Murine CD14 Gene Expression In Vivo: Extramyeloid Synthesis and Regulation by Lipopolysaccharide

By Colleen Fearns,* Vladimir V. Kravchenko,‡ Richard J. Ulevitch,‡ and David J. Loskutoff*

*From the *Department of Vascular Biology, and ‡Department of Immunology, The Scripps Research Institute, La Jolla, California 92037*

Summary

A murine model system was used to study the distribution and regulation of CD14 gene expression in vivo. Western blot analysis failed to detect CD14 in plasma from untreated CB6 (BALB/c × C57Bl6) mice, but showed markedly increased levels of CD14 in plasma from mice treated with lipopolysaccharide (LPS). Plasma levels of CD14 increased in a time- and dose-dependent manner, reaching a maximum between 8 and 16 h. Northern blot analysis of total RNA extracted from mouse tissues revealed low, but significant, levels of CD14 mRNA in many tissues of untreated animals with the highest levels in uterus, adipose tissue, and lung. After intraperitoneal injection of LPS, induction of CD14 gene expression was detected in all organs examined with the extent of induction varying between organs. Induction of CD14 mRNA was both time and dose dependent. Maximum induction in the heart and lung was observed 2–4 h after injection of LPS, while liver and kidney showed maximal induction between 8 and 16 h. In situ hybridization showed that CD14 mRNA was expressed in myeloid cells in many tissues, and that expression in these cells was upregulated by LPS. Unexpectedly, CD14 mRNA was also detected in other cells within tissues, including epithelial cells, and expression in these cell types also was upregulated by LPS. Immunochemical analysis revealed that CD14 antigen colocalized to the cytoplasm of cells expressing CD14 mRNA. These studies demonstrate that CD14 gene expression is not restricted to myeloid cells, and that the level of expression of CD14 is influenced by exposure to LPS.

LPS, an endotoxin found in the outer cell wall of gram-negative bacteria, is a particularly potent activator of myeloid cells, resulting in the production and release of immunoregulatory and inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, interleukin 6, and prostaglandins (1). The excessive production of these mediators contributes to the pathophysiologic effects of LPS (2). The existence of membrane-bound leukocyte receptors for LPS has been postulated and an increasing number of membrane-bound LPS-binding proteins have been identified (3). Of these, CD14, a 55-kD glycoprotein found on the cell surface of myeloid cells, is the only protein of fully defined structure that mediates LPS-induced cell activation (3).

CD14 exists in two distinct forms: as a glycosyl-phosphatidylinositol (GPI)[1]-anchored membrane protein (mCD14) and as a soluble plasma protein lacking the GPI anchor (sCD14) (4). Both forms appear to be involved in LPS signaling. The cell surface form serves as a high affinity receptor for complexes of LPS and lipopolysaccharide-binding protein, a serum protein which binds LPS (5). Expression of human mCD14 in transfected murine 70Z/3 B cells (6), Chinese hamster ovary cells (7), and in transgenic mice (8) has been shown to increase sensitivity to LPS. For example, responsiveness to LPS was increased by as much as 1,000-fold in the 70Z/3 B cells (6) and by approximately threefold in the transgenic mice (8). The soluble form of CD14 appears to potentiate responsiveness to LPS in cells which do not express mCD14, such as endothelial and epithelial cells. LPS-responsiveness was abrogated by immunodepletion of sCD14 from serum and could be restored by addition of sCD14 (9–11). These findings indicate that CD14 plays a crucial role both in the recognition of LPS, and in the initiation of cellular responses by LPS.

Immunological analysis has shown that mCD14 is expressed primarily on cells of the monocyte/macrophage lineage (reviewed in reference 4), with early progenitor stem cells being CD14 negative and mature blood monocytes expressing high levels of CD14. Neutrophils also express CD14 but at lower levels (12). Differential expression of CD14 is also found among resident tissue macrophages. For example, peritoneal macrophages stain strongly for CD14, while alveolar macrophages and microglial cells stain only weakly. CD14 antigen has also been detected in non-myeloid cells, namely, B cells and mammary cells (4).

Previous studies using in vitro systems and measuring cell

[1] *Abbreviation used in this paper:* GPI, glycosyl-phosphatidylinositol.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/03/0857/10 $2.00
Volume 181 March 1995 857–866

surface expression only, have shown that LPS can modulate CD14 expression but that the results obtained vary with the in vitro system used. For example, in purified human monocytes, LPS treatment decreases the expression of CD14 on the cell surface by inducing shedding of the receptor into the conditioned medium (13). In whole blood assays, however, cell surface expression of CD14 on neutrophils and monocytes is upregulated by LPS (14, 15). Upregulation is rapid and independent of protein synthesis (14, 15).

To date, the potential role of CD14 in determining cellular responsiveness to LPS has only been studied using in vitro systems. Moreover, the tissue distribution of CD14 mRNA in vivo and its regulation by LPS have not been studied. In this paper, we demonstrate that CD14 mRNA is detected in many murine tissues where it is produced both by myeloid cells and, unexpectedly, by non-myeloid cells. CD14 mRNA expression in these cells is stimulated by LPS.

Materials and Methods

Experimental Protocols. Adult CB6 mice (BALB/c/ByJ × C57Bl6/J; Scripps Clinic Rodent Breeding Colony), aged 6 to 8 wk, were injected intraperitoneally with 50 μg LPS (*Escherichia coli* serotype 0111:B4; Sigma Chemical Co., St. Louis, MO) diluted in 100 μl sterile saline (Baxter, Deerfield, IL). Control animals were injected with an equivalent volume of saline alone. At the conclusion of experiments, the mice were anesthetized by inhalation anesthesia with metofane (methoxyflurane; Pitman-Moore, Mundelein, IL) and exsanguinated. The blood was collected into 20 mM EDTA (final concentration) to prevent clotting. Tissues were rapidly removed by standard dissection techniques, and either minced and immediately frozen in liquid nitrogen for preparation of total RNA or fixed in paraformaldehyde for in situ hybridization and immunohistochemistry.

Production and Characterization of Anti-murine CD14 Antibodies. Polyclonal anti-murine CD14 antibodies were prepared as described in Pugin et al. (16). Briefly, murine CD14 cDNA was obtained from RAW 264.7 cells cDNA by polymerase chain reaction. When the cDNA was expressed in the pDSpv3 prokaryotic expression vector, it gave rise to a protein with the NH_2-terminal amino acid sequence and molecular weight expected of murine CD14 (16). The solubilized recombinant protein, purified from bacterial lysates by reverse-phase HPLC chromatography, was used to immunize New Zealand White rabbits. The resulting antiserum appeared to be specific for CD14 since FACS analysis of murine macrophage cell lines showed staining of $CD14^+$ J774 and RAW 264.7 cells (16). Moreover, Western blot analysis of supernatants from these cell lines treated with phosphoinositol-specific phospholipase C detected a strongly positive band with the mobility expected for CD14 (16). Finally, immunohistochemical analysis showed strong staining of J774 cells, whereas LR-9 cells which lack cell surface CD14 showed no staining (data not shown).

Western Blot Analysis. Plasma (2 μl per lane) was fractionated on 9% SDS-PAGE and transferred to Immobilon-P membranes (Millipore Corp., Bedford, MA) (17). The membranes were soaked in PBS containing 5% casein for 1 h at room temperature to block additional protein-binding sites and then incubated at room temperature for 1 h with the polyclonal rabbit antiserum specific for murine CD14 (1:100 dilution in PBS containing 1% casein). The membranes were washed three times with PBS containing 1% casein and then incubated for 1 h at room temperature with ^{125}I-labeled donkey anti-rabbit antibody (affinity purified $F(ab)_2$ fragment; Amersham Corp., Arlington, IL). The membranes were washed, dried, and autoradiography performed at −80°C, using Kodak XAR-5 film with intensifying screens.

