

The U937 cell line induced to express CD14 protein by 1,25-dihydroxyvitamin D3 and be sensitive to endotoxin stimulation

Hai-Zhong Liu, Jian-Ping Gong, Chuan-Xin Wu, Yong Peng, Xu-Hong Li and Hai-Bo You

Chongqing, China

BACKGROUND: CD14 was first described as a differentiation antigen on the surface of myeloid lineage cells. It acts as a glycosylphosphatidylinositol (GPI)-anchored receptor for the complex of lipopolysaccharide (LPS) and plays a key role in the activation of LPS-induced monocytes. The purpose of this study was to observe the expression of CD14 protein and its gene in the human U937 promonocytic cell line when these cells were exposed to 1,25-dihydroxyvitamin D3 (VitD3) and investigate their sensitivity to endotoxin stimulation.

METHODS: U937 cells were exposed to (0.1 μmol) VitD3 for 24 hours and were induced to express the CD14 mRNA gene and CD14 protein, then their responses were observed when they were stimulated with different concentrations of LPS for different time.

RESULTS: The U937 cells induced by VitD3 were found to stably express CD14 mRNA and CD14 protein. And CD14 protein enhanced the sensitivity of U937/CD14 cells to lipopolysaccharide (LPS) stimulation. NF- κ B in U937/CD14 cells can be activated with low concentration of LPS (1 ng/ml-10 ng/ml), the TNF- α mRNA gene was induced, and then TNF- α was produced and released into the supernatant of culture.

CONCLUSION: VitD3 can induce U937 cell to express the CD14 gene and CD14 protein and enhance the response of this type of cells to LPS stimulation.

(*Hepatobiliary Pancreat Dis Int* 2005; 4: 84-89)

KEY WORDS: CD14; lipopolysaccharide;

Author Affiliations: Department of Hepatobiliary Surgery, Second College of Clinical Medicine & Second Affiliated Hospital of Chongqing University of Medical Sciences, Chongqing 400010, China (Liu HZ, Gong JP, Wu CX, Peng Y, Li XH and You HB)

Corresponding Author: Jian-Ping Gong, MD, Department of General Surgery, Second College of Clinical Medicine & Second Affiliated Hospital of Chongqing University of Medical Sciences, Chongqing 400010, China (Tel: 86-23-63766701; Fax: 86-23-63822815; Email: haizhong97@21cn.com, or gongjianping11@hotmail.com)

This work was supported by grants from the National Natural Science Foundation of China (No. 39970719, 30170919).

© 2005, Hepatobiliary Pancreat Dis Int. All rights reserved.

U937 cell line; VitD3; endotoxin stimulation

Introduction

CD14 has been identified as a receptor for lipopolysaccharide (LPS) on the surface of the mononuclear phagocyte system (MPS), and it plays an important role in the activation of LPS-mediated information transmission.^[1-7] Human U937 promonocytic cells as the precursors of MPS have many characteristics of the MPS, and are the important cell line for the study of the MPS *in vitro*.^[8,9] None of the CD14 gene has been detected in the DNA of this cell line. Thus the CD14 gene doesn't express CD14 protein and is not respond to the stimulation of LPS.^[10,11] As an important regulator to induce differentiation and proliferation of various cells, VitD3 may induce U937 cells to express CD14 protein.^[12] The purpose of this study was to observe the expression of CD14 mRNA and CD14 protein in U937 cells when they were exposed to VitD3 and whether they were sensitive to LPS stimulation.

Methods

Reagents

LPS (*Escherichia coli* O111:B4) and VitD3 were purchased from Sigma Chemical Company (St. Louis, Mo., USA). Goat anti-rat CD14 polyclonal antibody and goat anti-rat NF- κ B polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif, USA). Fluorescein isothiocyanate (FITC)-IgG was purchased from Zhongshan Biotechnology Company, Beijing, China. One-way reverse transcription polymerase chain reaction (RT-PCR) kit was bought from Roche Biotechnology Company, Roche, USA. U937 cells were presented by Dr Liu Xin from the Department of Molecular Inheritance, Third Military Medical University, Chongqing, China.

VitD3 inducing U937 cells to express CD14 protein

The method recommended by Jack et al^[13] included the following procedures. U937 cells (1×10^6 /ml) and VitD3 (0.1 μ mol) were added into the bottle of cell culture, which was coated with agarose, then incubated at 37 °C in a CO₂ (5%) cell incubator for 24 hours. The cells induced to express CD14 by VitD3 were U937/CD14 cells, whereas the control group comprised U937/Con1 cells.

