GPI-linked endothelial CD14 contributes to the detection of LPS

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Lloyd, Katie L., and Paul Kubes. GPI-linked endothelial CD14 contributes to the detection of LPS. Am J Physiol Heart Circ Physiol 291: H473–H481, 2006. First published January 27, 2006; doi:10.1152/ajpheart.01234.2005.—The inflammatory endothelial response to LPS is critical to the host’s surviving a gram-negative bacterial infection. In this study we investigated whether human endothelial cells express the functional coreceptor for LPS, CD14, and, most importantly whether it is glycosylphosphatidylinositol (GPI) linked. We also examined whether plasma proteins could reconstitute an LPS response in CD14-inhibited endothelium. RT-PCR- and CD14-specific MAbs demonstrated CD14 expression on primary human umbilical vein endothelial cells (HUVEC) but not passaged HUVEC. The amino acid sequence of endothelial CD14 was 99% homologous to CD14 on monocytes. Endothelium responded to relatively low levels of LPS in the absence of plasma, and this was entirely dependent on CD14. Removal of GPI-linked proteins with phosphatidylinositol-phospholipase C prevented LPS detection and subsequent protein synthesis (E-selectin expression). Endothelial CD14 was sufficient to initiate functional leukocyte recruitment, an event inhibited by blocking its LPS binding epitope and also by removing CD14 from the endothelial surface. Plasma proteins restored only ~30% of the LPS response in CD14-inhibited endothelium. In conclusion, our results strongly support an important role for endothelial membrane CD14 in the activation of endothelium for leukocyte recruitment.

LPS is a major outer membrane constituent of gram-negative bacteria, highly immunogenic and shed during infection. This shed LPS is used as a detection system for local infection by our immune system, leading to important inflammatory responses, including leukocyte recruitment. However, under some conditions, LPS can enter the circulation, causing a systemic inflammatory response that is detrimental to the host. The human immune system is able to detect LPS by its trimeric protein sequence is 99% identical to monocyte CD14. Moreover, LPS is lost on passaging. Last, we demonstrate that primary HUVEC can synthesize and express CD14 reconstitute GPI-anchored proteins reconstituted ~30% of the LPS response in CD14-inhibited endothelium. We therefore suggest that GPI-linked CD14 is present on endothelium and that its presence is important to the endothelial response to LPS.

MAP MATERIALS AND METHODS

Reagents. CD14 antibody used for ELISA and blocking studies was unconjugated clone MEM-18 monoclonal antibody supplied by Hydult Biotechnology. The isotype (IgGl) control MOPC-21 was from

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Sigma (St. Louis, MO). FITC-conjugated CD14 antibody (clone LeuM3) and phycoerythrin (PE)-conjugated CD18 and CD54 (intercellular adhesion molecule, ICAM-1) and their matched IgG2a isotype controls were used for the flow cytometry data and supplied by Becton Dickinson Biosciences. The E-selectin MAb clone 1.2B6 (IgG1 isotype) for the E-selectin ELISA was purchased from DAKO, and peroxidase-labeled secondary antibody was obtained from Kirkegaard and Perry Laboratories. The human monoblastic leukemia cell line U937 and the EBV negative lymphoma B cell line BJAB were generous donations from Dr. S. Robbins and Dr. J. Deans, respectively (Univ. of Calgary, Calgary, Alberta, Canada). The U937 cells were matured with 50 ng/ml of PMA, and adherent cells were collected after 2 days’ incubation. Smooth E. coli O111:B4 LPS was purchased from Calbiochem.

**HUVEC isolation.** HUVEC were isolated from freshly obtained umbilical cords, as previously described (20). In brief, umbilical cord veins were rinsed of blood products with warm PBS, pH 7.4, and the vein was filled with warm collagenase A (Roche Diagnostics; used at a concentration of 320 U/ml in PBS). After 20 min of incubation in warm PBS, the digest was collected into centrifuge tubes containing heat-inactivated FBS (supplied by Hyclone) to neutralize the collagenase activity, and cells were pelleted. The supernatant was discarded, and the pellet was resuspended in M199 medium supplemented with 20% FBS, l-glutamine, and an antibiotic cocktail (all supplied from Gibco), but with no endothelial mitogen. The cells were seeded into bovine fibronectin (Biomedical Technologies)-coated T25 culture flasks and grown to confluence in a 37°C humidified incubator for 4–5 days. Cells were heavily seeded to minimize cell growth and thereby permit HUVEC to express CD14, which is otherwise lost. Consequently, only first passaged HUVEC were used for all experiments.