Northern Blot Analysis. Total RNA was prepared from frozen tissues by the acid guanidium thiocyanate-phenol-chloroform method (18) and its concentration determined by measurement of absorbance at 260 nm. Total RNA (10 μg) was analyzed for CD14 mRNA by Northern blotting using standard procedures. A 1,022-base pair, BglII/HindIII fragment of the murine CD14 cDNA was isolated and radiolabeled by the random primer technique (19) using [α-^{32}P]dGTP (>3,000 Ci/mmol; Amersham Corp.). Autoradiography was performed at −80°C, using Kodak XAR-5 film with intensifying screens. To verify equal loading and transfer of the RNA, Northern blots were rehybridized with a radiolabeled plasmid probe carrying cDNA encoding rat 18S RNA. The level of CD14 mRNA was quantitated by densitometric analysis of Northern blot autoradiograms using a LKB densitometer (Ultrosan XL; LKB, Bromma, Sweden).

Tissue Preparation for In Situ Hybridization. After removal from the animal, the tissues were immersed in chilled 4% (wt/vol) paraformaldehyde (Sigma Chem. Co.) in 0.1 M Na phosphate, pH 7.4, and fixed at 4°C overnight. The fixed tissues were embedded in paraffin blocks and sectioned at 2–5-μm thickness using a microtome. The sections were mounted onto Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA) and stored at room temperature pending analysis.

Riboprobe Preparation. A BglII/HindIII fragment of the mouse CD14 cDNA, containing nucleotides 527–1549, was subcloned into the vector pSP73 (Promega Corp., Madison, WI). This vector was linearized and used as a template for in vitro transcription of radiolabeled antisense or sense riboprobes employing T7 or SP6 RNA polymerase, respectively, in the presence of [^{35}S]UTP (>1,200 Ci/mmol; Amersham Corp.). Templates were removed by digestion with RQ1 DNAse (Promega) for 15 min at 37°C, and the riboprobes were purified by phenol extraction and ethanol precipitation.

In Situ Hybridization. In situ hybridizations were performed as described previously (20). Briefly, the paraffin-embedded tissues were pretreated sequentially with xylene (3 × 5 min), 2× SSC (1 × 10 min), paraformaldehyde (1 × 10 min, 4°C), and proteinase K (1 μg/ml, 1 × 10 min). Slides were prehybridized for 2 h in 100 μl of prehybridization buffer at 42°C. An additional 20 μl of prehybridization buffer, containing 2.5 mg/ml transfer RNA and 600,000 cpm of the ^{35}S-labeled riboprobe, was added and the slides hybridized for 16 to 18 h at 55°C. After hybridization, the slides were washed with 2× SSC (2 × 10 min), treated with RNAse (20 μg/ml, 1 × 30 min), washed in 2× SSC (2 × 10 min), 0.1× SSC (1 × 2 h) and 0.5× SSC (2 × 10 min). Finally, the slides were dehydrated by immersion in a graded alcohol series containing 0.3 M NH_4Ac, dried, coated with NTB2 emulsion (1:2 in water; Kodak), and exposed in the dark at 4°C for 4 to 12 wk. Slides were developed for 20 min in D19 developer (Kodak), fixed, washed in water, and counterstained with hematoxylin and eosin. Photomicrographs were taken using bright field or polarized light epiluminescence. The ability of the riboprobe to recognize myeloid CD14 was tested using J774 cells. Strong hybridization was detected in CD14 positive cells (i.e., J774 cells) after 4 wk exposure but not in CD14 negative cells (i.e., 3T3 cells), even after 12 wk exposure (data not shown).

Immunohistochemistry. Immunohistochemical staining was performed as described previously with minor modifications (20). Briefly, the paraffin-embedded tissues were deparaffinized with xy-

lene (3 × 5 min, 25°C), treated with 2% hydrogen peroxide to quench endogenous peroxidase activity, and rehydrated by immersion in a graded series of ethanol washes. The sections were then permeabilized by sequential treatment with 0.2 and 0.5% Triton X-100 in TBS. After incubation with 10% normal goat serum for 30 min, the slides were incubated with the polyclonal rabbit antiserum specific for murine CD14 (diluted 1:50 in TBS containing 0.1% normal goat serum) for 16 to 18 h at 4°C, followed by 1 h at room temperature. In control experiments, tissue sections were incubated with normal rabbit serum instead of primary antibody. The slides were then washed and treated sequentially with biotinylated goat anti-rabbit IgG (Zymed Labs., South San Francisco, CA) diluted 1:50 in PBS containing 0.05% Tween-20, streptavidin-peroxidase conjugate (Zymed Labs.) and aminoethylcarbazole chromogen containing 0.03% hydrogen peroxide (Zymed Labs.). After rinsing in distilled water for 3 min, the slides were counterstained with Gill modified hematoxylin for 20 s, rinsed well with tap water, and mounted in GVA-mount (Zymed Labs.).

Results

Effect of LPS on Plasma Levels of CD14. Normal human serum has been shown to contain sCD14, a form of the receptor which migrates in SDS-PAGE as a doublet with an apparent molecular mass of 48–50 kD (13). Moreover, increased levels of sCD14 have been observed in sera from septic or polytraumatized patients (21). Experiments were performed to determine whether murine plasma contained sCD14 and whether it too was increased in the presence of LPS. Plasma was collected from control and LPS-treated mice, fractionated by electrophoresis in SDS–polyacrylamide gels under non-reducing conditions and electroblotted onto Immobilon-P membranes. CD14 was detected with a polyclonal rabbit antibody specific for murine CD14 (16). In contrast to results with human serum (13), no CD14 was apparent in plasma from control animals (Fig. 1). However, there was strong induction of CD14 in plasma from LPS-treated mice (Fig. 1). In these studies, a single CD14 positive band with an apparent molecular mass of 48-kD was observed. The level of CD14 antigen increased in response to LPS in a dose- (Fig. 1 *B*) and time- (Fig. 1 *A*) dependent manner, reaching a maximum between 8 and 16 h.

Tissue Distribution of Murine CD14 mRNA and Its Regulation by LPS. To examine the tissue-specific expression of the CD14 gene in vivo, and to assess the effect of LPS on CD14 gene expression, mice were injected intraperitoneally either with 50 μg LPS (2.0 mg/kg) in saline, or with saline vehicle alone. Tissues were removed 4 h later, and total RNA was prepared and analyzed for CD14 mRNA by Northern blot analysis. Results from the combined tissues of two mice are presented in Fig. 2, with the optimal autoradiographic exposure time shown for each tissue. In agreement with previous reports (22), a single RNA transcript of ~1.5 kb was detected. In control tissues, low, but detectable, levels of CD14 mRNA were observed in uterus, adipose, and lung, followed by spleen, heart (note the longer exposure time), thymus, and testes. In contrast, CD14 gene expression was barely detectable in kidney, liver, brain, muscle, and gut. Thus, the level of CD14 mRNA was relatively low in control tissues and varied considerably between organs. After LPS treatment, CD14 mRNA was induced in all organs examined, with the extent of induction again varying between organs. For example, while there was moderate to strong induction in many tissues, there was relatively weak induction in brain, muscle and gut. Variations in sample loading were small as assessed by rehybridizing the blots with a rat 18S cDNA probe.

