INFECTION AND IMMUNITY, Nov. 1997, p. 4822–4831 Vol. 65, No. 11
0019-9567/97/$04.00+0

Role of Tumor Necrosis Factor Alpha in Induction of Murine CD14 Gene Expression by Lipopolysaccharide

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Received 11 April 1997/Returned for modification 5 August 1997/Accepted 21 August 1997

We previously demonstrated CD14 gene expression in myeloid and epithelial cells of the mouse and showed that expression of the CD14 gene in both is modulated by lipopolysaccharide (LPS). Here we test the hypothesis that the induction of CD14 in these cells is an indirect effect of LPS, one mediated by tumor necrosis factor alpha (TNF-α). TNF-α induced a transient increase in levels of CD14 in plasma with a peak at 6 to 8 h, and this increase in levels of CD14 antigen in plasma was accompanied by increased levels of CD14 mRNA in lung, liver, and kidney. Moreover, in situ hybridization studies revealed that CD14 mRNA was induced in both myeloid cells and epithelial cells, the same cells that respond to LPS. Pretreatment of mice with anti-TNF antiserum reduced the LPS-mediated increase in levels of CD14 in plasma and significantly reduced the level of induction of CD14 mRNA in selected epithelial cells in the kidney and liver. The antiserum did not appear to block LPS-mediated induction in myeloid cells in the tissues examined. In C3H/HeJ mice, the epithelial response to LPS was markedly attenuated whereas the response to TNF-α was normal. Thus, regulation of CD14 gene expression by LPS differs in epithelial and myeloid cells, with the epithelial responses in kidney and liver being mediated, in part, by TNF-α.

Bacterial lipopolysaccharide (LPS) is a potent activator of myeloid cells, endothelial cells, and other immune cells, initiating a cascade of events that can result in a systemic inflammatory response (4, 31). Many LPS-binding proteins have been identified on cell surfaces (e.g., on the surfaces of CD11/ CD18 and scavenger receptors) or in plasma (e.g., LPS-binding protein and bactericidal/permeability-increasing protein). Cellular activation, however, is thought to be initiated principally through binding of LPS to CD14, a 55-kDa glycoprotein found as a glycosylphosphatidylinositol-anchored protein on the surfaces of myeloid cells and as a soluble protein in plasma (sCD14). Both the membrane-bound form of CD14 (16, 35, 40–42) and the soluble form (12, 14, 19, 33, 34) appear to be involved in LPS signaling.

In vivo, LPS induces a highly predictable temporal order of cytokine appearance, with induction of tumor necrosis factor alpha (TNF-α) being a very early event, followed by induction of interleukin-1 (IL-1) and IL-6 (6, 27, 29). Both TNF-α and IL-1 induce IL-6, and both have the capacity to regulate their own synthesis (11, 27, 29, 43). Moreover, TNF-α and IL-1 have been shown to mediate many of the biologic effects of LPS (3, 5, 27). Thus, upregulation of gene expression by LPS may be the sum of both direct and indirect (i.e., cytokine-mediated) effects. Indeed, such an indirect effect has been shown for U937 cells and Mono-Mac-6 cells, where induction of expression of surface CD14 by gram-negative bacteria or LPS is mediated by an IL-6 autocrine mechanism (23, 26). In addition, administration of exogenous TNF-α has been shown to upregulate murine CD14 expression in vivo (37).

Previous studies (9, 30) have shown that although CD14 expression in myeloid and epithelial cells in vivo is rapidly upregulated by LPS, the increase in epithelial cells was delayed relative to that seen in myeloid cells. The slower response in epithelial cells suggests that induction within these cells may be an indirect effect of LPS, one mediated by endogenous cytokines. This hypothesis is addressed in this paper. Our results demonstrate that TNF-α induces CD14 in epithelial cells and that pretreatment of mice with an anti-TNF antiserum significantly reduces the level of induction of CD14 mRNA in selected epithelial cells of the kidney and liver. The antiserum did not appear to block LPS-mediated induction in myeloid cells in the tissues examined. Thus, regulation of CD14 gene expression by LPS in vivo is complex and the processes of regulation differ in cells and tissues.

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MATERIALS AND METHODS

Tissue preparation. Female CB6 mice (BALB/c/ByJ × C57Bl6/J) or C3H/HeJ mice (The Scripps Research Institute Rodent Breeding Colony), aged 6 to 8 weeks, were used for all experiments. LPS (50 μg, *Escherichia coli* serotype O111:B4; Sigma Chemical Co., St. Louis, Mo.) or recombinant murine TNF-α (10^7 U/mg, 4 μg; kind gift of Richard Ulevitch, The Scripps Research Institute) was diluted in 100 μl of sterile saline (Baxter, Deerfield, Ill.) and injected intraperitoneally into mice anesthetized by inhalation of metofane (methoxyflurane; Pitman-Moore, Mundelein, Ill.). The recombinant TNF-α was tested for LPS contamination by the *Limulus* amebocyte lysate assay (BioWhittaker, Walkersville, Md.) and was shown to contain 0.25 ng of LPS/μg of TNF-α (i.e., 1 ng of LPS per TNF injection). This concentration of LPS had no measurable effect on CD14 gene expression in vivo. Control animals were injected with the same volume of saline alone. At the conclusion of experiments, the mice were anesthetized by inhalation of metofane and exsanguinated. The blood was collected into 20 mM (final concentration) EDTA, pH 8.0, 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 chilled (4°C) 4% (wt/vol) paraformaldehyde in 0.1 M Na phosphate (pH 7.4) for in situ hybridization and immunohistochemistry. The paraformaldehyde-fixed (overnight) tissues were embedded in paraffin blocks and sectioned at 2- to 5-μm thickness with a microtome. The sections were mounted onto Superfrost/Plus slides (Fisher Scientific, Pittsburgh, Pa.) and stored at room temperature pending analysis.

Western blot analysis. Plasma (5 μl per lane) was fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred to nitrocellulose (Schleicher and Schuell, Keene, N.H.). The membranes were soaked in phosphate-buffered saline (PBS) containing 5% powdered milk for 1 h at room temperature to block additional protein binding sites and then incubated at room temperature for 1 h with a polyclonal rabbit antiserum specific for murine CD14 (1:2,000 dilution in PBS containing 0.1% Tween 20) (9). The membranes were washed three times with PBS containing 0.1% Tween 20 and then incubated for 1 h at room temperature with horseradish peroxidase-labeled donkey anti-rabbit antibody (Amersham Corp., Arlington, Ill.). Transferred pro-

teins were detected with ECL Western blot detecting reagents (Amersham Corp.) according to the manufacturer's instructions.