**Passaged HUVEC.** Confluent HUVEC were removed from primary fibronectin-coated culture flasks by trypsinizing (0.05% Trypsin-EDTA; Gibco) for 30–100 s followed by the addition of M199 medium containing 10% FBS. The cells were washed, and the pellet was resuspended in passage M199 medium (M199 medium with antibiotic cocktail plus 10% FBS and endothelial mitogen) and seeded into new fibronectin-coated culture tissue flasks until confluent. HUVEC passing was made by using population doubling, i.e., passage of one flask into two flasks. This process was repeated twice more for third-passage HUVEC.

**ELISA for cell surface molecules.** ELISAs were carried out as described (20). In brief, HUVEC were seeded at confluence into fibronectin-coated wells in a sterile 48-well plate (Costar, NY). Once described (20). In brief, HUVEC were seeded at confluence into new fibronectin-coated culture tissue flasks until confluent.

**Flow cytometry.** Cells were removed from culture flasks by using 0.05% Trypsin-EDTA and washed in cold PBS. Cells were aliquoted into 1 × 10^6 cells per flow tube and kept on ice. Appropriate antibodies (20 μl per flow tube) were added to the cells and incubated in the dark and on ice for 30 min. Cells were then washed with cold PBS and either fixed with 1% formaldehyde or read immediately. The cell populations were gated to avoid debris and analyzed on a flow cytometer (BD FACScan cytometer using Cell quest pro software).

**RT-PCR and real time RT-PCR.** HUVEC, BJAB cells, and matured U937 cells were washed with sterile PBS, and total RNA was isolated (RNAesy kit, Qiagen), dissolved in diethyl pyrocarbonate (DEPC)-water, and reverse transcribed (Omniscript, Qiagen). PCR analysis was performed with the following specific primers: GAPDH as described (25); CD14 fragment as described (34); CD141 kb, forward primer 5’ ACC ACG CCA GAA CCT TGT GAG 3’ and reverse primer 5’ CAG CAC CAG GGT TCC CG 3’; and CD18 primers as described (18). PCR conditions included a 94°C hot start for 4 min followed by 35 cycles of denaturing at 94°C for 1 min, annealing temperature at 55°C (for CD18 and GAPDH) and annealing temperature 60°C (for CD14 and GAPDH), and extension at 72°C for 2 min. Samples were run on a 1% agarose gel containing ethidium bromide. Real-time RT-PCR was carried out with the use of the same CD14 fragment primers and GAPDH primers as for the RT-PCR.

**Quantitative analysis.** Quantitative analysis was performed by monitoring in real time the increase in the fluorescence of the SYBR-green dye (Molecular Probes) on a Bio-Rad i-Cycler (Bio-Rad Laboratories, Hercules, CA). Proper amplification was confirmed by performing melting curve analysis. Real-time fluorescence measurements were performed, and a threshold cycle value for each gene of interest was determined as previously reported (12). All data were normalized against the GAPDH mRNA level and expressed as fold increases relative to positive control, matured U937.

**Polymyxin B treatment of LPS.** Polymyxin B (PMB) (Sigma) was incubated at a concentration of 5 μg/ml with smooth E. coli O111:B4 LPS for 20 min at room temperature before the treated LPS was added to HUVEC.

**Flow chamber assay.** To study LPS-induced leukocyte recruitment on HUVEC under shear conditions, a laminar flow chamber was used as previously described (20). Fibronectin-coated glass coverslips with confluent monolayers of HUVEC were mounted into a polycarbonate chamber with parallel-plate geometry. The flow chamber was placed on an inverted microscope stage, which was enclosed with a warm air cabinet, and the temperature was maintained at 37°C. A syringe pump (Harvard Apparatus) was used to draw freshly donated heparinized blood from a healthy donor over monolayers at a shear of 2 dyn/cm². The blood drawing protocol was approved by an independent University Ethics Committee. The tissue was then perfused with warm HBSS to enable visualization of the leukocytes (HBSS has no added phenol red). Interactions of leukocytes with the endothelium were visualized and recorded by using phase-contrast microscopy (×100 magnification). Transendothelial leukocytes were observed as phase dark cells (×200 magnification). The microscope was linked to a VCR machine, and video tapes of the experiments were recorded for later play-back analysis. Interactions were analyzed for rolling cells (defined as leukocytes moving at a slower rate than red blood cells), adhering cells (cells stationary for 10 s or longer), and transendothelial cells (phase dark cells).