To evaluate the time course of CD14 mRNA induction by LPS, mice were injected with 50 μg LPS and selected tissues

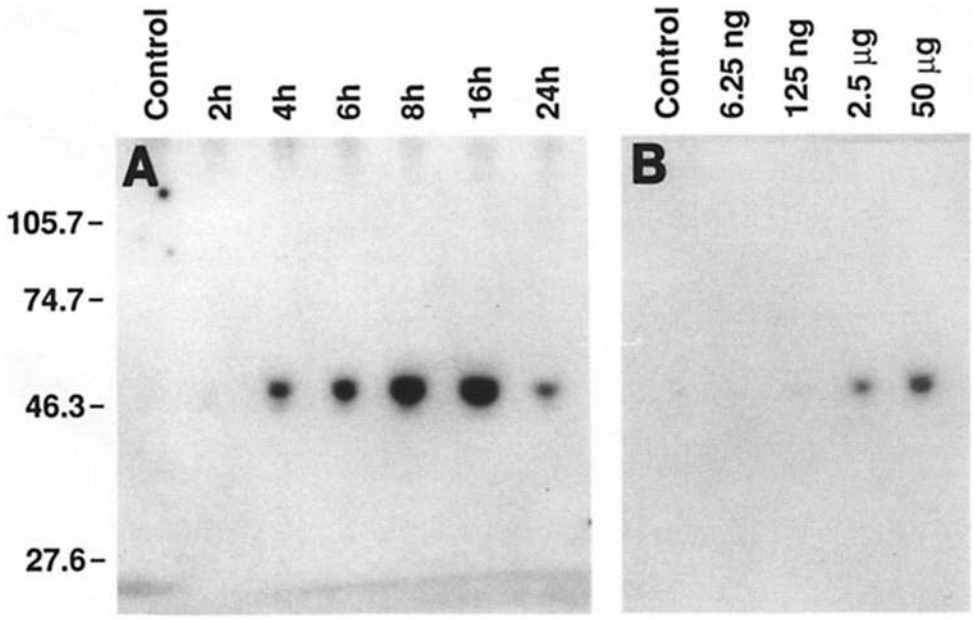


Figure 1. Effect of LPS on plasma levels of CD14. Plasma samples (2 μl per lane) were electrophoresed under non-reducing conditions on a 9% SDS-PAGE gel and transferred to Immobilon-P membranes. The membranes were analyzed by immunoblotting using a polyclonal rabbit anti-murine CD14 antiserum, followed by an ^{125}I-labeled anti-rabbit antibody, and subjected to autoradiography. (*A*) Mice were injected intraperitoneally with saline containing 50 μg LPS or with saline vehicle alone. The animals were killed at the indicated times and blood collected into 20 mM EDTA (final concentration). After centrifugation, the plasma was decanted, stored at −80°C, and then analyzed for CD14. (*B*) Mice were injected with saline alone as control or with saline containing increasing amounts of LPS as indicated. Blood was collected 8 h after LPS injection and analyzed for CD14 by immunoblot.

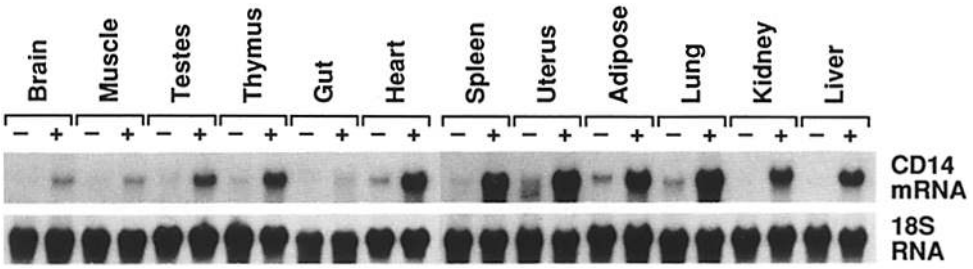


Figure 2. Induction of CD14 mRNA by LPS. Mice were injected intraperitoneally with either saline (−) or saline containing 50 μg LPS (+). The animals were killed 4 h later, total RNA was prepared from the indicated tissues, and 10 μg was analyzed for CD14 mRNA by Northern blot analysis. Variations in sample loading were assessed by rehybridizing the blots with a rat 18S cDNA probe. Autoradiographic exposure times for CD14 mRNA were varied between tissues to permit optimal visualization of control and induced signals and were as follows: brain, muscle, testes, thymus, gut, and heart, 14 d; spleen, uterus, adipose, lung, kidney, and liver, 3 d.

(heart, lung, liver, and kidney) were removed at various times and analyzed by Northern blotting (Fig. 3). The level of CD14 mRNA in the blots was quantitated by densitometric analysis of the autoradiograms. In all cases, the increase in CD14 mRNA was transient. A detectable increase was observed in heart and lung within 1 h, with maximal induction between 2 and 4 h. Although increased levels also were detected in liver and kidney within 1 h, maximal levels were not reached until 8 to 16 h after LPS injection. By 24 h, the level of CD14 mRNA had returned to near basal levels in all tissues examined.

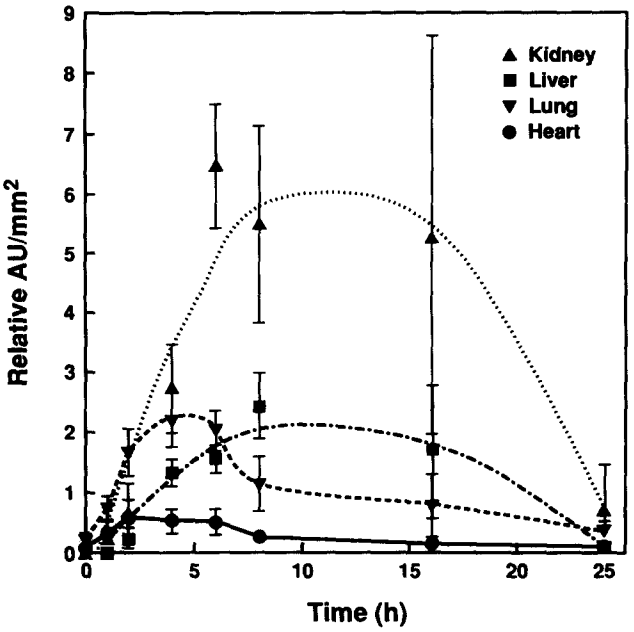


Figure 3. Time course of induction of CD14 mRNA by LPS. Mice were injected intraperitoneally with 50 μg LPS and selected tissues (kidney [▲], liver [■], lung [▼] and heart [●]) removed at the indicated times. Total RNA was prepared and analyzed for CD14 mRNA by Northern blot analysis. The concentration of CD14 mRNA was determined by densitometric analysis of the blot autoradiograms. Variations in sample loading were assessed by rehybridizing the blots with a rat 18S cDNA probe. The relative absorbance units (AU)/mm² were calculated by dividing the AU/mm² for CD14 mRNA by the AU/mm² for 18S RNA. Points and bars represent the means ± SD of four mice.

It was difficult to determine accurate CD14 mRNA levels in control tissues because of the very low levels of CD14 mRNA present, and thus, the fold induction could only be approximated. In spite of these limitations, it is clear that the greatest fold induction occurred in the kidney (40- to 80-fold), followed by liver (30- to 40-fold), lung (15- to 41-fold) and heart (four- to eightfold).