Northern Blot analysis. Total RNAs were prepared from frozen tissues by the acid guanidinium thiocyanate-phenol-chloroform method (7), and their concentrations were determined by measurement of absorbance at 260 nm. Total RNAs (10-μg samples) were analyzed for CD14 mRNA by Northern blotting by standard procedures. The blots were hybridized with a 331-bp fragment of murine CD14 cDNA (nucleotides 847 to 1178) radiolabeled by PCR with primers specific for murine CD14 in the presence of [α-^{32}P]dGTP (>3,000 Ci/mmol; Amersham Corp.). Autoradiography was performed at −80°C with Kodak XAR-5 film and intensifying screens. To verify equal loadings and transfer of the RNAs, Northern blots were rehybridized with a radiolabeled 495-bp probe specific for mouse 18S RNA (nucleotides 583 to 1078). The levels of CD14 mRNAs were quantitated by densitometric analysis of Northern blot autoradiograms with an Ultroscan XL densitometer (LKB, Bromma, Sweden).

Riboprobe preparation. A *Bgl*II/*Hin*dIII fragment of the mouse CD14 cDNA subcloned into the vector pSP73 (9) was linearized and used as a template for in vitro transcription of radiolabeled antisense or sense riboprobes with T7 and SP6 RNA polymerasees, respectively, in the presence of ^{35}S-UTP (>1,200 Ci/mmol; Amersham Corp.). Templates were removed by digestion with RQ1 DNase for 15 min at 37°C, and the riboprobes were purified by phenol extraction and ethanol precipitation.

In situ hybridization. In situ hybridization was performed as described previously (9). Briefly, paraffin-embedded tissues were pretreated sequentially with xylene (three times for 5 min each time), 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (once for 10 min), paraformaldehyde (once for 10 min, 4°C), and proteinase K (1 μg/ml, once for 10 min). Tissue sections 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 of transfer RNA per ml and 600,000 cpm of the ^{35}S-labeled riboprobe, was added, and the tissues were hybridized for 16 to 18 h at 55°C. After hybridization, the tissues were washed with 2× SSC (twice for 10 min each time); treated with RNase (20 μg/ml, once for 30 min); and washed in 2× SSC (twice for 10 min each time), 0.1× SSC (once for 2 h, 60°C), and 0.5× SSC (twice for 10 min each time). Finally, the tissues were dehydrated by immersion in a graded alcohol series containing 0.3 M NH_4 acetate, dried, coated with NTB2 emulsion (1:2 in water; Kodak) and exposed in the dark at 4°C for 4 to 12 weeks. Slides were developed for 2 min in D19 developer (Kodak), fixed, washed in water, and counterstained with hematoxylin and eosin. Photomicrographs were taken by bright-field or polarized-light epiluminescence. To check the specificity of the ^{35}S-labeled CD14 antisense riboprobe, tissue sections from LPS-treated animals were hybridized with a ^{35}S-labeled sense riboprobe. No specific hybridization was detected in any instance (data not shown).

RESULTS

Effect of TNF-α on CD14 gene expression in vivo. In order to examine the potential role of TNF-α in LPS-mediated induction of CD14 gene expression, we first determined whether TNF-α induced CD14 in vivo. CB6 mice received an intraperitoneal injection of recombinant murine TNF-α (160 μg/kg of body weight), while control animals received saline vehicle alone. At various times, whole blood was collected and plasma was prepared for analysis of CD14 protein by Western blotting and selected tissues (lung, liver, and kidney) were removed and analyzed for CD14 mRNA by Northern blotting. The relative levels of CD14 mRNA in the blots were quantitated by densitometric analysis of the autoradiograms. The results of a representative experiment are shown in Fig. 1. Low levels of a single CD14-positive band with an apparent molecular mass of approximately 48 kDa was observed in the plasma from control animals (Fig. 1A). TNF-α induced a transient increase in CD14 in plasma that was first evident 4 h after TNF-α treatment, reached a peak at 6 to 8 h, and declined thereafter (Fig. 1A). A similar, transient induction of CD14 mRNA was detected, with the extent and kinetics of induction differing slightly among tissues (Fig. 1B).

In order to examine the cell-specific expression of CD14 mRNA, tissues from TNF-treated mice were removed and fixed in paraformaldehyde and then analyzed by in situ hybridization with a riboprobe specific for murine CD14 (9). Representative sections from the various tissues are shown in Fig. 2. In agreement with results of a previous study (32), TNF-α administration triggered massive interstitial and intravascular

