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FEMS Microbiology Letters 178 (1999) 235–239

FEMS MICROBIOLOGY LETTERS

Production of tissue factor in CD14-expressing human umbilical vein endothelial cells by lipopolysaccharide

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Received 13 May 1999; received in revised form 9 July 1999; accepted 16 July 1999

Abstract

Production of tissue factor (TF) in response to lipopolysaccharide (LPS) was examined in human umbilical vein endothelial cells (HUVECs) transfected with human CD14 DNA. The expression of CD14 on HUVECs dramatically enhanced the production of TF at a low concentration of LPS in the absence of fetal calf serum (FCS). On the other hand, mock-transfected HUVECs did not respond to even a high concentration of LPS. TF production in CD14-expressing HUVECs was significantly inhibited by anti-CD14 monoclonal antibody. Addition of FCS to the culture of CD14-expressing HUVECs markedly augmented the LPS-induced TF production, whereas only a marginal effect was observed in mock-transfected HUVECs. The findings suggested that the integration of membrane CD14 rendered HUVECs highly sensitive to LPS in the production of TF irrespective of the presence of FCS.

Keywords: Tissue factor; CD14; Human umbilical vein endothelial cell; Lipopolysaccharide; Endotoxin

1. Introduction

Tissue factor (TF) is the primary initiator protein on the cell surface, which promotes blood coagulation via the extrinsic pathway [1,2]. TF-initiated coagulation disorders are commonly associated with Gram-negative sepsis [3,4]. The expression of TF on the surface of vascular endothelial cells and monocytes is closely linked to the initiation of the coagulation disorders [5,6]. Bacterial lipopolysaccharide (LPS) induces the expression of TF on vascular endothelial cells [7–9]. Soluble CD14 in the serum seems to play a pivotal role in LPS-induced TF expression of vascular endothelial cells since they do not express membrane CD14 normally [10–13]. It is of interest to determine how the integration of membrane CD14 into vascular endothelial cells affects LPS-induced TF expression. In this communication, we have investigated the effect of integrated membrane CD14 on LPS-induced TF expression of human umbilical vein endothelial cells (HUVECs).

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0378-1097/99/$20.00
PII: S0378-1097(99)00362-6

2. Materials and methods

2.1. Cells and cell culture

HUVECs were obtained from Clonetics (Walkersville, MD, USA) and were processed according to the manufacturer's instructions. They were maintained in endothelial growth medium (Clonetics) and cells from passage three to 10 were used for the experiments.

2.2. Expression of human CD14 on HUVECs

The plasmid encoding human CD14 and the glycophosphatidyl inositol attachment site from decay accelerating factor was a kind gift from Dr R.J. Ulevitch, the Scripps Research Institute, San Diego, CA, USA. HUVECs were transfected with the human CD14 plasmid or the empty vector by the method using lipofectin reagent (Gibco BRL, Grand Island, NY, USA). Transfectants were monitored routinely for the expression of CD14 by immunoblotting or immunofluorescence. The soluble form of human CD14 was not detected in the culture supernatant of transfectants with immunoblotting.

2.3. LPS stimulation and measurement of TF

CD14- or mock-transfected HUVECs were stimulated with various concentrations of LPS (*Escherichia coli* O55:B5, Difco Laboratories, Detroit, MI, USA) in 12-well plastic plates. In some experiments, 1% native or heat-inactivated fetal calf serum (FCS) from Gibco BRL was supplemented. Heat inactivation was performed at 60°C for 30 min. At various time points after LPS treatment, cells were lysed by phosphate-buffered saline containing 1% Triton X-100. TF in the cell lysates was measured by a TF ELISA kit (American Diagnostica, Greenwich, CT, USA) according to the manufacturer's instructions. Enzymatic activity of TF was determined by factor X activation using S-222 as the substrate as described elsewhere [14]. S-222 was obtained from Daiichi Chemicals, Tokyo, Japan. Factor VIIa, X and Xa were purchased from Sigma Chemical (St. Louis, MT, USA). The amount and functional activity of TF were expressed as the mean value of triplicates ± S.D.