The dose dependency of the response to LPS was then examined. Mice were injected with doses of LPS ranging from 0.00625–50 μg (0.25 μg–2.0 mg/kg), and selected tissues removed either at 2 h (heart and lung) or at 8 h (liver and kidney) for analysis. Results from a representative experiment are shown in Fig. 4. Although CD14 mRNA increased in each tissue in a dose-dependent manner, differences in sensitivity were evident between the four organs examined. For example, in heart, lung, and liver, induction of CD14 mRNA could be seen with as little as 125 ng LPS, while induction in kidney was only evident at the higher doses of LPS (2.5 and 50 μg).

Cellular Localization of CD14 mRNA and Antigen in Murine Tissues. In order to examine the cell-specific expression of CD14 mRNA in vivo, tissues from saline-injected control mice or LPS-treated mice were removed and fixed in paraformaldehyde, and then analyzed by in situ hybridization with a riboprobe specific for murine CD14. Representative sections from the various tissues are shown in Figs. 5–7. As expected, CD14 mRNA was expressed in monocyte-macrophage derived cells in a number of tissues and its expression in these cells was upregulated by LPS. Examples shown here include Kupffer cells in control liver (Fig. 5 *a*), interstitial cells in control kidney (Fig. 5 *b*), and Kupffer cells in LPS-treated liver (Fig. 5 *c*). In additional experiments (not shown), upregulation was rapid, with increased expression detected as early as 15 min after injection of LPS and maximal expression within 2–4 h. Note the absence of CD14 mRNA in hepatocytes in LPS-treated liver at this early time (Fig. 5 *c*). In lymphoid tissues, a positive LPS-inducible signal was observed in the red pulp of spleen and in isolated cells within the cortex of thymus. In connective tissue, CD14 mRNA was detected in adipose tissue and in cells within adventitial tissue surrounding vessels (data not shown).

CD14 mRNA was not detected in neutrophils from control animals, but was strongly expressed in neutrophils after LPS treatment (Fig. 5 *d*). LPS is known to cause a profound

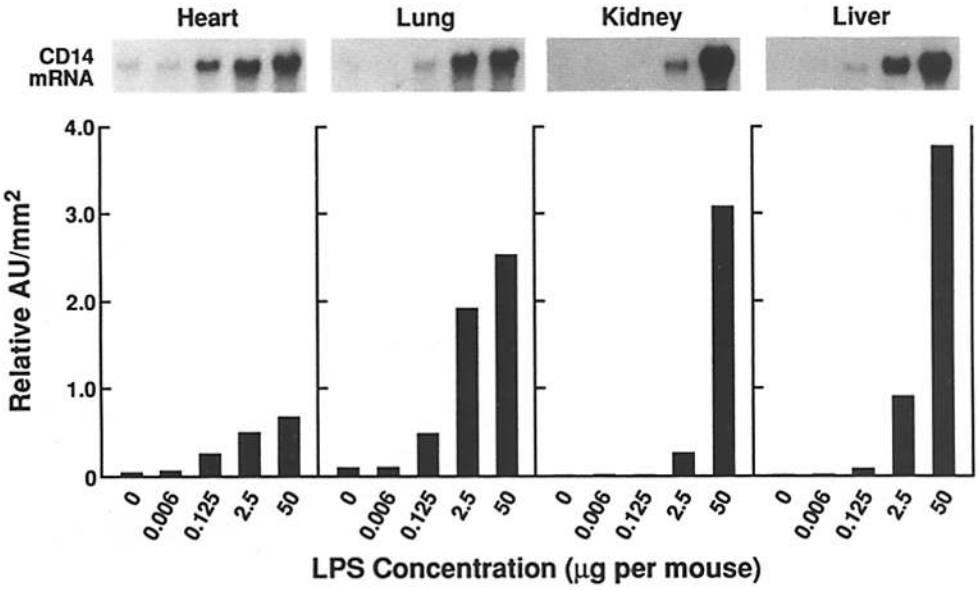


Figure 4. Dose dependency of induction of CD14 mRNA by LPS. Mice were injected with saline alone as the control (0 dose) or with saline containing increasing amounts of LPS. Tissues were removed after 2 (heart and lung) or 8 h (liver and kidney) and total RNA was prepared and analyzed for CD14 mRNA by Northern blotting. The concentration of CD14 mRNA was determined by densitometric analysis of the blot autoradiograms. Variations in sample loading were assessed by rehybridizing the blots with a rat 18S cDNA probe. The normalized levels of CD14 mRNA are shown in the histogram and are expressed as relative AU/mm². Autoradiographic exposure times for CD14 mRNA were varied between tissues and were as follows: heart, 7 d; lung, 2 d; kidney and liver, 3 d.

neutropenia as a result of sequestration of the neutrophils within capillaries, particularly in the lung (1). Neutrophil sequestration was seen in a number of organs (e.g., adrenals, bladder, uterus, and liver) and these neutrophils were shown to express CD14 mRNA (data not shown). Neutrophil sequestration was particularly prominent in lung, where the massive influx of neutrophils after LPS injection showed strong induction of CD14 expression (Fig. 5 *d*). Induction in neutrophils probably accounts, in part, for the increase in CD14 mRNA seen in the various tissues in response to LPS.

Unexpectedly, CD14 mRNA also was detected within epithelial cells from various organs (Fig. 6), and the response of these cells to LPS could be divided into three broad categories. Bronchiolar epithelium in lung (Fig. 6 *a*) is an example of the first category. In this group, epithelium from control animals showed a weak hybridization signal for CD14, and this signal was not increased after LPS treatment. Similarly, epithelium in bladder, stomach, and gut showed weak positivity that was not stimulated by LPS (data not shown).

The second category includes epithelium that was weakly positive in control tissue but was markedly induced by LPS treatment. Examples of this are transitional epithelium in kidney (Fig. 6, *b* and *c*, respectively) and bile duct epithelium in liver (Fig. 7, *a* and *c*, respectively). Increased expression in these cell types in response to LPS was first evident at 2 h, peaked at 4–8 h and returned to near basal levels by 24 h (data not shown).

In the last group, there was induction of CD14 mRNA expression in cells which do not appear to express CD14 under basal conditions. This was observed in hepatocytes (compare Fig. 6 *d* to 5 *a*) and in tubular epithelium in kidney (compare Fig. 6 *e* to 5 *b*). CD14 mRNA was markedly increased in the tubules at the cortex-medulla junction but was also seen in the parietal cells that line the Bowman's capsule and in proximal convoluted tubules (not shown). The response in hepatocytes and tubular epithelium was delayed relative to that seen in myeloid cells (compare early times Fig. 5 *c* to later times 6 *d*) and was only evident 4 h after LPS treatment. In these cells, CD14 mRNA increased to maximum levels at about 8 h, and then declined slowly over the next 16 h. This slower epithelial response may account, in large part, for the extent of induction seen in liver and kidney and for the slower kinetics seen in these tissues.

To check the specificity of the ^{35}S-labeled CD14 antisense riboprobe, tissue sections from liver taken 3 h after LPS treatment were hybridized with a ^{35}S-labeled sense riboprobe. No specific hybridization was detected (compare Fig. 7 *g* to *d*). Immunohistochemical staining of tissue sections from liver for CD14 antigen showed weak staining of Kupffer cells in control sections (Fig. 7 *b*). Increased staining of Kupffer cells and bile duct epithelium was observed after LPS treatment (Fig. 7 *e*). No specific staining was apparent in a parallel section incubated with normal rabbit serum instead of primary antibody (Fig. 7 *h*). Similarly, immunohistochemical analysis of sections of control kidney showed staining for CD14 antigen in interstitial cells (Fig. 7 *c*) and the transitional epithelium (data not shown). Increased staining was observed in these cells (Fig. 7 *f*) 3 h after LPS treatment. In addition, CD14 antigen could be detected in parietal epithelial cells and in tubular epithelium 16 h after LPS treatment (Fig. 7 *i*).