Treating cells with LPS

LPS of different concentrations (0 ng/ml, 1 ng/ml, 10 ng/ml, 100 ng/ml and 1000 ng/ml) and U937/CD14 cells or U937/Con1 cells were incubated together respectively for 60 minutes. LPS (1000 ng/ml) and U937/CD14 cells or U937/Con1 cells were incubated together for different minutes (0, 30, 60, and 120 minutes).

Indexes for observation

ELISA method was used in detecting changes of TNF- α levels in culture supernatants of U937 cells. The expression of TNF- α mRNA in U937 cells was shown by the *in situ* hybridization method. The expression of CD14 protein on the surface of U937 cells was detected by laser scanning confocal microscopy. The activity of NF- κ B in nucleus of U937 cells was assessed by the EMSA method, and was shown with relative optical density (ROD) titer \times area (mm²). The expression of CD14 mRNA and TNF- α mRNA in U937 cells was assayed by RT-PCR method. The changes of CD14 protein content on the surface of U937 cells were detected by the Western blot method.

Statistical analysis

The data were expressed as mean \pm standard deviation. Statistical difference was analyzed by means of the analysis of variance (ANOVA) using SAS software. A *P* value less than 0.05 was considered statistically significant, and a *P* value less than 0.01 was considered extraordinarily significant.

Results

Identification of the expression of CD14 in U937 cells

Stained U937 cells with goat anti-CD14 polyclonal antibody and fluorescein isothiocyanate (FITC)-IgG, the expression of CD14 protein in U937 cells was then examined by FCM (Fig. 1). The positive rate of FITC-CD14 cells was 95.40% in the U937/CD14 cells induced by VitD3, whereas it was 4.49% in the control group (U937/Con1 cells).

Changes of TNF- α level in the culture supernatant of U937 cells

Changes of TNF- α levels were observed in the culture supernatant of U937 cells under the stimulation of different concentrations of LPS. In the U937/CD14 group, the level of TNF- α started to increase after stimulation with 10 ng/ml LPS for 60 minutes and continued to increase with the elevation of concentrations of LPS. Extraordinarily significant difference was seen when compared with the 0 point concentration of LPS and the U937/Con1 cells group (*P* < 0.01). The level of TNF- α in the U937/Con1 cells group started to increase only after stimulation with 1000 ng/ml LPS, and significant difference was found when compared with the 0 LPS point concentration (*P* < 0.05, Table 1, Fig. 2).

Table 1. TNF- α levels in the culture supernatant of U937 mediated with various concentrations of LPS for 60 minutes

| Group (n=3) | TNF- α levels (pg/ml) | | | |
|-------------|------------------------------|----------------------|----------------------|-----------------------|
| | 0 ng/ml | 10 ng/ml | 100 ng/ml | 1000 g/ml |
| U937/CD14 | 48 \pm 7.74 | 167.43 \pm 25.53 * | 254.48 \pm 34.72 * | 394.73 \pm 59.58 * |
| U937/Con1 | 49 \pm 8.57 | 55.31 \pm 8.44 | 67.32 \pm 10.46 | 133.47 \pm 16.69 ** |

*: *P* < 0.0, 1 vs U937/Con1 or 0 point; **: *P* < 0.05, vs 0 point.

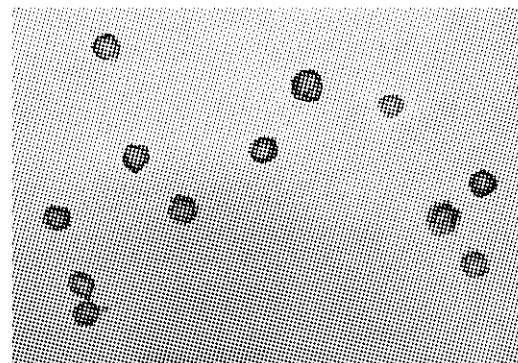


Fig. 1. U937 cells stained with goat anti-CD14 polyclonal antibody (SP, original magnification \times 400).

