology. Adhesion shapes were seen in treated cells. Figure 1 demonstrates that cells completed the macrophage-monocyte differentiation characteristics.

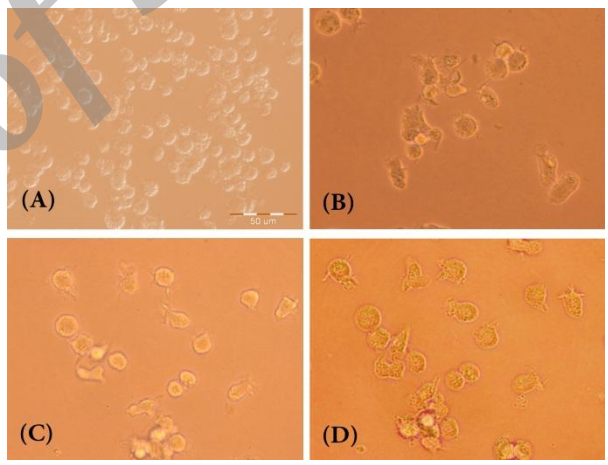

Figure 1. Morphology of U937 cells. A) Control, B) DMSO, C) 1, 25-D3 and D) LPS treated cells. The LPS, DMSO, and 1, 25-D3 effect on Differentiation of U937 cells shift from a round shape to a macrophage-like morphology, from suspension to adhesion growth and acquire phagocytic activity with amoeboid morphology.

The expression of CD14 molecule in the membrane of U937 and HL-60 cells was detected using ELISA assay by mouse monoclonal antibodies against human CD14 to confirmed Differentiation of the cells. ELISA analysis explained that expression of CD14 in cells surface significantly enhance by treatment with DMSO + LPS, [1,25(OH)2D3], LPS, DMSO, and with LPS + [1,25(OH)2D3], altogether. As Figure 2 shows, LPS and LPS + [1,25(OH)2D3] treatment, determined the highest expression of CD14 in HL-60 cell line but DMSO group showed little CD14 expression in U937 and HL-60 cells.

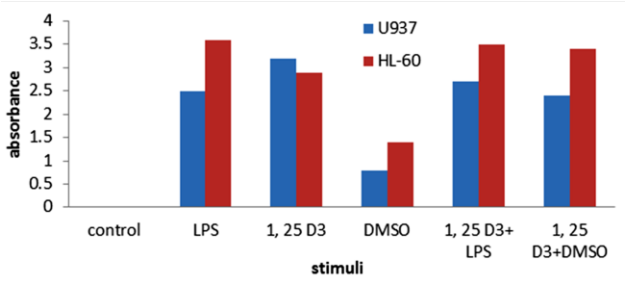

Figure 2. ELISA analysis, expression of CD14 in cells surface was significantly enhanced by treatment with DMSO + LPS, [1,25(OH)2D3], LPS, DMSO, and with LPS + [1,25(OH)2D3], altogether.

Discussion

Human U937 promonocytic and HL-60 leukemic cell lines as the precursors of the mononuclear phagocyte system are the important cell line to study the cells shift from a round shape to a macrophage-like morphology.[12] In addition, CD14 protein expression in membrane of HL-60 is slight too. Furthermore, [1,25(OH)2D3] and DMSO can induce U937 and HL-60 cells expressing the CD14, and make them take reaction to the stimulation of LPS. The result of this study showed that the way inducing U937 and HL-60 to express CD14 protein is a stable, reliable and practicable method, and it can satisfy the demand of experiments.[13] U937 cells can be induced to differentiation by a large number of substances (TPA, retinoic acid, DMSO, Zn++ and low concentration of glutamine). In their differentiated stage, these cells assume a typical macrophage aspect, adhere to substrate, and change morphology: they assume a more irregular flat shape with long pseudopodia and cytoplasmic protrusions, and acquire phagocytic capability.[14] [1,25(OH)2D3] is an important regulator of inducing differentiation and proliferation of many cells, and it can induce U937 cells to express CD14 protein. Some researchers have reported that [1,25(OH)2D3] can induce 40% of U937 cells to express CD14 protein in 8 hours and the percentage of cells expressing CD14 protein reached 100% in 24 hours.[15,16]

Sun et al found that some intermediated molecular are required in the process of inducing U937 cell to express the CD14 gene, shown by [1,25(OH)2D3] using FCM, Northern blotting and run-on transcription analysis. They detected the inducing process needed for the synthesis of new protein called SP1, and found that the sequencing U937 cell to express CD14 protein by [1,25(OH)2D3].[16] Baek et al reported that retinoic acid could increase the expression of CD14 in U937 cell after [1,25(OH)2D3] treatment. This aspect needs further study. The present study, induction of CD14 membrane expression with different stimuli was assayed in vitro. LPS, DMSO and [1,25(OH)2D3], alone and together as inducers of U937 and HL-60 differentiation were compared and then found that LPS and LPS + [1,25(OH)2D3] groups treatment expressed the highest amount of CD14.[17]

Conclusion

According to the our results, it is speculated that CD14 gene expression may be induced in human U937 and HL-60 cell lines by different factors including [1,25(OH)2D3], and LPS.

Acknowledgements

The authors would like to thank the support of immunology Research Center of Tabriz University of medical sciences and kind assistance of who contribute for this research.

Conflict of Interest

The authors report no conflicts of interest.

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