In addition to expression in epithelium, CD14 mRNA was detected in scattered cells within the heart under basal conditions (not shown) and was increased in these cells after LPS treatment (Fig. 6 *f*). Once again, induction by LPS was rapid, with increased expression detected as early as 15 min after injection of LPS and maximal expression within 2–4 h. The positive signal was seen in cells in the connective tissue that surrounds the muscle fibers, and often in close proximity to erythrocytes. Their location and proximity to erythrocytes is consistent with the possibility that they are capillary endothelial cells. However, we have not excluded the possibility that they may be mononuclear leukocytes.

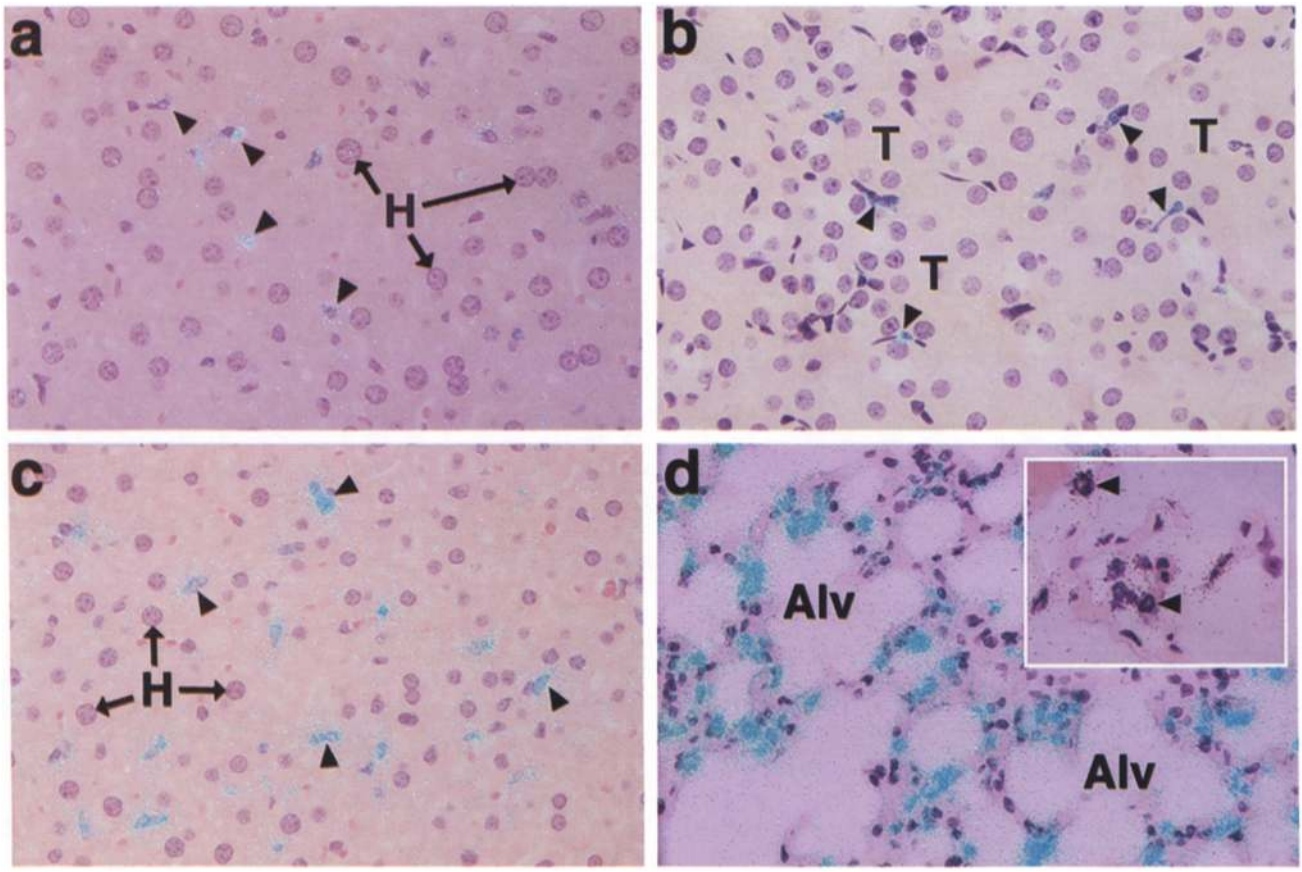


Figure 5. Localization of CD14 mRNA in myeloid cells. Sections of liver, kidney, and lung were analyzed by in situ hybridization for CD14 mRNA as described in Materials and Methods. (*a* and *c*) Liver sections from an untreated (×400, 8-wk exposure) and an LPS-treated (×400, 1 h LPS, 12-wk exposure) animal, respectively. Arrowheads indicate CD14 mRNA in Kupffer cells and H refers to hepatocytes. (*b*) Section of kidney from an untreated animal (×400, 6-wk exposure). Arrowheads indicate positive interstitial cells and T identifies tubules. (*d*) Lung section from an LPS-treated mouse (×400, 2 h LPS, 12-wk exposure). Alveolar spaces are indicated by Alv. Inset, lung section at ×1,000. Arrowheads indicate positive neutrophils within the alveolar septa.

Discussion

Experimental evidence to date has shown that CD14 antigen is expressed primarily on cells of the monocyte/macrophage lineage (reviewed in reference 4). In this paper, we have extended these studies by showing that, in the mouse, CD14 gene expression also is detected within a number of other cells including epithelial cells from various organs. Although the level of CD14 gene expression in untreated control animals was relatively low, a specific and reproducible signal was detected in many of the organs examined (Fig. 2), and in situ hybridization analysis frequently localized it to isolated interstitial/adventitial cells within these tissues. In addition, CD14 mRNA could be detected in epithelial cells in the respiratory tract (Fig. 6 *a*), urinary tract (Fig. 6 *b*), and liver (Fig. 7 *a*), as well as in the gastrointestinal and reproductive tracts (data not shown).

CD14 expression in the presence of LPS has been shown by some to be upregulated on monocytes (15) and neutrophils (14), while others have shown downregulation on monocytes or monocyte-derived macrophages (13). These studies measured effects on cell surface expression only and did not address the effects of LPS on synthesis of CD14 mRNA. In a recent paper, Matsuura et al. (23) showed a time- and dose-dependent induction of CD14 mRNA in murine liver in response to LPS. Our results confirm and extend these studies. Our studies show that LPS is able to induce CD14 gene expression in most tissues (Fig. 2), with highest induction in kidney followed by liver and lung (Fig. 3). This increase occurs in a time- and dose-dependent manner (Figs. 3 and 4, respectively) and is reflected by a corresponding increase in plasma (Fig. 1) and tissue (Fig. 7) levels of CD14 antigen. Our results differed from those of Matsuura et al. (23) in that maximal induction in the liver was seen at 8–16 h compared to 3 h in their system. In addition, they were unable to detect CD14 antigen within the parenchyma of the liver. These differences may reflect differences between mouse strains and LPS and antibody preparations, or may result from differences in fixation procedures.