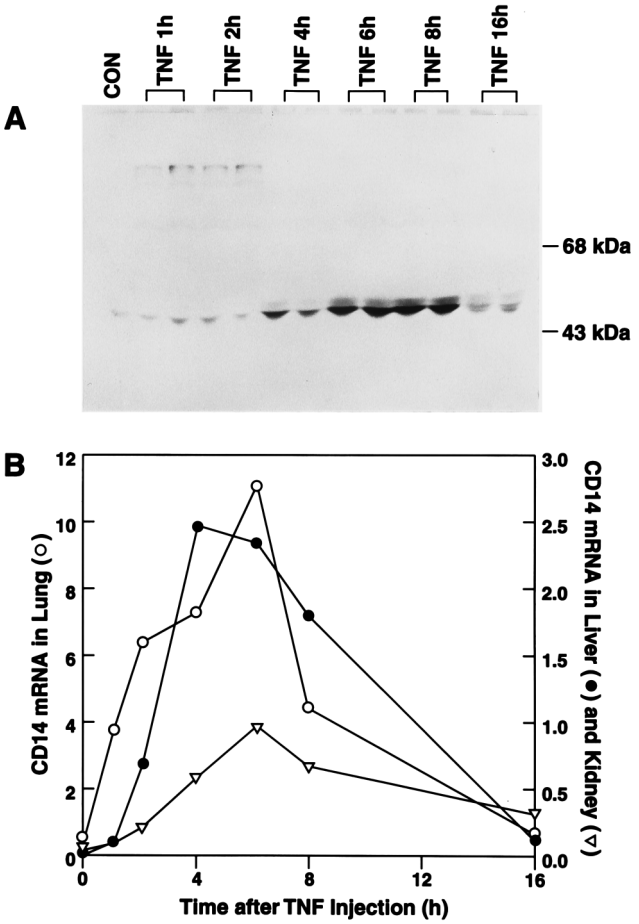

FIG. 1. Time course of induction of CD14 by TNF-α. Mice were injected intraperitoneally with 4 μg of TNF-α, and at the indicated times the animals were sacrificed. Whole blood was collected into 20 mM (final concentration) EDTA, and plasma was prepared. The lungs, livers, and kidneys were removed, and total RNAs were prepared. (A) Plasma samples (5 μl per lane) were electrophoresed under reducing conditions on an SDS–9% polyacrylamide gel, and the proteins were transferred to nitrocellulose membranes. The membranes were then analyzed for CD14 by immunoblotting with a polyclonal rabbit anti-murine CD14 antiserum. The positions of prestained SDS-polyacrylamide gel electrophoresis standards (Bio-Rad Laboratories, Hercules, Calif.) are indicated on the right. CON, control. (B) Total RNAs (10 μg) from the various tissues were analyzed for CD14 mRNA by Northern blot analysis. The relative concentrations of CD14 mRNA were determined by densitometric analysis of the blot autoradiograms. Variations in sample loading were assessed by rehybridizing the blots with a murine 18S cDNA probe. Relative CD14 mRNA levels were determined by dividing the absorbance units per square millimeter for CD14 mRNA by the absorbance units per square millimeter for 18S RNA. Each data point represents the mean of results obtained with two mice.

infiltration of neutrophils into the lungs. The infiltrating neutrophils showed strong induction of CD14 mRNA (Fig. 2A and B). Neutrophil infiltration and expression of CD14 were also observed in the liver (data not shown). Induction of CD14 mRNA was detected also within cells of monocyte/macrophage origin, such as Kupffer cells in the liver and interstitial cells in the kidney (data not shown).

CD14 mRNA was also detected within epithelial cells from the various tissues examined. For example, there was strong induction of CD14 mRNA in the transitional epithelium of the kidney (Fig. 2C) and the tubular epithelium, particularly in the tubules at the cortex-medulla junction (Fig. 2D). In the liver, increased expression of CD14 was observed within the bile duct epithelium (Fig. 2E) and hepatocytes (Fig. 2F). In con-

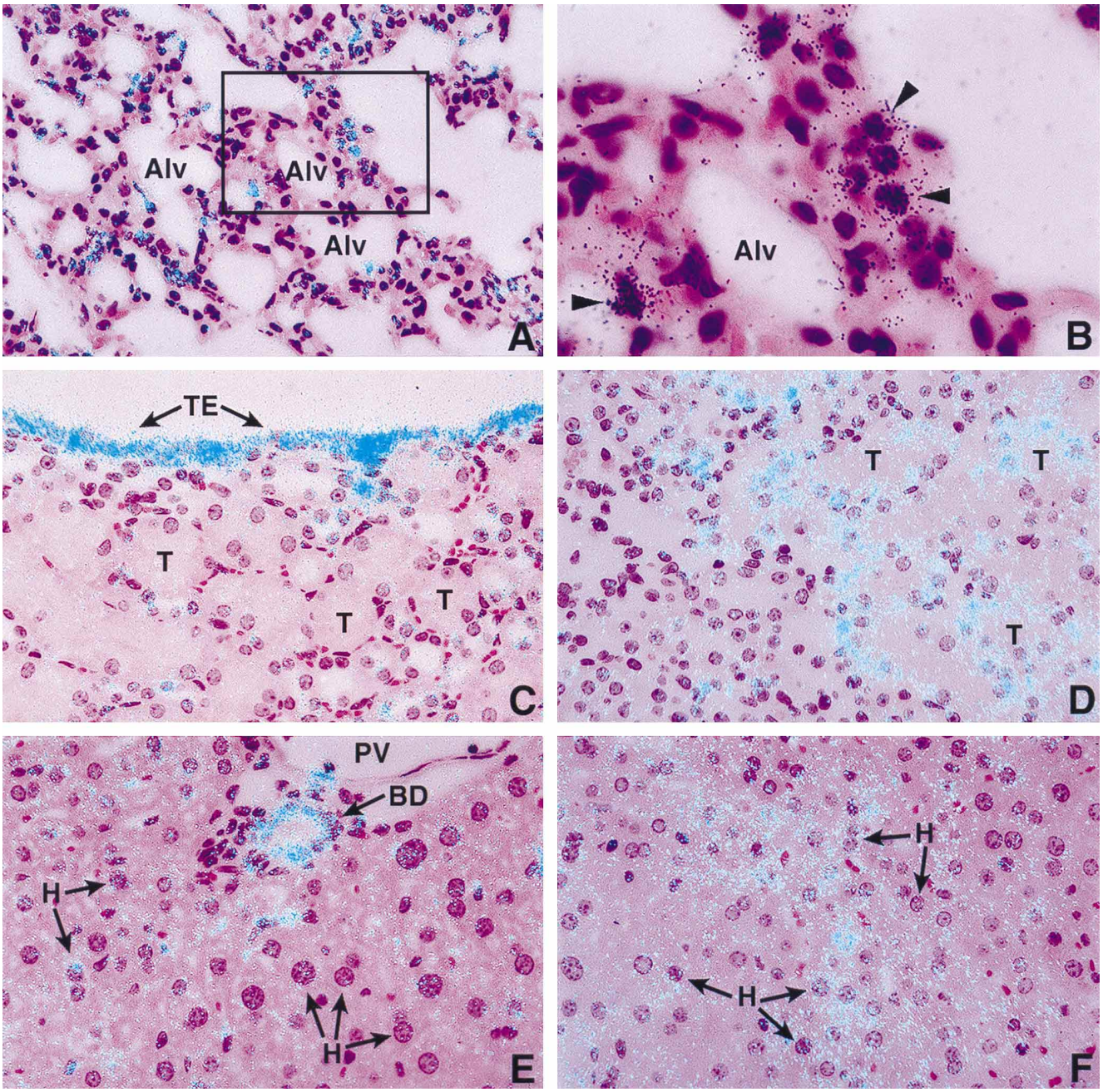

FIG. 2. Localization of CD14 mRNA in murine tissues after TNF-α treatment. Sections of lung, kidney, and liver from mice treated with recombinant TNF-α (4 μg) were analyzed by in situ hybridization for CD14 mRNA as described in Materials and Methods. (A) Section of lung from a mouse treated with TNF-α for 1 h. Alv, alveolar spaces. (B) Magnification (×1,000) of lung section within the boxed area in panel A. Arrowheads indicate positive polymorphonuclear cells within the alveolar septa. (C and D) Sections of kidneys from mice treated with TNF for 3 h (C) or 8 h (D). TE, transitional epithelium; T, tubules. (E and F) Liver sections from mice treated with TNF for 3 h (E) or 8 h (F). In the portal triad, PV identifies the portal vein and BD identifies the bile duct. H refers to hepatocytes. Original magnification, except where indicated, ×400. The exposure time was 8 weeks.

trast, a weak hybridization signal for CD14 was observed in the bronchiolar epithelium in lungs from control animals, and this signal was not increased by TNF-α treatment (data not shown). Thus, TNF-α modulation of CD14 gene expression is analogous to that observed after LPS treatment, with a time-dependent induction in both myeloid cells and epithelial cells.