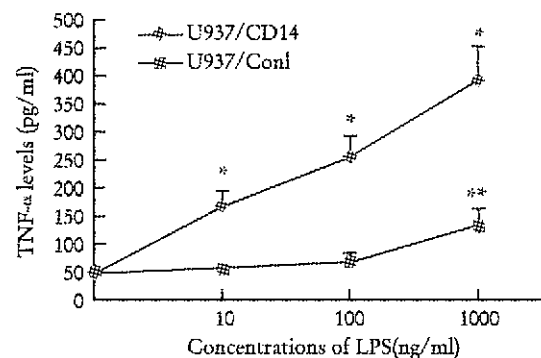


Fig. 2. TNF- α levels in the culture supernatant of U937 cells mediated with various concentrations of LPS for 60 minutes. *: *P* < 0.01, vs U937/Con1 or 0 point; **: *P* < 0.05, vs 0 point.

Table 2. TNF- α levels in the culture supernatant of U937 mediated with 1000 ng/ml of LPS for various times

| Group (n=3) | TNF- α levels (pg/ml) | | | |
|-------------|--------------------------------|----------------------|-----------------------|-----------------------|
| | 0 min | 30 min | 60 min | 120 min |
| U937/CD14 | 49 \pm 7.47 | 173.79 \pm 22.36 * | 258.64 \pm 38.25 * | 463.57 \pm 63.43 * |
| U937/Con1 | 48 \pm 6.59 | 63.42 \pm 10.54 | 134.49 \pm 16.46 ** | 157.38 \pm 22.05 ** |

* : P<0.01, vs U937/Con1 or 0 point; ** : P<0.05, vs 0 point.

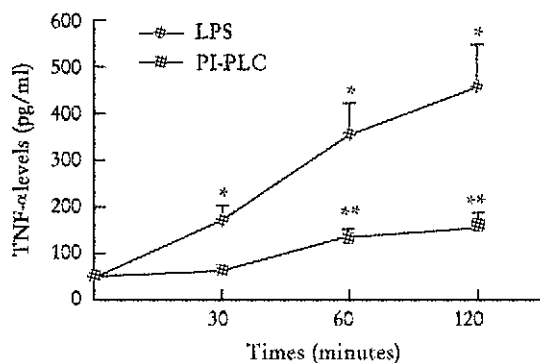


Fig. 3. TNF- α levels in the culture supernatant of U937 cells mediated with 1000 ng/ml of LPS for various times. * : P < 0.01, vs U937/Con1 or 0 point; ** : P < 0.05, vs 0 point.

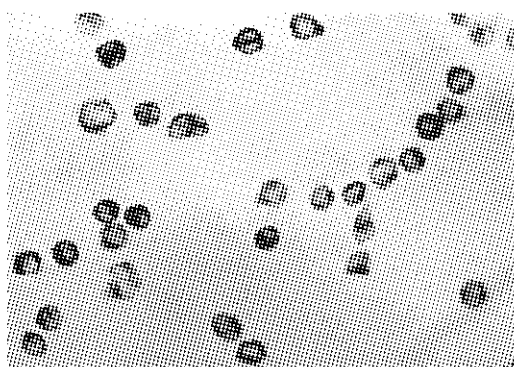


Fig. 4A. Result of *in situ* hybridization of TNF- α mRNA in U937/CD14 cells (original magnification \times 400). The expression of TNF- α mRNA in U937/CD14 cells was positive and was mainly distributed in cytoplasm.

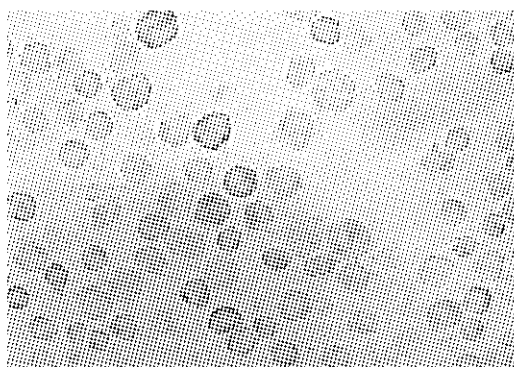


Fig. 4B. Result of *in situ* hybridization of TNF- α mRNA in U937/Con1 cells (original magnification \times 400). The expression of TNF- α mRNA in U937/Con1 cells was not obvious.