Our experiments also demonstrate that the sensitivity to LPS and the kinetics of induction by LPS varied from tissue to tissue. For example, in lung, where induction was detected

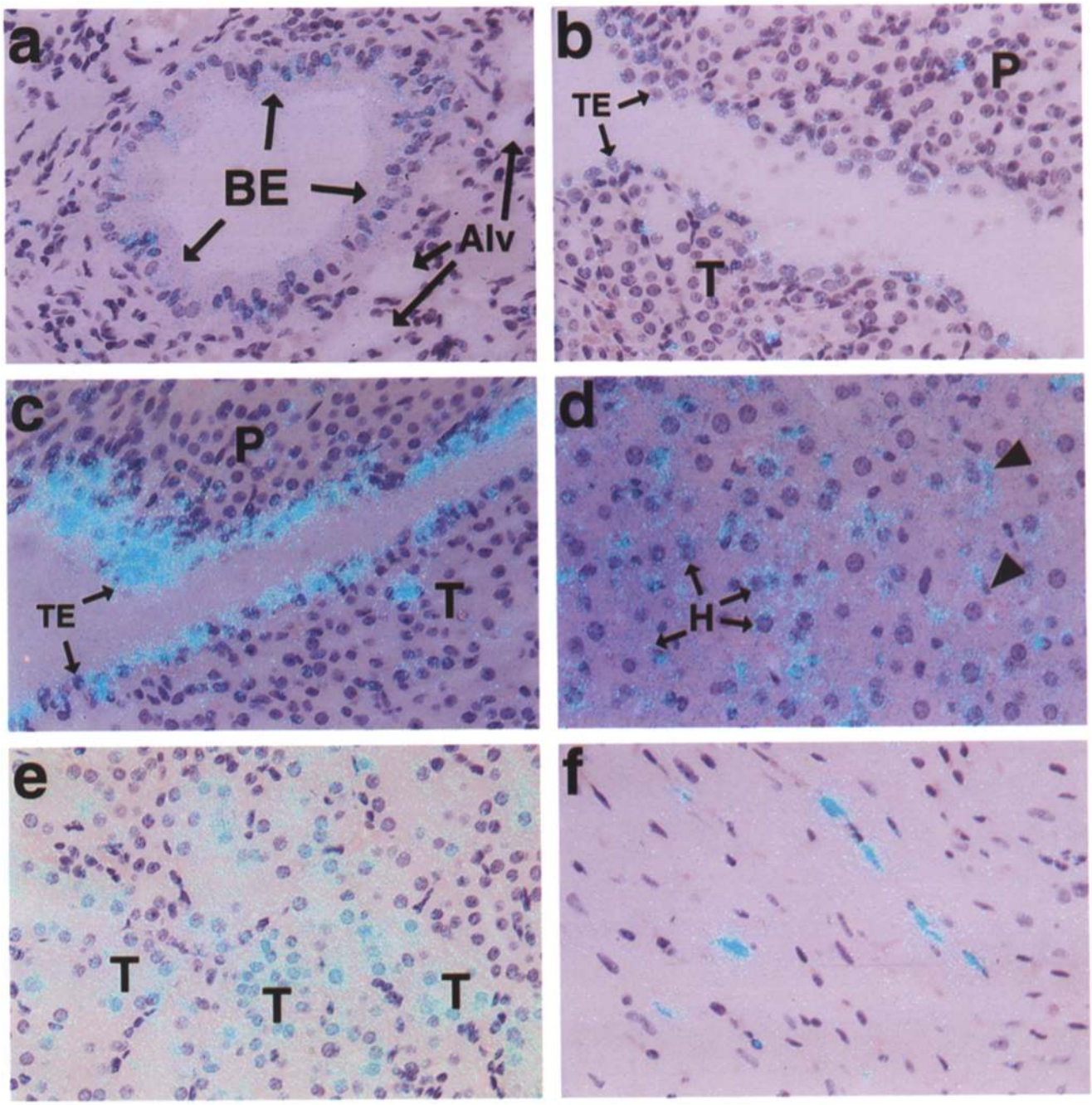
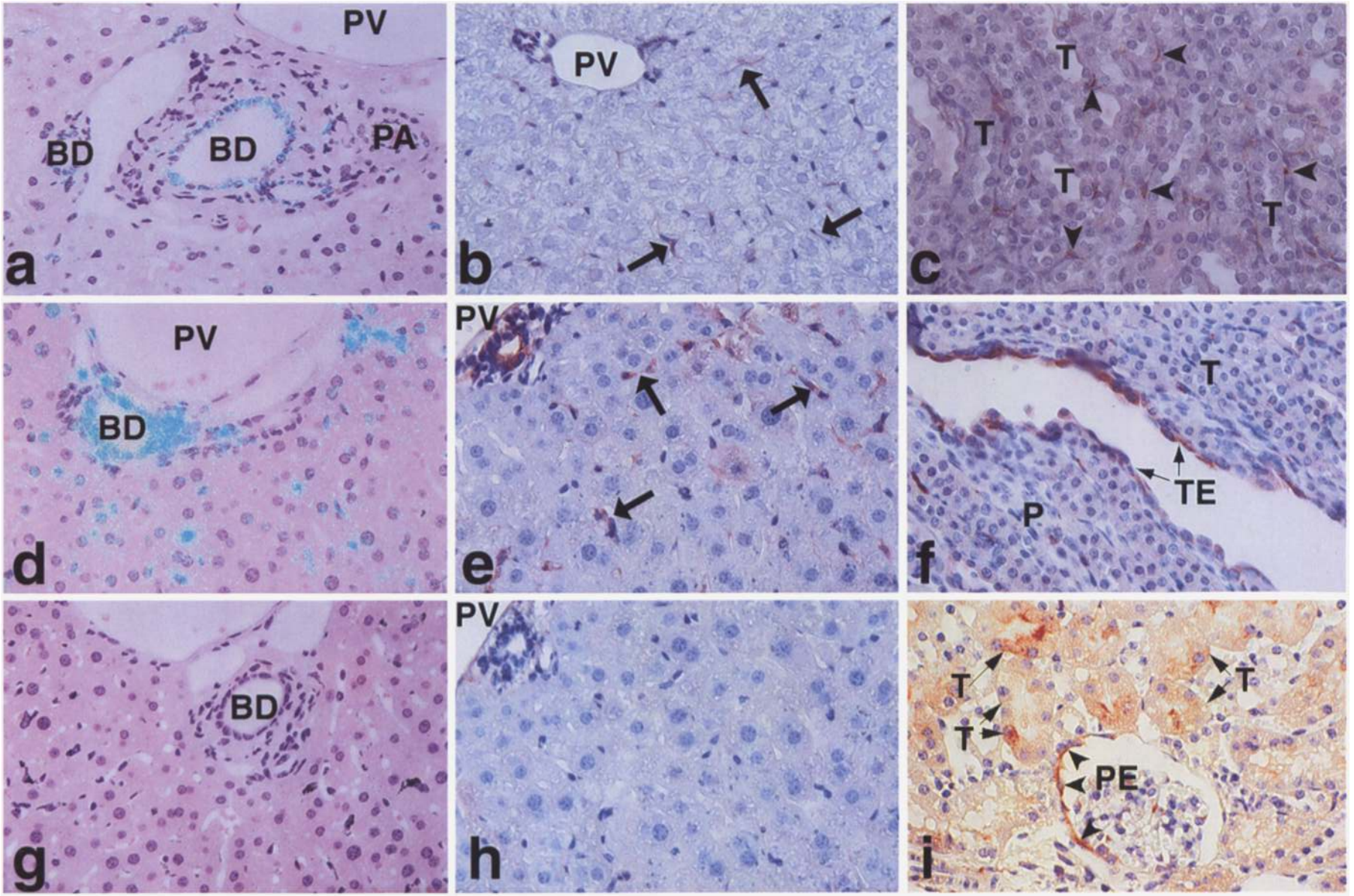