Effect of an anti-TNF-α antiserum on induction of CD14 gene expression by LPS. Experiments were performed to determine the effects of neutralizing antibodies to TNF-α on the induction of CD14 by LPS. In these experiments, mice received an intraperitoneal injection of a neutralizing rabbit antiserum specific for murine TNF-α (100 μl of serum/mouse, approximately 10^6 neutralization units/ml; supplied by J. Mathison, The Scripps Research Institute) 2 h prior to challenge with 50 μg of LPS. At various times, blood was collected and analyzed for CD14 protein and selected tissues (lung, liver, and kidney) were removed and analyzed for CD14 mRNA.

Pretreatment of mice with the anti-TNF-α antiserum abol-

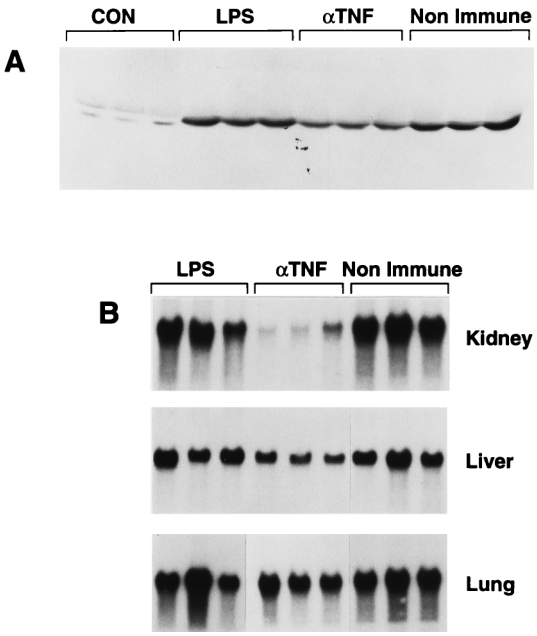

FIG. 3. Effect of neutralizing anti-TNF serum on induction of CD14 by LPS. Two hours prior to challenge with 50 μg of LPS, mice received an intraperitoneal injection of either a neutralizing rabbit antiserum specific for murine TNF-α or nonimmune rabbit serum (100 μl of serum/mouse). (A) The animals were sacrificed at 8 h, blood was collected into 20 mM (final concentration) EDTA, and plasma was prepared and analyzed for CD14 by immunoblot analysis. CON, control. (B) Total RNAs (10 μg) from kidney, liver, and lung were analyzed for CD14 mRNA by Northern blot analysis. Kidney and liver were removed 8 h after LPS treatment, and lung was removed 3 h after LPS treatment. The experiment was performed in triplicate.

ished the increase in TNF-α observed in the plasma 1 h after injection of LPS, reducing levels of TNF-α activity in plasma from 1,650 to 70 U/ml (data not shown). Pretreatment of the mice with the anti-TNF-α antibody reduced levels of CD14 in plasma by approximately 40% at 8 h, while pretreatment with nonimmune serum had no effect (Fig. 3A). Moreover, pretreatment of the mice with the antiserum markedly inhibited induction of CD14 mRNA in kidney and liver but only slightly reduced induction of CD14 mRNA in lung (Fig. 3B). Pretreatment with the antiserum resulted in a greater than 85% reduction in CD14 mRNA levels in kidney 8 h after the LPS injection and in an approximately 50% reduction in CD14 mRNA levels in liver (Table 1). Nonimmune serum had little effect on induction of CD14 mRNA in any of the organs.

In situ hybridization studies demonstrated that the effect of the antibody was relatively restricted, inhibiting induction of CD14 mRNA within some epithelial cells without blocking induction in myeloid cells or in other epithelial cells (Fig. 4 and 5). For example, pretreatment with the anti-TNF antiserum appeared to have no effect on induction of CD14 mRNA in neutrophils in the lung (cf. Fig. 4A with 4C). Similarly, pretreatment appeared to have no effect on CD14 gene expression in cells of monocyte/macrophage origin, such as Kupffer cells in liver (data not shown). In contrast, the immune serum appeared to reduce markedly the signal in hepatocytes 8 h after the LPS injection (cf. Fig. 4D with 4B) and slightly reduced expression in the bile duct epithelium (cf. Fig. 4D with 4B). Nonimmune serum had little or no effect on either the hepatocytes or the bile duct epithelium (cf. Fig. 4F with 4B).

Similarly, pretreatment appeared to have little or no effect on induction in the transitional epithelium in the kidney (cf. Fig. 5C with 5A), whereas the response in tubular epithelial cells at 8 h was greatly diminished after pretreatment with the immune serum (cf. Fig. 5D with 5B). Pretreatment with nonimmune serum appeared to have no effect on CD14 mRNA expression in both the transitional epithelium and the tubular epithelium (Fig. 5E and F, respectively). Thus, the epithelial response to LPS in kidney and liver appears to be mediated, at least in part, by TNF-α.

Induction of CD14 mRNA in C3H/HeJ mice by LPS or TNF-α. C3H/HeJ mice have an autosomal gene defect, *Lps*^{d}, which results in a greatly diminished capacity to respond to LPS (reviewed in reference 39). This global defect is detected in all cells tested and is reflected by an impaired ability to produce prostaglandins or cytokines such as TNF, IL-1, and IL-6 in response to LPS. The attenuated response does not appear to be due to defective receptor function, because there are equal levels of expression of all LPS-binding proteins, e.g., CD11/CD18, CD14, and scavenger receptors, on macrophages from *Lps*^{n} and *Lps*^{d} mice. We, therefore, compared the levels of expression of CD14 in C3H/HeJ mice in response to LPS and to TNF-α. In these experiments, CB6 or C3H/HeJ mice received an intraperitoneal injection of LPS (2 mg/kg) or recombinant TNF-α (160 μg/kg). Blood was collected and selected tissues were removed 1, 3, or 8 h later and analyzed for CD14 protein and mRNA, respectively. The results of a representative experiment are shown in Fig. 6.