**PI-PLC treatment of cells.** PI-PLC (Sigma) was added into the corresponding tissue culture medium and incubated with cells for 1 h at 37°C. To determine the effective concentration, the PI-PLC was titrated from 0.001 U/ml up to 0.1 U/ml on matured U937 cells (see Fig. 7A). PI-PLC at 0.1 U/ml was used for all subsequent experiments.

**Statistical analyses.** Values are expressed as means ± SE. Where appropriate, results were analyzed for statistical significance by using a Student’s t-test. A P value of <0.05 was taken to be significant.

**RESULTS**

**CD14 is present on primary-passage HUVEC but not tertiary passaged cells.** We examined whether CD14 is present on monolayers of cultured primary-passage HUVEC. Furthermore, we investigated the impact of cell passage on this
expression (Fig. 1). Two flasks of HUVEC were cultured from the same umbilical cord, and cell surface expression for CD14 was examined by using a cell-surface ELISA. Filled bars represent CD14 as detected by CD14 antibody MEM-18, and open bars represent appropriate (IgG1) isotype controls. Data are expressed as arithmetic means + SE from 3 separate experiments. OD, optical density. *P < 0.005.

**Fig. 1.** CD14 is present on primary-passage but not on tertiary-passage human umbilical vein endothelial cells (HUVEC). HUVEC were isolated from a single cord and examined at 1st and 3rd passage for CD14 expression with a cell-surface ELISA. Filled bars represent CD14 as detected by CD14 antibody MEM-18, and open bars represent appropriate (IgG1) isotype controls. Data are expressed as arithmetic means + SE from 3 separate experiments. OD, optical density. *P < 0.005.

To further characterize the level of CD14 expression on HUVEC, we compared percentage and mean fluorescence intensity of CD14. Figure 2 reveals that a very significant proportion of both monocytes (Fig. 2A) and endothelium (Fig. 2B) displays CD14 on the membrane surface. The histogram profiles from the flow cytometry data demonstrate the variability of CD14 on HUVEC from the same cord (Fig. 2B). Figure 2, C and D, shows that on average 92% of monocytes and 50% of HUVEC were positive for CD14, respectively. The density of CD14 per cell was analyzed, and monocytes showed average mean fluorescence intensity (MFI) of 90 compared with an MFI of 49 on HUVEC. Therefore mean expression per HUVEC was approximately half of mean expression of monocytes. Interestingly, the highest expressing HUVEC had an MFI of 124, which is more than the highest expressing monocytes, which had an MFI of 97 (Fig. 2, E and F).

**HUVEC are capable of synthesizing CD14.** There are reports in the literature to suggest that molecules can originate from one cell type and transfer on to another cell type (5, 15). To examine if HUVEC are producing CD14-specific RNA, RT-PCR was carried out (Fig. 3A). A band corresponding to the expected 425-bp product from the CD14 primers was detected in four different HUVEC samples (Fig. 3A, lanes 5–8), as well as in a matured monocyte cell line (Fig. 3A, lane 4). There was minimal product in a B cell line (BJAB) consistent with low expression (Fig. 3A, lane 2), and the water control showed no contamination (Fig. 3A, lane 2).

**HUVEC synthesize large amounts of CD14-specific mRNA.** To demonstrate that there are physiological quantities of CD14-specific mRNA present in HUVEC, a comparison was made of the quantities detected compared with a matured monocyte cell line (Fig. 3B). Real-time RT-PCR analysis for three different HUVEC samples showed CD14 mRNA at similar and even higher levels compared with the matured monocyte cell line control. Again, variability was noted. BJAB expressing low levels of CD14 protein confirmed a minimal amount of CD14-specific mRNA.

The specific CD14 mRNA detected was not due to contaminating monocyte mRNA. To ensure that the specific CD14 bands observed in endothelium were not due to contamination of blood cells during the isolation procedure, PCR was carried out on the same cDNA (Fig. 3C) to detect CD18 message, which is known to be present on leukocytes but not on HUVEC. The primers used were designed to amplify a 462-bp fragment of CD18. The results from the RT-PCR demonstrate that there was no CD18 message detected in the HUVEC total RNA (Fig. 3C, lanes 5–8) compared with the positive controls U937 (Fig. 3C, lane 4) and the B cell line (Fig. 3C, lane 3). The water controls were negative (Fig. 3C, lane 2), demonstrating that no other contamination was detected. These data demonstrate that the HUVEC lysates used were not significantly contaminated with leukocytes and so could not account for the CD14 mRNA detected.