3. Results

3.1. Transfection of HUVECs with human CD14 plasmid initiated TF production in a serum-independent fashion

HUVECs were transfected with either human CD14 plasmid or vector alone. The expression of CD14 on the transfected HUVECs was confirmed by an immunoblotting method (Fig. 1). TF production in CD14-expressing HUVECs was determined by cultivation with LPS (10 μg ml^{-1}) in the absence of FCS. As shown in Fig. 2A, LPS induced a dramatic increase in TF production of CD14-expressing HUVECs. TF production gradually increased from 1 to 8 h after LPS stimulation and decreased after 12 h. Mock-transfected cells did not show any increment in TF production. There was a close parallelism between the TF amount and functional TF activity as determined by factor Xa generation (Fig. 2B).

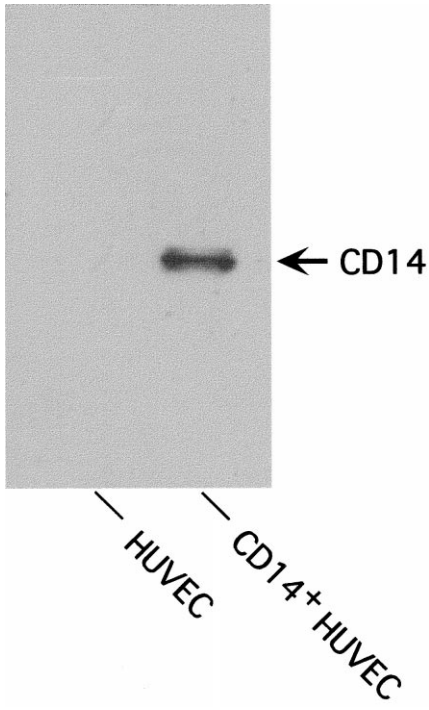

Fig. 1. Detection of CD14 on HUVECs transfected with human CD14 DNA. The expression of CD14 was detected by the immunoblotting method with anti-CD14 monoclonal antibody.

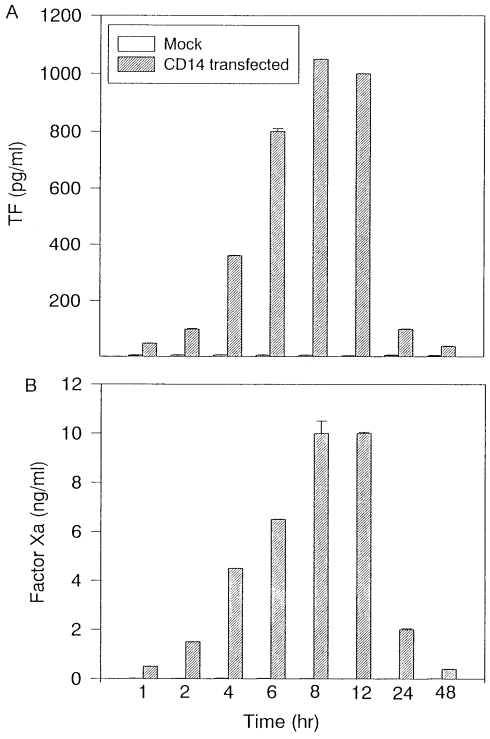

Fig. 2. Time course of TF production in CD14-expressing HUVECs in response to LPS. CD14- or mock-transfected HUVECs were stimulated with LPS (10 μg ml^{-1}) in the absence of FCS. The TF amount (A) and functional TF activity (B) were determined various hours after LPS stimulation. Data are expressed as the mean values of triplicates ± S.D. of five independent experiments.

3.2. *CD14-expressing HUVECs were susceptible to a low concentration of LPS*

TF production of CD14-expressing HUVECs at various concentrations of LPS was examined in the absence of FCS (Fig. 3). Expression of membrane CD14 rendered HUVECs susceptible to as low as 0.001 μg ml^{-1} LPS. TF production of CD14-expressing HUVECs increased in a dose-dependent fashion within a range from 0.001 to 10 μg ml^{-1} LPS. On the other hand, mock-transfected HUVECs did not show TF production at any concentration of LPS.