Substantial induction of CD14 antigen in plasma was observed in CB6 mice 8 h after treatment with either LPS or TNF-α. Although little or no induction was observed in C3H/HeJ mice after LPS treatment, strong induction was observed 8 h after administration of TNF-α (Fig. 6). In kidney and liver, there was a time-dependent induction of CD14 mRNA in CB6 mice treated with either agent, with the extent of induction being slightly less after TNF-α administration than after LPS administration (Fig. 6). A similar time-dependent induction was observed in C3H/HeJ mice after TNF-α treatment. In this case, induction was equivalent to or slightly greater than that observed when TNF-α was administered to CB6 mice (Fig. 6). In contrast to these results, CD14 mRNA levels in the kidneys of C3H/HeJ mice treated with LPS increased only marginally (approximately 4-fold) whereas those in the livers of these mice increased approximately 20-fold 3 h after the LPS injection (Fig. 6). Treatment of CB6 mice with either LPS or TNF-α increased CD14 mRNA levels in the lungs. Somewhat surprisingly, LPS induced a good CD14 response in the lungs of C3H/HeJ mice. TNF-α treatment resulted in very strong in-

TABLE 1. LPS-induced CD14 mRNA levels in kidney and liver after pretreatment with neutralizing antiserum to TNF-α

Sample (8 h)	Pretreatment antiserum	Mean CD14 level ± SD[a]	P^b
Kidney	None	6.183 ± 2.013	
	TNF-α	0.780 ± 0.615	0.011 (LPS vs TNF)
	Nonimmune	9.267 ± 1.082	0.080 (LPS vs nonimmune)
Liver	None	7.530 ± 1.657	
	TNF-α	3.897 ± 0.452	0.021 (LPS vs TNF)
	Nonimmune	7.350 ± 1.706	0.902 (LPS vs nonimmune)

[a] The relative concentrations of CD14 mRNAs were determined by densitometric analysis of the blot autoradiograms. Variations in sample loading were assessed by rehybridizing the blots with a murine 18S cDNA probe. Relative CD14 mRNA levels were determined by dividing the absorbance units per square millimeter for CD14 mRNA by the absorbance units per square millimeter for 18S RNA.

[b] *P* values were determined by an unpaired *t* test.

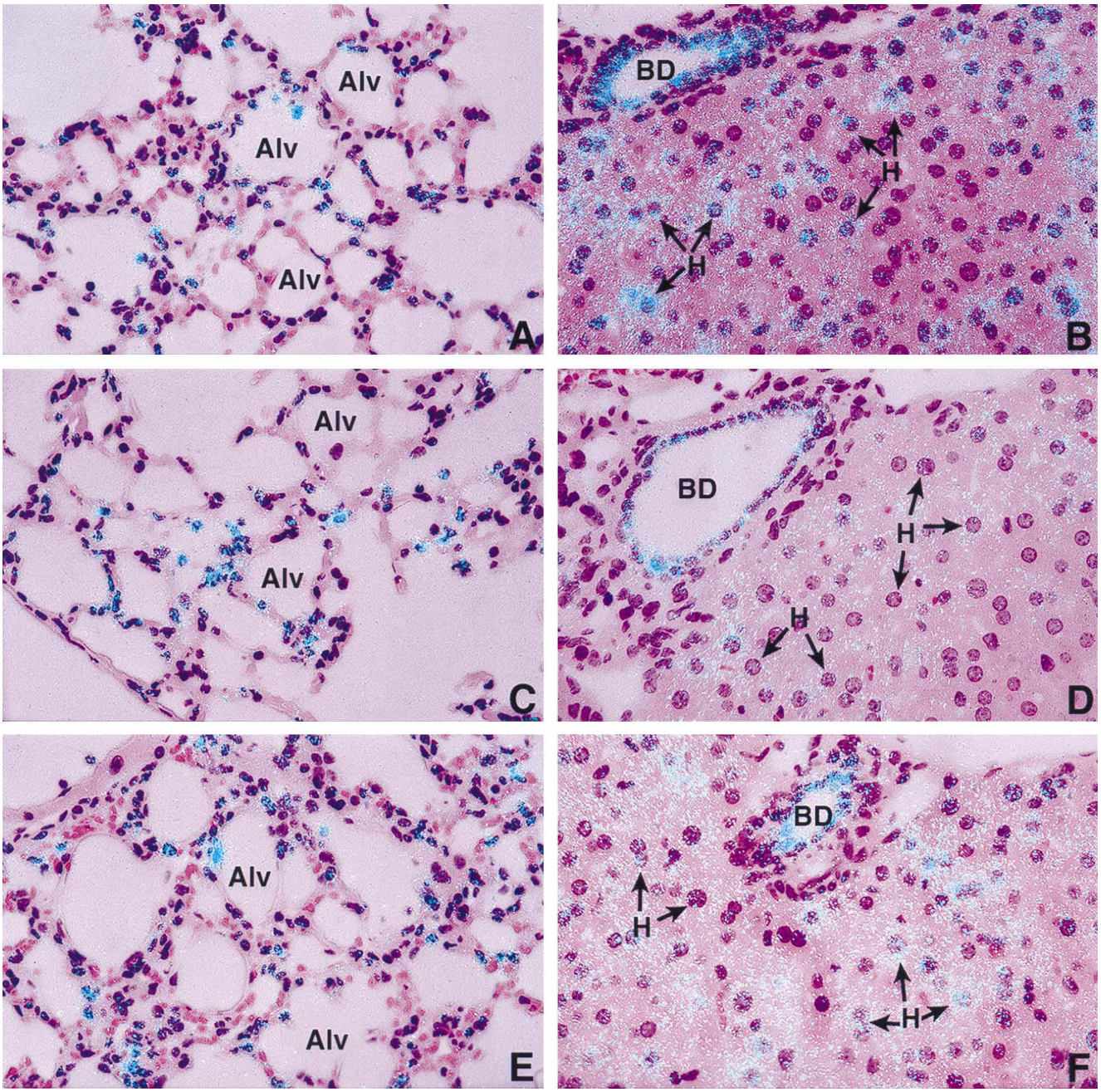

FIG. 4. Effect of neutralizing anti-TNF serum on induction of CD14 mRNA in lung and liver. Mice received an intraperitoneal injection of either a neutralizing rabbit antiserum specific for murine TNF-α or nonimmune rabbit serum (100 μl of serum/mouse) 2 h prior to challenge with LPS (50 μg). Lung (A, C, and E) and liver (B, D, and F) were removed 3 and 8 h, respectively, after LPS treatment and analyzed by in situ hybridization for CD14 mRNA. (A and B) Treatment with LPS alone; (C and D) pretreatment with anti-TNF antiserum; (E and F) pretreatment with nonimmune serum. Slides were exposed for 8 weeks at 4°C. Original magnification, ×400. Alv, alveolar spaces; H, hepatocytes; BD, the bile duct.

duction of CD14 mRNA in the lungs of C3H/HeJ mice (Fig. 6).