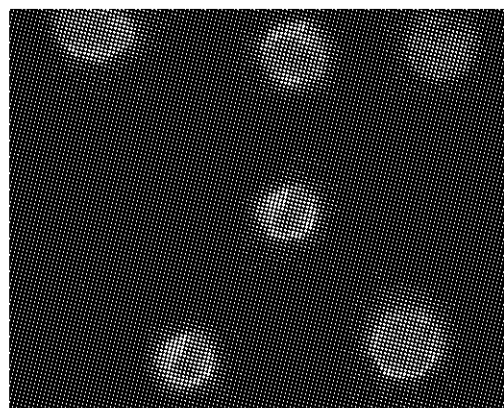


Fig. 5. CD14 protein on the surface of U937/CD14 cells expressed strong positive after the stimulation of LPS.

Changes of TNF- α levels in the culture supernatant of U937 cells at different time under the stimulation of LPS. Under the stimulation of 1000 ng/ml LPS, the level of TNF- α in the U937/CD14 group began to increase after 30 minutes, and continued to increase with time. Extraordinary significant difference was observed when compared with the 0 LPS point concentration and the U937/Con1 group (P<0.01). But in the U937/Con1 group, treated with the same concentrations of LPS, the level of TNF- α started to increase after 60 minutes, and increased significantly after 120 minutes. Significant difference was seen when compared with the 0 LPS point concentration (P<0.05, Table 2, Fig. 3).

In situ hybridization of TNF- α mRNA in U937 cells

In the U937/CD14 group, the expression of TNF- α mRNA was obvious after stimulation of 10 ng/ml LPS, and became more obvious after stimulation of 1000 ng/ml LPS, TNF- α mRNA was mainly distributed in the cytoplasm. But in the U937/Con1 group, TNF- α mRNA was expressed considerably after stimulation of 1000 ng/ml LPS. The expression of TNF- α mRNA was low without LPS stimulation (0 LPS point) in the U937/CD14 group and U937/Con1 group, and also the positive response was not significant (Fig. 4A,B).

Expression of CD14 on the surface of U937 cells under laser scanning confocal microscopy

After 60 minutes, the stimulation of 10 ng/ml LPS, the expression of CD14 on the cell surface in the U937/CD14 group was positive, showing that the surface was stained, and it was more significant after stimulation of 1000 ng/ml LPS. In the U937/Con1 group, however, the positive response was not significant after stimulation of 10 ng/ml LPS, CD14 was expressed considerably under the stimulation of 1000 ng/ml LPS (Fig. 5).

Changes of relative activity of NF- κ B in the nuclei of U937 cells

Table 3. Quantification of NF-κB in nuclear extracts of U937 cells mediated by different concentrations of LPS (ROD×mm²) (n=3)

| Group | 0 ng/ml | 1 ng/ml | 10 ng/ml | 100 ng/ml | 1000 ng/ml |
|-----------|-----------|------------|------------|------------|-------------|
| U937/CD14 | 0.14±0.2 | 2.37±0.32* | 3.12±0.42* | 4.49±0.65* | 4.38±0.72* |
| U937/Con1 | 0.17±0.15 | 0.24±0.34 | 0.35±0.22 | 0.54±0.21 | 4.26±0.42** |

*: P<0.01, vs U937/Con1 or 0 point; **: P<0.05, vs 0 point.

Table 4. Expression of CD14 mRNA by U937 cells mediated with various concentrations of LPS for 60 minutes(ROD×mm²) (n=3)

| Group | 0 ng/ml | 10 ng/ml | 100 ng/ml | 1000 ng/ml |
|-----------|------------|------------|------------|-------------|
| U937/CD14 | 3.69±0.19* | 5.84±0.92* | 8.92±1.37* | 13.59±2.46* |
| U937/Con1 | 1.56±0.25 | 1.65±0.18 | 2.25±0.42 | 7.84±1.52** |

*: P<0.01, vs U937/Con1; **: P<0.05, vs 0 point.

Table 5. Expression of TNF-α mRNA by U937 cells mediated with various concentrations of LPS for 60 minutes(ROD×mm²) (n=3)

| Group | 0 ng/ml | 10 ng/ml | 100 ng/ml | 1000 ng/ml |
|-----------|-----------|-------------|-------------|--------------|
| U937/CD14 | 2.54±0.37 | 13.78±2.41* | 35.45±4.36* | 39.51±5.83* |
| U937/Con1 | 2.35±0.41 | 2.87±0.45 | 4.84±7.02** | 10.27±1.62** |

*: P<0.01, vs U937/Con1 or 0 point; **: P<0.05, vs 0 point.