Figure 6. Localization of CD14 mRNA in epithelial cells. Sections of lung, kidney, liver, and heart were analyzed by in situ hybridization for CD14 mRNA. (*a*) Section of lung (×400, 12-wk exposure) from an untreated animal. BE indicates the bronchiolar epithelium and Alv identifies the alveolar spaces. (*b*) Sections of kidney from control (×400, 12-wk exposure) and (*c*) LPS-treated mice (×400, 2 h LPS, 8-wk exposure). Transitional epithelium is indicated by TE and the papilla region by P. T indicates tubules. (*d*) Section of LPS-treated liver (×400, 4 h LPS, 8-wk exposure). Arrowheads indicate Kupffer cells and H indicates hepatocytes. (*e*) Section of LPS-treated kidney (×400, 8 h LPS, 6-wk exposure). (*f*) Section of LPS-treated heart (×400, 2 h LPS, 12-wk exposure). T, tubules.

primarily within myeloid cells, the response to LPS was rapid. In contrast, induction in kidney and liver was observed predominantly within epithelial cells and was delayed relative to that seen in myeloid cells. These differences raise the possibility that induction of CD14 gene expression by LPS may be modulated by two distinct pathways. The initial response is rapid (peaks at 2 h) and can be triggered by low doses (125 ng of LPS per mouse). This may be the result of a direct effect of LPS on the myeloid cells leading to activation of these cells, upregulation of cell surface receptors and production of inflammatory mediators. Upregulation of a receptor by its own ligand has been observed with interleukin-4 (24) and


PV
BD
BD
PA
a
PV
b
T
T
T
T
c
PV
BD
d
PV
e
T
TE
P
f
BD
g
PV
h
T
T
T
PE
i

interleukin-6 (IL-6) (25), with various growth factors (26–28), and with hormones (29–31). According to this hypothesis, the initial, rapid induction in myeloid cells is followed by a second, slower response (peaks at 8–16 h) in cells that appear to be predominantly epithelial in origin. This response appears to require higher concentrations of LPS (2.5 μg per mouse). Previous studies have shown that induction of surface CD14 expression is mediated by an IL-6 autocrine mechanism in U937 cells (32) and Mono-Mac-6 cells (33). In this regard, we have preliminary evidence to suggest that tumor necrosis factor-α (TNF-α) is at least partially responsible for the epithelial response seen in liver and kidney (our unpublished observations). Thus, the delayed response may result from induction of endogenous mediators such as TNF-α, interleukin-1, or IL-6.

Apart from its apparent role as an LPS receptor that mediates activation of myeloid cells, CD14 also appears to serve as an opsonic receptor for LPS-coated particles. Particles opsonized with either LPS binding protein (34) or septin (35) bind to and are engulfed by phagocytes in a CD14-dependent manner, resulting in clearance of LPS. In both rats and rabbits, liver is the main clearance organ for intravenously injected LPS (36, 37). Clearance from the circulation is biphasic, with 40–80% of the injected LPS localizing to the liver and other organs within minutes of injection. In the liver, the LPS is taken up by Kupffer cells in rabbits (37) and sinusoidal cells, granulocytes, and hepatocytes in rats (36). In both species, there is evidence to suggest that the LPS may be cleared from the liver via the bile canicular system into the gut. Indeed, 3 h after LPS injection, bile samples taken from the gall bladder of rabbits contained substantial amounts of LPS, equivalent to that found in plasma (37). Of interest in this regard is our finding that CD14 is expressed in an LPS-inducible manner in Kupffer cells, neutrophils, hepatocytes, and bile duct epithelium, suggesting a possible role for CD14 in the uptake and clearance of LPS from the body.

Although sCD14 has been demonstrated in normal human serum (13) and is increased in sera from septic patients (21), the origin of sCD14 has yet to be determined. It has been assumed that sCD14 is derived from the membrane bound form on myeloid cells either by phospholipase cleavage of the GPI anchor or by protease digestion (13). It could also represent a secreted form of the protein as is suggested by the finding that patients with paroxysmal nocturnal hemoglobinuria have normal sCD14 levels in their sera, although monocytes from these patients do not express CD14 on their surface (38). Our observations that CD14 mRNA is detected in a variety of cells raise the possibility that sCD14 may also originate from non-myeloid cells. Experiments are in progress to determine whether the CD14 synthesized by epithelial cells is a GPI-anchored protein or a secreted protein.

The authors thank K. Roegner and T. Thinnes for their expert technical assistance.

This work was supported by National Institutes of Health grants HL-16411 and HL-31950 (D.J. Loskutoff), and AI-15236 and GM-28485 (R.J. Ulevitch).

Address correspondence to David J. Loskutoff, Dept. of Vascular Biology-VB3, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

Received for publication 23 August 1994 and in revised form 10 November 1994.

References

1. Morrison, D.C., and R.J. Ulevitch. 1978. The effects of bacterial endotoxins on host mediation systems. *Am. J. Pathol.* 93:525–617.
2. Bone, R.C. 1991. The pathogenesis of sepsis. *Ann. Intern. Med.* 115:457–469.
3. Ulevitch, R.J. 1993. Recognition of bacterial endotoxins by receptor-dependent mechanisms. *Adv. Immunol.* 53:267–289.
4. Ziegler-Heitbrock, H.W.L., and R.J. Ulevitch. 1993. CD14: cell surface receptor and differentiation marker. *Immunol. Today.* 14:121–125.
5. Wright, S.D., R.A. Ramos, P.S. Tobias, R.J. Ulevitch, and J.C. Mathison. 1990. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science (Wash. DC).* 249:1431–1433.
6. Lee, J.D., K. Kato, P.S. Tobias, T.N. Kirkland, and R.J. Ulevitch. 1992. Transfection of CD14 into 70Z/3 cells dra-

Figure 7. Localization of CD14 antigen in epithelial cells. Sections of liver and kidney analyzed by immunohistochemistry for CD14 antigen or by in situ hybridization for CD14 mRNA, as described in Materials and Methods. (*a*) Section of liver from a control mouse analyzed for CD14 mRNA (×400, 12-wk exposure). In the portal triad, PV identifies the portal vein; P, the portal artery; and BD, the bile duct. (*b*) Section of liver from a control mouse analyzed for CD14 antigen (×400). Arrows indicate positive Kupffer cells. (*c*) Section of kidney from an untreated mouse stained for CD14 antigen (×400). Arrowheads indicate positive interstitial cells and T denotes tubules. (*d*) Section of liver from an LPS-treated mouse analyzed for CD14 mRNA (×400, 2 h LPS, 8-wk exposure). (*e*) Section of liver from an LPS-treated mouse analyzed for CD14 antigen (×400, 8 h LPS). Arrows indicate Kuppfer cells. (*f*) Section of kidney from an LPS-treated mouse stained for CD14 antigen (×400, 3 h LPS). Transitional epithelium is indicated by TE and the papilla region by P. T indicates tubules. (*g*) Section of liver from an LPS-treated mouse hybridized with a CD14 sense probe (×400, 3 h LPS, 6-wk exposure). (*h*) Section of liver from an LPS-treated mouse incubated with normal rabbit serum instead of rabbit anti-CD14 antiserum (×400, 8 h LPS). (*i*) Section of kidney from an LPS-treated mouse stained for CD14 antigen (×400, 16 h LPS). PE denotes parietal cells and T indicates tubules.