In situ hybridization analysis revealed that control C3H/HeJ mice showed a cell-specific expression of CD14 mRNA similar to that described previously for CB6 mice (9) (Fig. 7). For example, there was a low level of expression in the bronchiolar epithelium (data not shown), transitional epithelium (Fig. 7C), and bile duct epithelium (Fig. 7F). Upon LPS treatment, CD14 mRNA was induced in myeloid cells. For example, neutrophils in lung were positive (Fig. 7A and B), as were cells of macrophage/monocyte origin, such as interstitial cells in kidney (data not shown) and Kupffer cells in liver (Fig. 7G). There was also induction in the transitional epithelium in kidney (cf. Fig. 7D with 7C) and bile duct epithelium in liver (cf. Fig. 7G with 7F). However, induction in tubular epithelial cells in kidney (cf. Fig. 7E with 5B) and hepatocytes (cf. Fig. 7H with 4B) was markedly attenuated. The cellular response to TNF-α in C3H/HeJ mice was very similar to that observed in CB6 mice treated with TNF-α. Thus, there was strong induction in both the transitional epithelium and the tubular epithelium in kidney

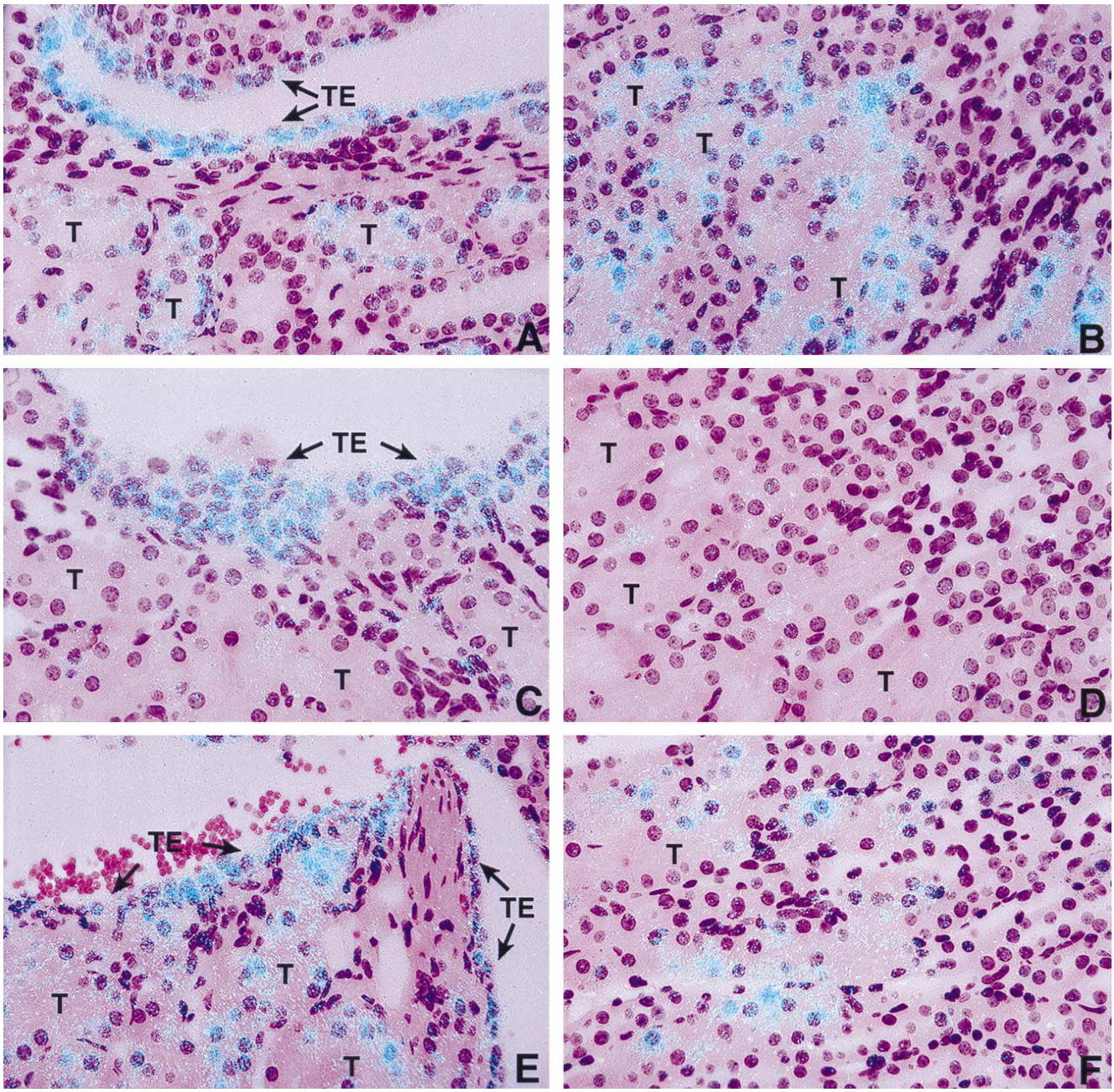

FIG. 5. Effect of neutralizing anti-TNF serum on induction of CD14 mRNA in kidney. Mice received an intraperitoneal injection of either a neutralizing rabbit antiserum specific for murine TNF-α or nonimmune rabbit serum (100 μl of serum/mouse). Two hours later the mice were challenged with 50 μg of LPS. The kidneys were removed 8 h after LPS treatment and analyzed by in situ hybridization for CD14 mRNA. (A and B) LPS alone; (C and D) pretreatment with anti-TNF antiserum; (E and F) pretreatment with nonimmune serum. Slides were exposed for 8 weeks at 4°C. Original magnification, ×400. TE, transitional epithelium; T, tubules.

and in the bile duct epithelium and hepatocytes in liver (data not shown).