3.3. *Anti-CD14 monoclonal antibody reduced LPS-induced TF expression on CD14-expressing HUVECs*

To verify the participation of membrane CD14 in the LPS-induced TF response, CD14-transfected HUVECs were pretreated with anti-CD14 monoclonal antibody (MEM-18, Monosan, Uden, The Netherlands) for 5 h, followed by the addition of LPS (1 μg ml^{-1}). The experimental result is shown in Fig. 4. Pretreatment with anti-CD14 monoclonal antibody (MEM-18), which is known to inhibit the binding of LPS to CD14 [15], significantly reduced the TF production on CD14-expressing HUVECs (approximately 90% reduction at 10 μg ml^{-1} of the antibody).

3.4. *FCS augmented LPS-induced TF expression on HUVECs*

The effect of FCS on LPS-induced TF expression of HUVECs was investigated (Fig. 5). The addition of native FCS to CD14-expressing HUVEC cultures at the concentration of 1% resulted in a 5-fold en-

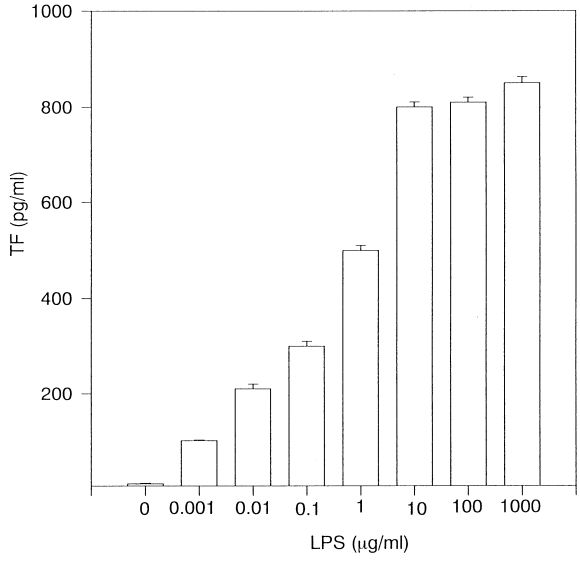

Fig. 3. Dose-dependent effect of LPS on TF production in CD14-expressing HUVECs. CD14-expressing HUVECs were stimulated with various concentrations of LPS for 6 h in the absence of FCS. The amount of TF is expressed as the mean value of triplicates ± S.D. of three independent experiments.

hancement of the LPS-induced TF response. However, the addition of heat-inactivated FCS induced the increase in TF production only 1.3-fold compared to that of CD14-expressing HUVECs without the addition of FCS (Fig. 5). Some heat-labile molecule(s) in FCS seemed to participate in the LPS-induced TF response. The addition of native FCS into the cultures of mock-transfected HUVECs induced only a weak TF response. The maximum level observed in mock-transfected cells was at most 200 pg ml^{-1}, even in the presence of 1 μg ml^{-1} LPS.

4. Discussion

In the present study, we demonstrated that the integration of membrane CD14 to HUVECs rendered those cells highly susceptible to LPS and induced a high level expression of functional TF on the cells. Moreover, a marked augmentation was observed by the addition of serum. It was, therefore, suggested that cell surface CD14 triggered the maximal TF response of HUVECs with the help of serum factors. CD14-negative HUVECs also exhibited a weak TF response in the presence of serum, suggesting the importance of serum factors. These findings are consistent with the reports that soluble CD14 present in serum plays a pivotal role in the TF response of vascular endothelial cells [10–13]. It was of particular interest that the integration of membrane CD14 resulted in higher TF expression than that observed by the addition of serum containing soluble CD14. This might suggest that membrane CD14 was more effective in the presentation of LPS to HUVECs than soluble CD14. The present result was consistent with the finding reported by Nishijima et al. [16] that small amounts of LPS do not activate CD14-negative macrophage-like cell mutants. Our idea was also supported by the fact that transfection of CD14 to pre-B-cells dramatically enhanced the sensitivity to LPS [17]. However, the molecule recognizing LPS subsequently after the interaction of LPS with membrane CD14 on HUVECs is still unclear.