The specific CD14 protein detected was not due to contaminating monocytes. To ensure that contaminating CD14 protein from infiltrating cells was not contaminating the endothelium, we again made use of the fact that monocytes and other leukocytes are CD18 positive, whereas HUVEC is CD14 positive but CD18 negative. Flow cytometric analysis was carried out, and Fig. 4, A and B, demonstrates our gating strategy to select for monocytes from whole blood and HUVEC. While the majority of our monocytes were double positive for CD14 and CD18 (Fig. 4C), <1% of our HUVEC were double positive (Fig. 4D) compared with the isotype controls (Fig. 4, E and F).

The amino acid sequence of HUVEC CD14 is 99% similar to that of monocyte CD14. A comparison was made between the sequences of HUVEC CD14 and monocyte CD14. We designed primers that amplified a region from amino acid 37 to amino acid 347 and sequenced the PCR product. The data show that there is a 99% homology between the detected CD14 sequence on HUVEC compared with the published monocyte sequence (www.ncbi.nlm.nih.gov accession number NM_000591.1) (data not shown).

**Fig. 1.** CD14 is present on primary-passage but not on tertiary-passage human umbilical vein endothelial cells (HUVEC). HUVEC were isolated from a single cord and examined at 1st and 3rd passage for CD14 expression with a cell-surface ELISA. Filled bars represent CD14 as detected by CD14 antibody MEM-18, and open bars represent appropriate (IgG1) isotype controls. Data are expressed as arithmetic means + SE from 3 separate experiments. OD, optical density. *P < 0.005.
Endothelium can detect LPS in the absence of plasma proteins. LPS invokes a response in endothelium (E-selectin synthesis). Figure 5A demonstrates that at an LPS concentration range from 0.01 to 0.1 ng/ml E-selectin expression was not detectable in the absence of plasma. By contrast, addition of plasma allowed LPS responses at 0.01 and 0.1 ng/ml of LPS. However, at 1 ng/ml of LPS a large response was detected in the absence of plasma compared with the isotype control. Maximum levels of E-selectin expression occur at an LPS concentration of 1 ng/ml or above in the absence of plasma, and addition of plasma did not increase maximal responses to LPS in this range.

The lipid A domain of LPS is the critical factor in activating endothelium. To study the specificity of the endothelial response to LPS, the critical activation domain, lipid A, was inhibited using PmB. Figure 5B demonstrates that the endothelial response to LPS was completely blocked by PmB. By contrast PmB had no effect on TNF-α-induced responses, suggesting an absence of nonspecific effects of PmB on endothelium. It is worth noting that high concentrations of PmB caused nonspecific cellular injury, making the TNF-α control very important.

Primary-passage HUVEC can recruit leukocytes in the absence of plasma proteins. To examine if the E-selectin endothelial response to LPS observed on ELISA was physiologically relevant, leukocyte interactions with endothelium were quantified. Figure 6 shows increased rolling, adhesion, and emigration (Fig. 6, A–C, respectively) in HUVEC stimulated with LPS in the absence of plasma proteins. Figure 6 also shows that pretreatment with a CD14 blocking antibody completely inhibited the LPS response for all three parameters investigated. This antibody did not affect TNF-α-induced leukocyte rolling and adhesion (data not shown). Addition of plasma to the CD14-inhibited HUVEC reconstituted ~30% of
the LPS response, suggesting that there are proteins in plasma that can function as a surrogate for membrane CD14 (data not shown).

**CD14 is attached to the endothelium via a GPI anchor.** To establish whether the CD14 detected by both ELISA and flow cytometry was fixed to the HUVEC cell surface and not due to contaminating soluble CD14 trapped on the HUVEC cell surface, HUVEC were treated with GPI-specific PI-PLC. GPI-specific PI-PLC has been previously used to inhibit GPI-linked CD14 on monocytes (32). Figure 7A shows that 0.1 U/ml PI-PLC removed known GPI-anchored CD14 on PMA-treated U937 cells. This same optimal concentration of PI-PLC also removed CD14 from HUVEC (Fig. 7B). Figure 7C shows that a transmembrane (non-GPI anchored) HUVEC surface protein, CD54 (ICAM-1), is not removed by PI-PLC. Finally, to ensure that the PI-PLC reagent was not contaminated with LPS, thus causing the downregulation of CD14, the PI-PLC was boiled to inactivate the enzyme. Once inactivated, the PI-PLC failed to remove CD14 expression (Fig. 7D).