DISCUSSION

In this paper we extend our previous studies on the regulation of CD14 gene expression in endotoxemic mice. We show that TNF-α, a major mediator of systemic responses in endotoxemia, induces CD14 gene expression in a time-dependent manner, with increases in CD14 antigen levels in plasma (Fig. 1A) and mRNA levels in tissues (Fig. 1B). In situ hybridization studies demonstrate induction of CD14 mRNA in both myeloid cells and epithelial cells (Fig. 2). We also provide evidence that induction of CD14 mRNA by LPS in some organs is mediated in part by endogenous TNF-α (Fig. 3 to 5). In these experiments, inhibition of induction of CD14 expression by antiserum to TNF-α was observed only in kidney and liver. These two organs demonstrate a delayed CD14 response to LPS within epithelial cells. Preadministration of the TNF-α neutralizing antiserum did not alter the LPS-mediated induction of CD14 mRNA in lung, an organ where CD14 expression is maximal at 2 to 4 h (9) and occurs predominantly within

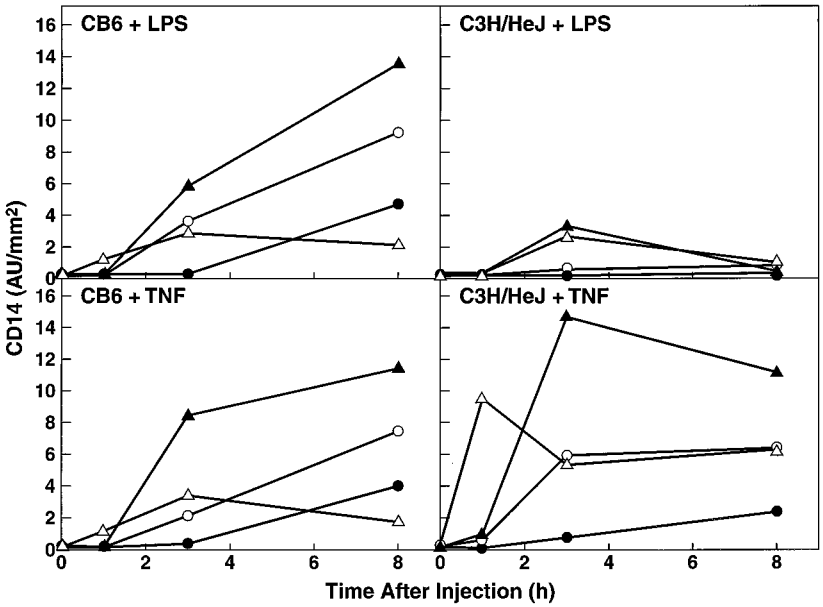

FIG. 6. CD14 induction by LPS and TNF-α in CB6 and C3H/HeJ mice. CB6 or C3H/HeJ mice were injected with either 50 μg of LPS or 4 μg of recombinant murine TNF-α. At the indicated times, blood was collected and various tissues were removed and analyzed for CD14 protein and mRNA, respectively. Plasma (5 μl) was fractionated on an SDS–9% polyacrylamide gel and analyzed for CD14 by immunoblot analysis. The relative concentrations of CD14 protein were then determined by densitometric analysis of the Western blot and are expressed as absorbance units per square millimeter (AU/mm^2). Total RNAs (10 μg) were analyzed for CD14 mRNA by Northern blot analysis. The concentrations of CD14 mRNA, expressed as absorbance units per square millimeter, were determined by densitometric analysis of the blot autoradiogram. Relative CD14 mRNA levels were determined by dividing the absorbance units per square millimeter for CD14 mRNA by the absorbance units per square millimeter for 18S RNA. Each data point represents the result from individual mice. ●, plasma; ○, kidney; ▲, liver; △, lung.

neutrophils. In situ hybridization studies confirmed that the effect of the antiserum appeared to be restricted to the tubular epithelium in kidney and to hepatocytes in liver. The antiserum did not significantly reduce the signal in myeloid cells in any of the organs examined or in the transitional epithelium in kidney and the bile duct epithelium in liver. The results of the C3H/HeJ mice studies corroborated those obtained with the neutralizing antiserum, since CD14 induction by LPS in the tubular epithelium and hepatocytes was attenuated, but a strong response to TNF-α was observed (Fig. 6 and 7). Thus, it appears that induction of CD14 expression may be independent of TNF in some cells (i.e., myeloid cells and some epithelial cells) but require TNF in other cells (i.e., hepatocytes and tubular epithelial cells).

These results confirm and extend the results of Takakuwa et al. (37). Those investigators showed increased levels of CD14 mRNA in murine liver, kidney, spleen, and lung 4 h after intravenous administration of recombinant murine TNF. The effects of TNF appeared to be mediated via TNF receptor (TNFR) 1, since TNFR1-deficient, but not TNFR2-deficient, mice failed to respond to TNF (37). It is interesting that in these studies, in contrast to our own (Fig. 3 to 5), the effect of LPS did not appear to require TNF signaling since LPS induced CD14 expression in TNFR1-deficient mice to approximately the same extent as in wild-type mice. This discrepancy may reflect differences between LPS preparations or routes of administration (intravenous versus intraperitoneal). However, it is more likely that the differences may simply reflect the different times at which the tissues were examined. In this regard, we showed that induction of CD14 by LPS occurred in a time-dependent manner, and, more importantly, that the population of cells producing CD14 changed over time (9). Thus, it is likely that Takakuwa et al. did not observe any TNF-dependent effects on LPS-mediated induction of CD14 mRNA because they measured expression 4 h after injection of LPS, a time when TNF-independent effects appear to predominate. One potentially interesting observation in this regard comes from a recent clinical study of sCD14 levels in the sera of septic patients (8). While no correlation was observed between plasma endotoxin levels and sCD14 levels, there was a good correlation between plasma TNF-α levels and sCD14 levels. These observations support the idea that TNF-α may be involved in sCD14 production. Similarly, in vivo blockade of TNF-α by a single infusion of a chimeric TNF-α-blocking antibody in patients with rheumatoid arthritis significantly diminished concentrations of sCD14 in serum (28).

Although the increase in TNF-α in the circulation in response to a bolus injection of endotoxin may originate from a variety of tissues, there is evidence to suggest that the splanchnic organs are the major sources. In humans, the liver accounts for approximately one-quarter to one-half of the total body TNF production, with maximal hepatic TNF efflux of approximately 7 μg (10). In situ hybridization and immunochemical analysis localized TNF production in liver not only to Kupffer cells but also to hepatocytes and the bile duct epithelium (21, 22). The kidney may also be a source of TNF-α. For example, when transgenic mice bearing the chloramphenicol acetyltransferase (CAT) reporter gene flanked by known TNF regulatory sequences were challenged with endotoxin, the highest level of CAT activity was observed in the kidney (13). No CAT activity could be demonstrated in the liver. Whether this difference is simply due to species differences or an artifact of the transgene is unclear. Other studies have demonstrated expression of TNF in both the transitional and the tubular epithelia in mouse kidney (22, 24). Thus, both liver and kidney appear to be major sources of TNF. This TNF-α may act in a paracrine or autocrine fashion to induce CD14 expression in the epithelia in those organs. These findings emphasize the potential influence that the local production of cytokines may have in determining the course of disease.