Vascular endothelial cells are normally CD14-negative. However, it has been reported that microvascular endothelial cells from patients with adult respiratory distress syndrome or cerebral malaria

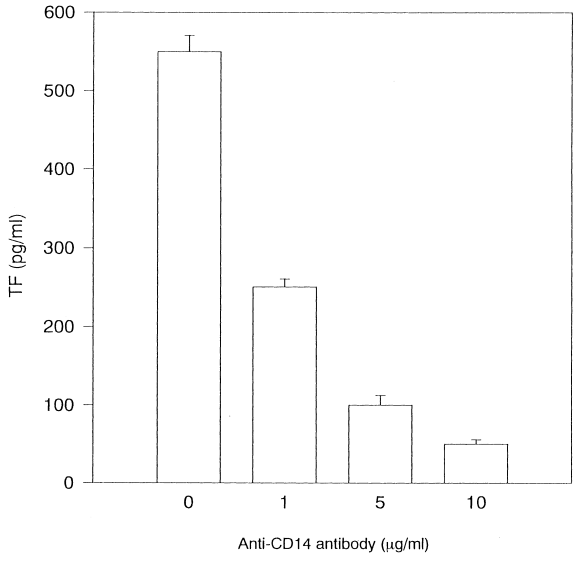

Fig. 4. Inhibitory action of anti-CD14 monoclonal antibody on LPS-induced TF production in CD14-expressing HUVECs. CD14-expressing HUVECs were pretreated with various concentrations of anti-CD14 antibody for 5 h, followed by the addition of LPS (1 μg ml^{-1}). The amount of TF is expressed as the mean value of triplicates ± S.D. of four independent experiments.

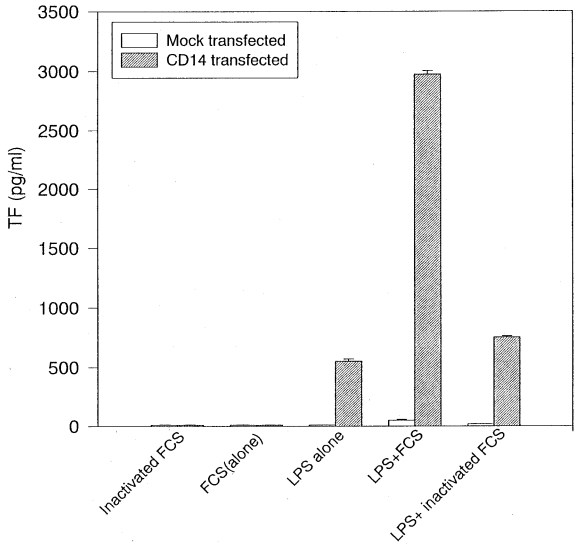

Fig. 5. Augmentation of the LPS-induced TF response in HUVECs by the addition of FCS. CD14- or mock-transfected HUVECs were stimulated with LPS (1 μg ml^{-1}) in the presence of 1% native or heat-inactivated FCS for 6 h. The amount of TF is expressed as the mean value of triplicates ± S.D. of three independent experiments.

express CD14 on their surface [18,19]. The present study indicated the possibility that the appearance of membrane CD14 on vascular endothelial cells might lead to excessive TF-induced coagulation in response to small amounts of LPS. This process might be involved in the pathogenesis of LPS-associated coagulation disorders.

In conclusion, this is the first study demonstrating that the expression of membrane CD14 on HUVECs plays a critical role in the LPS-induced TF response. On the other hand, the absence of membrane CD14 on vascular endothelial cells might protect them from over-activation or damage by a small amount of LPS.

Acknowledgements

This work was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. We thank A. Morikawa and K. Takahashi for their technical assistance.

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