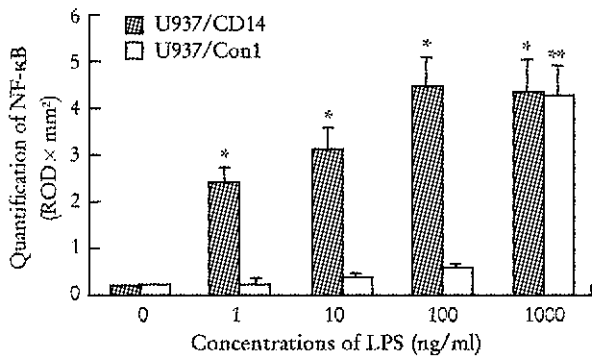


Fig. 6. Quantification of NF-κB in nuclear extracts of U937 cells mediated by different concentrations of LPS. *: P < 0.01, vs U937/Con1 or 0 point; **: P < 0.05, vs 0 point.

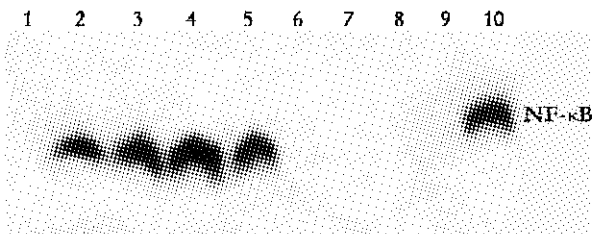


Fig. 7. Changes of relative activity of NF-κB in nucleus of U937 cells. Lanes 1, 6; 0 concentration LPS point in the U937/Con1 group; Lanes 2-5; after stimulation of different concentrations of LPS (1 ng/ml, 10 ng/ml, 100 ng/ml and 1000 ng/ml) in the U937/CD14 group; Lanes 7-10; after stimulation of different concentrations of LPS (1 ng/ml, 10 ng/ml, 100 ng/ml and 1000 ng/ml) in the U937/Con1 group.

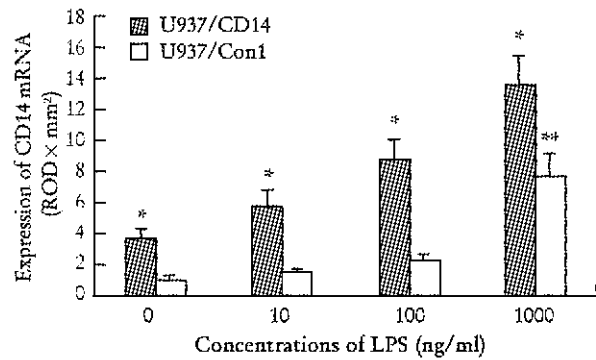


Fig. 8. Expression of CD14 mRNA by U937 cells mediated with various concentrations of LPS for 60 minutes. *: P < 0.01, vs U937/Con1; **: P < 0.05, vs 0 point.

Under the stimulation of 1 ng/ml LPS, the activity of NF-κB began to increase in the nuclei of U937 cells, and continued to increase with the increase of concentration of LPS. Their ROD titers were markedly higher than those of the 0 LPS point and the U937/Con1 group (P < 0.01). In the U937/Con1 group, the activity of NF-κB in the nucleus was not significant after the stimulation of LPS at the dose from 1 ng/ml to 100 ng/ml. Only when the LPS concentration reached 1000 ng/ml, did the cells express their activity of NF-κB, and their relative ROD titers were significantly different from those of the 0 LPS point (P < 0.05, Table 3, Figs 6,7).

Expression of CD14 mRNA and TNF-α mRNA in U937 cells

The PCR product of CD14 mRNA and TNF-α mRNA in U937 cells were 267bp and 692bp in length, respectively. CD14 mRNA was expressed considerably at the 0 point concentration of LPS in the U937/CD14 group. After the stimulation of 10 ng/ml LPS for 60 minutes, the expression of CD14 mRNA was increased significantly, continued to increase with the elevation of concentration of LPS, and peaked after stimulation of 1000 ng/ml LPS. Extraordinary significant difference was seen when compared to the U937/Con1 group (P < 0.01). The expression of TNF-α mRNA in the U937/CD14 group was low at the 0 LPS point concentration, but increased significantly after the stimulation of 10 ng/ml LPS, and continued to increase with the elevation of LPS concentration. Extraordinarily significant difference was noted compared to the U937/Con1 group (P < 0.01). No significant expression of CD14 mRNA was found at the 0 LPS point concentration in the U937/Con1 group, and TNF-α mRNA was low expressed. The expression of CD14 mRNA and TNF-α mRNA was increased after the stimulation of 100 ng/ml LPS, and increased significantly after the stimulation of 1000 ng/ml LPS. There were significant differences as compared to the 0 point concentration of LPS (P < 0.05, Table 4,5, Figs 8,9,10A,B).