Removing CD14 from the HUVEC surface is associated with a reduction in the HUVEC response to LPS. HUVEC were treated with LPS with or without prior treatment with GPI-specific PI-PLC (to remove GPI-anchored proteins, including CD14). Figure 8 demonstrates that HUVEC stimulated by 10 ng/ml of LPS caused the expression of E-selectin to be increased fourfold compared with either isotype levels or levels of E-selectin expression on untreated HUVEC. In HUVEC treated with PI-PLC to remove CD14, there was a reduction of E-selectin expression.

**DISCUSSION**

Over the past 10–15 years, it has become clear that neither passaged endothelium nor endothelial cell lines have the capacity to respond to LPS in the absence of plasma proteins. However, in the presence of plasma proteins and more specifically soluble CD14, endothelium responds to LPS by expressing adhesion molecules to allow for leukocyte recruitment. From this work it has become generally accepted that endothelium does not constitutively express CD14 but requires external sources of soluble CD14. However, for many years, numerous investigators using primary or first-passaged endothelium stimulated with LPS observed a robust adhesive response of leukocytes to LPS-stimulated endothelium (24, 30, 31). These LPS responses were observed in the absence of plasma, raising some potentially conflicting data. Additionally, von Asmuth and colleagues reported that anti-CD14 antibody blocked endothelial responses to LPS even in the absence of serum (30). Although these data do suggest the possibility that endothelium could express CD14, an alternative explanation could be that soluble CD14 from the fetal bovine serum in which the endothelium was grown was incorporated onto the surface of endothelium. However, quite recently, Jersmann and colleagues (11) reported CD14 immunoreactivity on the surface of endothelium that was lost upon passage, an observation that was suggestive that perhaps endothelium may have its own CD14. Indeed, we present data herein that demonstrate CD14 message and protein associated with endothelium. The CD14 was not due to contaminating leukocytes. Most importantly, removal of GPI-linked proteins from the endothelial surface eliminated CD14 immunoreactivity, suggesting for the first
time that it was the GPI-linked membrane CD14 that was on endothelium and functionally could induce adhesion molecule expression as well as recruit leukocytes to the endothelial surface.

The endothelium as the essential cell for leukocyte recruitment is well established. If endothelium is not activated, leukocytes cannot be recruited to the leukocyte-endothelial interface regardless of the state of activation of the leukocytes. Under flow conditions, there is a requirement for endothelial activation by LPS, leading to E-selectin synthesis and subsequent leukocyte recruitment. Our data demonstrate that in the absence of any exogenous source of CD14, endothelium has the capacity to detect low levels of LPS (1 ng/ml), leading to E-selectin synthesis and leukocyte recruitment. Moreover, the endothelium has message for CD14, and Jersmann et al. (11) were able to reduce expression of endothelial CD14 by inhibiting protein synthesis. By blocking CD14 function with PI-PLC, a molecule that removes all GPI-linked proteins from the cell membrane surface, we also support the view that the endothelial CD14 is the membrane form found on monocytes. Finally, removal of this CD14 inhibited endothelial E-selectin production and leukocyte recruitment, further supporting a functional role for membrane CD14 on endothelium.

Our data are also entirely in line with Frey and colleagues (4) who reported that at very low concentrations of LPS (1 ng/ml or less), endothelium was unable to detect LPS without the presence of plasma and more specifically soluble CD14. Our data also demonstrate that plasma was required to detect 0.1 ng/ml LPS; however, we cannot discount the possibility that membrane-bound CD14 is necessary for this process. In
In this regard, two studies have reported that serum was not sufficient to induce LPS responsiveness in cells devoid of membrane CD14, but on transfection of CD14, LPS responsiveness (in the presence of plasma) was then restored (7, 17). One possibility may be that soluble CD14 transfers LPS to membrane CD14. Indeed, Hailman et al. (8) reported that soluble CD14 can facilitate rapid transfer and efficient presentation of LPS to membrane CD14. However, preliminary data from our laboratory (data not shown) suggest that following inhibition of membrane CD14, plasma can reconstitute ~30% of the LPS response. These data would suggest that plasma independent of membrane CD14 can directly aid in endothelial LPS detection.