matically enhances the sensitivity to complexes of lipopolysaccharide (LPS) and LPS binding protein. *J. Exp. Med.* 175: 1697–1705.
7. Golenbock, D.T., Y. Liu, F.H. Millham, M.W. Freeman, and R.A. Zoeller. 1993. Surface expression of human CD14 in Chinese hamster ovary fibroblasts imparts macrophage-like responsiveness to bacterial endotoxin. *J. Biol. Chem.* 268:22055–22059.
8. Ferrero, E., D. Jiao, B.Z. Tsuberi, L. Tesio, G.W. Rong, A. Haziot, and S.M. Goyert. 1993. Transgenic mice expressing human CD14 are hypersensitive to lipopolysaccharide. *Proc. Natl. Acad. Sci. USA.* 90:2380–2384.
9. Frey, E.A., D.S. Miller, T.G. Jahr, A. Sundan, V. Bazil, T. Espevik, B.B. Finlay, and S.D. Wright. 1992. Soluble CD14 participates in the response of cells to lipopolysaccharide. *J. Exp. Med.* 176:1665–1671.
10. Read, M.A., S.R. Cordle, R.A. Veach, C.D. Carlisle, and J. Hawiger. 1993. Cell-free pool of CD14 mediates activation of transcription factor NF-kB by lipopolysaccharide in human endothelial cells. *Proc. Natl. Acad. Sci. USA.* 90:9887–9891.
11. Pugin, J., C.-C. Schurer-Maly, D. Leturcq, A. Moriarty, R.J. Ulevitch, and P.S. Tobias. 1993. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. *Proc. Natl. Acad. Sci. USA.* 90:2744–2748.
12. Wright, S.D., R.A. Ramos, A. Hermanowski-Vosatka, P. Rockwell, and P.A. Detmers. 1991. Activation of the adhesive capacity of CR3 on neutrophils by endotoxin: dependence on lipopolysaccharide binding protein and CD14. *J. Exp. Med.* 173:1281–1286.
13. Bazil, V., and J.L. Strominger. 1991. Shedding as a mechanism of down-modulation of CD14 on stimulated monocytes. *J. Immunol.* 147:1567–1574.
14. Weingarten, R., L.A. Sklar, J.C. Mathison, S. Omidi, T. Ainsworth, S. Simon, R.J. Ulevitch, and P.S. Tobias. 1993. Interactions of lipopolysaccharide with neutrophils in blood via CD14. *J. Leukocyte Biol.* 53:518–524.
15. Marchant, A., J. Duchow, J.-P. Delville, and M. Goldman. 1992. Lipopolysaccharide induces up-regulation of CD14 molecules on monocytes in human whole blood. *Eur. J. Immunol.* 22:1663–1665.
16. Pugin, J., D. Heumann, A. Tomasz, V.V. Kravchenko, Y. Akamatsu, M. Nishijima, M.P. Glauser, P.S. Tobias, and R.J. Ulevitch. 1994. CD14 is a pattern recognition receptor. *Immunity.* 1:509–516.
17. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocelluose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.
18. Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
19. Feinberg, A.P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6–13.
20. Keeton, M., Y. Eguchi, M. Sawdey, C. Ahn, and D.J. Loskutoff. 1993. Cellular localization of type 1 plasminogen activator inhibitor messenger RNA and protein in murine renal tissue. *Am. J. Pathol.* 142:59–70.
21. Kruger, C., C. Schutt, U. Obertacke, T. Joka, F.E. Muller, J. Knoller, M. Koller, W. Konig, and W. Schonfeld. 1991. Serum CD14 levels in polytraumatized and severely burned patients. *Clin. Exp. Immunol.* 85:297–301.
22. Setoguchi, M., N. Nasu, S. Yoshida, Y. Higuchi, S. Akizuki, and S. Yamamoto. 1989. Mouse and human CD14 (myeloid cell-specific leucine-rich glycoprotein) primary structure deduced from cDNA clones. *Biochim. Biophys. Acta.* 1008:213–222.
23. Matsuura, K., T. Ishida, M. Setoguchi, Y. Higuchi, S. Akizuki, and S. Yamamoto. 1994. Upregulation of mouse CD14 expression in Kupffer cells by lipopolysaccharide. *J. Exp. Med.* 179: 1671–1676.
24. Dokter, W.H., P. Borger, D. Hendriks, I. Van Der Horst, M.R. Halie, and E. Vellenga. 1992. Interleukin-4 (IL-4) receptor expression on human T cells is affected by different intracellular signaling pathways and by IL-4 at transcriptional and posttranscriptional level. *Blood.* 80:2721–2728.
25. Bauer, J., G. Lengyel, T.M. Bauer, G. Acs, and W. Gerok. 1989. Regulation of interleukin-6 receptor expression in human monocytes and hepatocytes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 249:27–30.
26. Miller, F.D., T.C. Mathew, and J.G. Toma. 1991. Regulation of nerve growth factor receptor gene expression by nerve growth factor in the developing peripheral nervous system. *J. Cell Biol.* 112:303–312.
27. Eriksson, A., M. Nister, P. Leveen, B. Westermark, C.H. Heldin, and L. Claesson-Welsh. 1991. Induction of platelet-derived growth factor alpha- and beta-receptor mRNA and protein by platelet-derived growth factor BB. *J. Biol. Chem.* 266:21138–21144.
28. Saito, H., S. Kasamaya, H. Kouhara, K. Matsumoto, and B. Sato. 1991. Up-regulation of fibroblast growth factor (FGF) receptor mRNA levels by basic FGF or testosterone in androgen-sensitive mouse mammary tumor cells. *Biochem. Biophys. Res. Commun.* 174:136–141.
29. Nilsson, A., B. Carlsson, L. Mathews, and O.G. Isaksson. 1990. Growth hormone regulation of the growth hormone receptor mRNA in cultured rat epiphyseal chondrocytes. *Mol. Cell. Endocrinol.* 70:237–246.
30. Tilly, J.L., P.S. Lapolt, and A.J. Hsueh. 1992. Hormonal regulation of follicle-stimulating hormone receptor messenger ribonucleic acid levels in cultured rat granulosa cells. *Endocrinology.* 130:1296–1302.
31. Hussein, M.O., and W.B. Zipf. 1988. Temporal relationship of the prolactin-dependent LH-induced LH receptor to the LH stimulus. *J. Cell. Physiol.* 134:137–142.
32. Ikewaki, N., and H. Inoko. 1991. Induction of CD14 antigen on the surface of U937 cells by an interleukin-6 autocrine mechanism after culture with formalin-killed Gram-negative bacteria. *Tissue Antigens.* 38:117–123.
33. Labeta, M.O., J.-J. Durieux, G. Spagnoli, N. Fernandez, J. Wijdenes, and R. Herrmann. 1993. CD14 and tolerance to lipopolysaccharide: biochemical and functional analysis. *Immunology.* 80:415–423.
34. Wright, S.D., P.S. Tobias, R.J. Ulevitch, and R.A. Ramos. 1989. Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. *J. Exp. Med.* 170:1231–1241.
35. Wright, S.D., R.A. Ramos, M. Patel, and D.S. Miller. 1992. Septin: a factor in plasma that opsonizes lipopolysaccharide-bearing particles for recognition by CD14 on phagocytes. *J. Exp. Med.* 176:719–727.
36. Freudenberg, M.A., and C. Galanos. 1988. The metabolic fate of endotoxins. *Prog. Clin. Biol. Res.* 272:63–75.
37. Mathison, J.C., and R.J. Ulevitch. 1979. The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. *J. Immunol.* 123:2133–2143.
38. Duchow, J., A. Marchant, A. Crusiaux, C. Husson, C. Alonso-Vega, D. De Groote, P. Neve, and M. Goldman. 1993. Impaired phagocyte responses to lipopolysaccharide in paroxysmal nocturnal hemoglobinuria. *Infect. Immun.* 61:4280–4285.