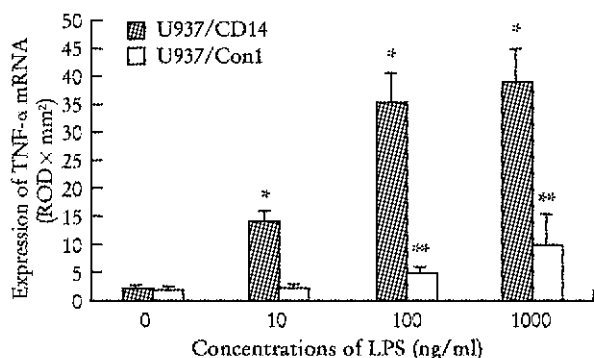


Fig. 9. Expression of TNF- α mRNA by U937 cells mediated with various concentrations of LPS for 60 minutes. *: $P < 0.01$, vs U937/Con1 or 0 point; **: $P < 0.05$, vs 0 point.

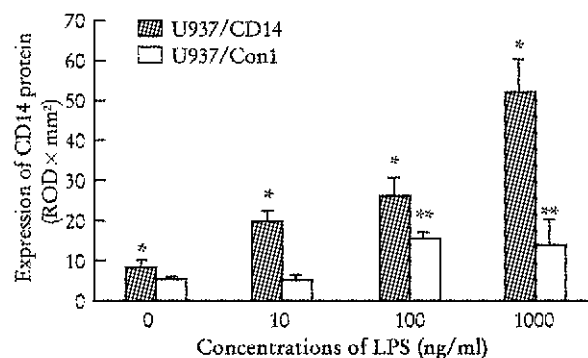


Fig. 11. Expression of CD14 protein by U937 cells mediated with varying concentrations of LPS. *: $P < 0.01$ vs U937/Con1; **: $P < 0.05$, vs 0 point.

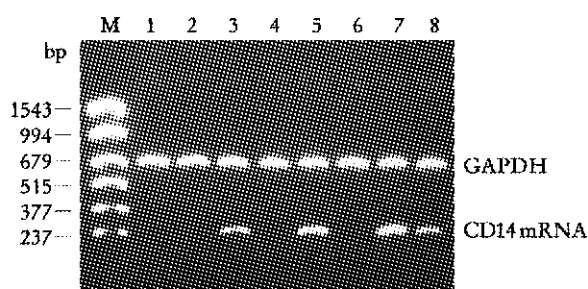


Fig. 10A. Expression of CD14 mRNA in U937 cells. M: Marker; Lanes 1, 2: 0 concentration LPS point in the U937/Con1 group. Lanes 3, 5, 7: after stimulation of different concentrations of LPS (10 ng/ml, 100 ng/ml, and 1000 ng/ml) in the U937/CD14 group. Lanes 4, 6, 8: after stimulation of different concentrations of LPS (10 ng/ml, 100 ng/ml, and 1000 ng/ml) in the U937/Con1 group.

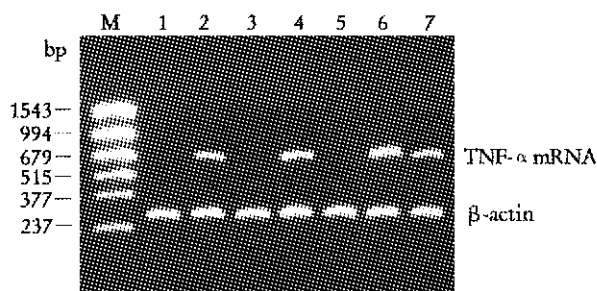


Fig. 10B. Expression of TNF- α mRNA in U937 cells. M: Marker; Lanes 2, 4, 6: after stimulation of different concentrations of LPS (1 ng/ml, 10 ng/ml, 100 ng/ml, and 1000 ng/ml) in the U937/CD14 group. Lanes 1, 3, 5, 7: after stimulation of different concentrations of LPS (0, 1 ng/ml, 10 ng/ml, 100 ng/ml, and 1000 ng/ml) in the U937/Con1 group.