As previous studies have shown endothelium to be negative for CD14, we investigated whether the absence of CD14 was due to passaging of HUVEC. Our results demonstrate that CD14 expression on endothelium is indeed lost on passaging, an observation consistent with one other study (11). Passaged HUVEC are routinely used in many laboratories, and the loss of expression of surface molecules due to passaging of endothelium has been observed for other proteins, including von Willibrand factor and P-selectin (13, 19). We also report that CD14 expression is heterogeneous on HUVEC. Indeed, we have seen heterogeneity of CD14 expression in our samples, which range from as high as 85% to as low as 20% of that detected on monocytes (Fig. 2D). It is well known that mono-

Fig. 5. HUVEC response to LPS is independent of plasma proteins at LPS concentrations of 1 ng/ml and above, and this response is specific to LPS. Serial dilutions of LPS were added to HUVEC either in the presence or absence of plasma proteins and incubated for 4 h at 37°C. The E-selectin response was measured by using a cell-surface ELISA. E-selectin in the presence or absence of plasma is depicted by open and filled ovals, respectively, and isotype controls in the presence and absence of plasma are depicted in gray squares and gray circles, respectively (A). Polymyxin B (PmB) was used to study the specificity of the LPS response. E-selectin was used as a measure of LPS responses. Filled bars denote E-selectin, and open bars denote isotype control. TNF-α was used as a control to ensure specificity of the PmB response (B). Data are expressed as arithmetic means ± SE of 3 separate experiments. *P < 0.05, †P < 0.005.

Fig. 6. Under physiological conditions, primary HUVEC respond to LPS by recruiting leukocytes into tissue, and this response is CD14 specific. HUVEC on glass coverslips were either treated with 10 ng/ml of LPS for 4 h or left untreated. Blood was then perfused across the coverslip, and leukocyte recruitment was analyzed. Flow chamber data demonstrate that the treatment of LPS induced leukocyte rolling (A), adhesion (B), and transmigration (C) (left and middle bars). When the HUVEC was pretreated with blocking antibody (ab) for 30 min before LPS stimulation, cell recruitment did not occur (A–C; right bars). Data are expressed as arithmetic means ± SE of 2–4 separate experiments. *P < 0.05.
cytes vary in their expression of CD14. Interestingly, variable CD14 expression has also been reported on human gingival fibroblasts from different sources as well as the same source cultured in separate flasks (28). Therefore, variable CD14 expression on HUVEC could be due to time of culture or maturation state. Alternatively, it could be due to differences within the human population, as suggested by Sugawara and colleagues (28). Nonetheless, our data clearly show that CD14 protein can be detected on endothelium.

Previously, it has been suggested that the plasma protein LPS binding protein (LBP) is also required for endothelium to respond to LPS. Analysis of the LBP knockout mouse has revealed less sensitivity to LPS, but nevertheless a potent response is still seen (33). It has been hypothesized that LBP is required for CD14-negative cells to respond to LPS (3). Our data would suggest that LBP is not essential for LPS concentrations of 1 ng/ml and higher because all these responses were inhibited with a CD14 MAb, as well as GPI-linkage inhibition. Nevertheless, we do not discount the possibility that LBP, much like soluble CD14, could increase sensitivity of endothelium to LPS.

This is the first report that endothelial CD14 is linked to the membrane via a GPI anchor. This study suggests that there is functional, GPI-linked CD14 on endothelium, in contrast to the generally accepted view that CD14 is absent from endothelium. Moreover, we support the view that a lack of CD14 on endothelium likely reflects issues related to cell culture rather than a physiological phenomenon. In this study, we show that primary HUVEC cultured in the absence of mitogen express GPI-linked CD14 and that it originates from the endothelium. The identification of endothelial CD14 is important as it further suggests endothelium as a critical cell in the inflammatory response.

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Fig. 8. CD14 is important in the HUVEC response to LPS. Cell surface ELISA data demonstrate that HUVEC removed of CD14 show a significantly reduced response to LPS compared with CD14-positive HUVEC. Data shown are arithmetic means of 3 experiments + SE. Filled bars indicate E-selectin response; open bars indicate appropriate isotype controls. *P < 0.05.
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