Table 6. Expression of CD14 protein by U937 cells mediated with various concentrations of LPS (ROD \times mm²)

| Group | 0 ng/ml | 10 ng/ml | 100 ng/ml | 1000 ng/ml |
|-----------|------------------|-------------------|--------------------|--------------------|
| U937/CD14 | 8.69 \pm 0.94* | 22.36 \pm 1.62* | 26.69 \pm 3.27* | 52.56 \pm 4.78* |
| U937/Con1 | 5.72 \pm 1.03 | 5.78 \pm 0.73 | 16.02 \pm 0.94** | 28.55 \pm 2.37** |

*: $P < 0.01$, vs U937/Con1; **: $P < 0.05$, vs 0 point.

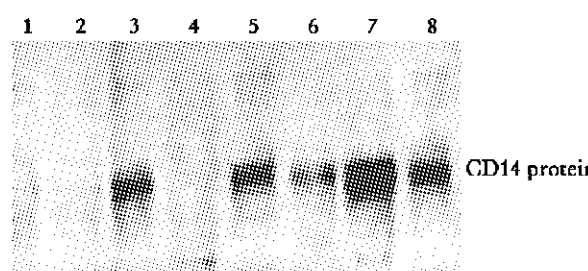


Fig. 12. Western blot analysis of CD14 protein on the surface of U937 cells. Lanes 1, 3, 5, 7: after stimulation of different concentrations of LPS (0, 10 ng/ml, 100 ng/ml, 1000 ng/ml) in the U937/CD14 group; Lanes 2, 4, 6, 8: after stimulation of different concentrations of LPS (0, 10 ng/ml, 100 ng/ml, 1000 ng/ml) in the U937/Con1 group.

Western blot analysis of CD14 protein

CD14 protein was found as a single ribbon in U937 cells by Western blot analysis, and its molecular weight was 60 Kda. It was expressed considerably at the 0 concentration point of LPS in the U937/CD14 group. The expression was increased after the stimulation of 10 ng/ml LPS, and continued to increase with the elevation of the concentration of LPS peaked at last after the stimulation of 1000 ng/ml LPS. Extraordinarily significant difference was found when compared with the U937/Con1 group ($P < 0.01$). CD14 protein in the U937/Con1 group was expressed considerably after the stimulation of 100 ng/ml LPS, and expressed significantly after the stimulation of 1000 ng/ml LPS. Significant difference was shown when compared with the 0 point concentration of LPS ($P < 0.05$, Table 6, Figs 11,12).

Discussion

Human U937 promonocytic cells as the precursors of the mononuclear phagocyte system (MPS) are the important cell line to study the MPS *in vitro*. Since the CD14 gene is absent in the DNA of this cell line, CD14 pro-

tein is not expressed, and the reactivity of the cell line to the stimulation of LPS is about 1000 times lower than other MPS members.^[10,11] VitD3 can induce U937 cells expressing the CD14 gene, then let their cytomembrane produce CD14 protein stably, and make them take reaction to the stimulation of LPS. The results of this study showed that the way inducing U937/CD14 cells to express CD14 protein is a stable, reliable and practicable method, and it can satisfy the demands of experiments.

VitD3 is an important regulator of inducing differentiation and proliferation of many cells, and it can induce U937 cells to express CD14 protein. Some researchers^[13] have reported that VitD3 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. The U937/CD14 cells induced to express CD14 protein by VitD3 can increase the adherence to endothelial cells, induce reaction to LPS stimulation, produce and release various kinds of cytokines.^[14] In this study, VitD3 induced 95.40% of U937 cells to express CD14 protein in 24 hours, and can evoke reaction to the stimulation of low concentration of LPS, expressed TNF- α mRNA, and produced TNF- α cytokines. RT-PCR analysis suggested that VitD3 induce U937 cells to express CD14 protein at the gene level, because the expression of CD14 mRNA is increased more significantly in U937/CD14 cells than in U937/Con1 cells.

The mechanism by which VitD3 induces U937 cells to express CD14 protein is unclear. In the present study, the induction of VitD3 to U937/CD14 cells was realized at the gene level through their nucleus receptor.^[15,16] VitD3 receptor VDR is one of the members of the steroid receptor family. Gene map analysis of CD14 protein has shown that its gene is located on the fifth chromosome, which is the gene site of many growth factors and their receptors. Zhang et al^[15] found that some intermediate molecules are required in the process of inducing U937 cells to express the CD14 gene, shown by VitD3 using FCM, Northern blotting and run-on transcription analysis. They detected the inducing process needed for the synthesis of a new protein called SP1, and found that the sequence of bp-128 to -70 in SP1 DNA is essential to inducing U937 cells to express CD14 protein by VitD3. Nakajima et al^[17] reported that retinoic acid can increase the expression of CD14 in U937 cells after VitD3 treatment. This aspect needs further study.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

- Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995;13:437-457.
- Fenton MJ, Golenbock DT. LPS-binding proteins and receptors. *J Leukoc Biol* 1998;64:25-32.
- Koo DJ, Chaudry IH, Wang P. Kupffer cells are responsible for producing inflammatory cytokines and hepatocellular dysfunction during early sepsis. *J Surg Res* 1999; 83:151-157.
- Gong JP, Liu CA, Wu CX, Li SW, Shi YJ, Li XH. Nuclear factor κ B activity in patients with acute severe cholangitis. *World J Gastroenterol* 2002;8:346-349.
- Parker SJ, Watkins PE. Experimental models of gram-negative sepsis. *Br J Surg* 2001;88:22-30.
- Li SW, Gong JP, Wu CX, Shi YJ, Liu CA. Lipopolysaccharide induced synthesis of CD14 protein and its gene expression in hepatocytes during endotoxemia. *World J Gastroenterol* 2002;8:124-127.
- Gong JP, Dai LL, Liu CA, Wu CX, Shi YJ, Li SW, et al. Expression of CD14 protein and its gene in liver sinusoidal endothelial cells during endotoxemia. *World J Gastroenterol* 2002;8:551-554.
- Kim J, Feldman RA. Activated Fes protein tyrosine kinase induces terminal macrophage differentiation of myeloid progenitors (U937 cells) and activation of the transcription factor PU. 1. *Mol Cell Biol* 2002;22:1903-1918.
- Hallbeck AL, Walz TM, Wasteson A. Interleukin-6 enhances transforming growth factor- α mRNA expression in macrophage-like human monocytoid (U-937-1) cells. *Biosci Rep* 2001;21:325-339.
- Fan X, Stelter F, Menzel R, Jack R, Spreitzer I, Hartung T, et al. Structure in *Bacillus subtilis* are recognized by CD14 in a lipopolysaccharide binding protein-dependent reaction. *Infect Immun* 1999;67:2964-2968.
- Oberg F, Botling J, Nilsson K. Functional antagonism between vitamin D3 and retinoic acid in the regulation of CD14 and CD23 expression during monocytic differentiation of U-937 cells. *J Immunol* 1993;150:3487-3495.
- Landmann R, Link S, Sansano S, Rajacic Z, Zimmerli W. Soluble CD14 activates monocytic cells independently of lipopolysaccharide. *Infect Immun* 1998;66:2264-2271.
- Jack RS, Gruunwald U, Stelter F, Workalemahu G, Schutt C. Both membrane-bound and soluble forms of CD14 bind to gram-negative bacteria. *Eur J Immunol* 1995;25:1436-1441.
- Tenno T, Oberg F, Nilsson K, Siegbahn A. Induction of differentiation in U-937 and NB4 cells is associated with inhibition of tissue factor production. *Eur J Haematol* 1999;63:112-119.
- Zhang DE, Hetherington CJ, Gonzalez DA, Chen HM, Tenen DG. Regulation of CD14 expression during monocytic differentiation induced with 1 α ,25-dihydroxyvitamin D3. *J Immunol* 1994;153:3276-3283.
- Tomura K, Narumi S. Differential induction of interferon (IFN)-inducible protein 10 following differentiation of a monocyte, macrophage cell lineage is related to the changes of nuclear proteins bound to IFN stimulus response element and kappaB sites. *Int J Mol Med* 1999;3:477-84.
- Nakajima H, Kizaki M, Ueno H, Muto A, Takayama N, Matsushita H. All-trans and 9-cis retinoic acid enhance 1,25-dihydroxyvitamin D3-induced monocyte differentiation of U937 cells. *Leuk Res* 1996;20:665-676.

Received September 16, 2004

Accepted after revision December 1, 2004