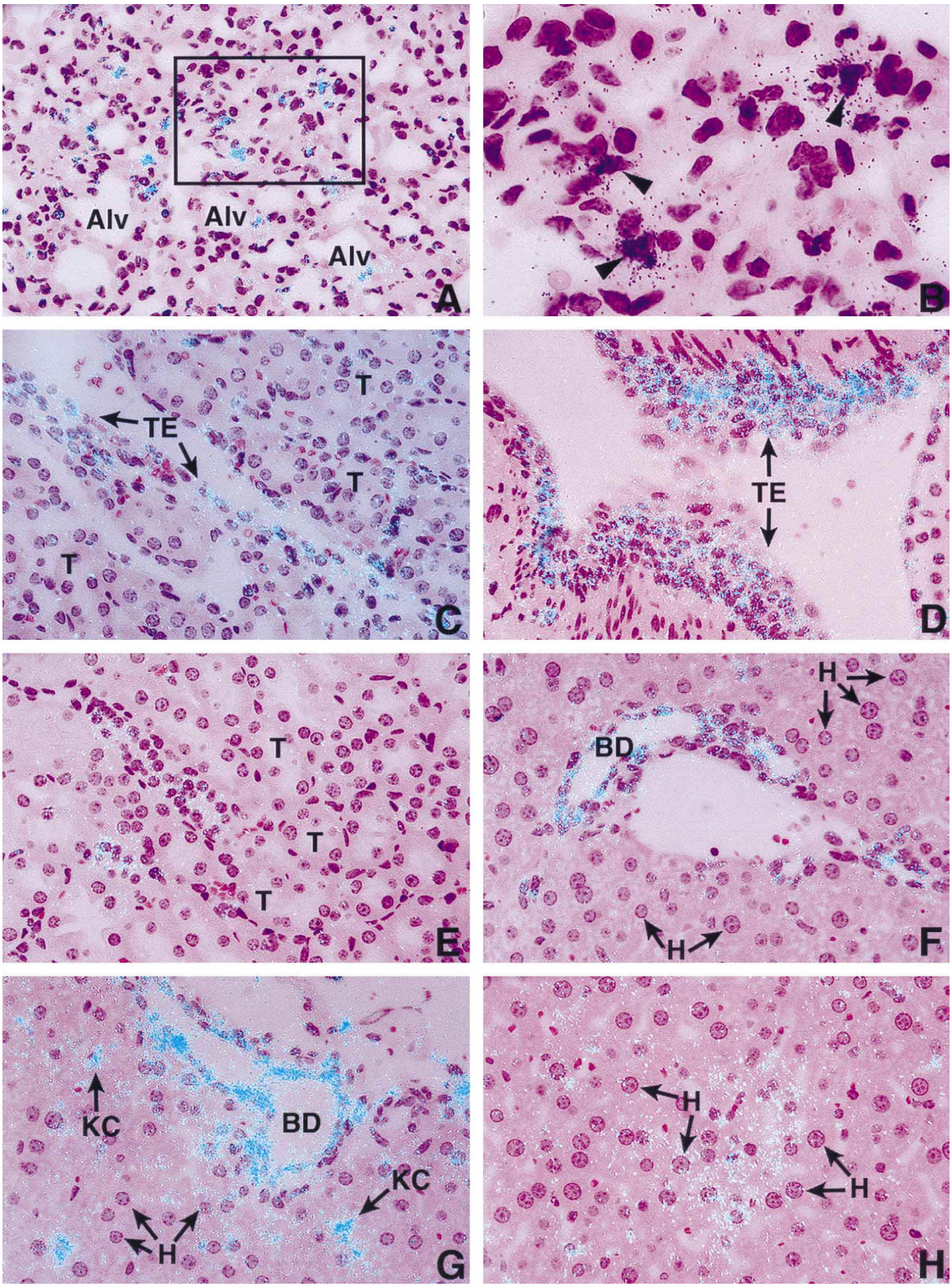

FIG. 7. Localization of CD14 mRNA in tissues of C3H/HeJ mice. Sections of lung, kidney, and liver from control or LPS-treated C3H/HeJ mice were analyzed by in situ hybridization for CD14 mRNA. (A) Section of lung from a mouse treated with LPS (50 μg) for 1 h. Alv, alveolar spaces. (B) Magnification (×1,000) of lung section within the boxed area in panel A. Arrowheads indicate positive neutrophils within the alveolar septa. (C to E) Sections of kidneys from control mice (C) or mice treated with LPS for 3 h (D) or 8 h (E). TE, transitional epithelium; T, tubules. (F to H) Sections of livers from control animals (F) or mice treated with LPS for 3 h (G) or 8 h (H). KC, Kupffer cells; BD, bile duct; H, hepatocytes. Original magnification, except where indicated, ×400. The exposure time was 8 weeks.

Induction of CD14 gene expression by LPS and endogenous TNF-α may have implications in the pathogenesis of systemic inflammatory responses. For example, sCD14 appears to play a dual role in regulating responsiveness to LPS. On the one hand, sCD14 serves to broaden the cellular basis of the response by enabling cells which do not express membrane CD14, such as endothelial and epithelial cells, to respond to LPS (12, 14, 19, 33, 34). In addition, sCD14 has been shown to promote LPS responsiveness in macrophages and neutrophils, cells which do express membrane CD14 (14, 17, 25). On the other hand, sCD14 at high concentrations inhibits release of TNF-α and reactive oxygen intermediates from human mononuclear cells (18, 36) and blocks the binding of fluorescein isothiocyanate-LPS to bovine monocytes (15). In addition, recombinant sCD14 given together with or 10 min after LPS protected mice from LPS-induced mortality (20).

A similar dichotomy of function has been shown with soluble TNF receptors. These protease cleavage products of the high-affinity membrane receptors for TNF are shed after LPS treatment and are present in sera of critically ill patients (38). The function of the soluble TNF receptors appears to be concentration specific. For example, at low concentrations, they enhance TNF bioactivity by stabilizing the trimeric form of TNF-α, thus preventing clearance from serum (1). However, at high concentrations, they neutralize TNF activity in animal models of septic shock (2, 38). By analogy with soluble TNF receptors, therefore, it may be that the low concentrations of CD14 in plasma demonstrated early in the response to LPS may serve to broaden the response to LPS and that the higher concentrations found later in the response, when induction in epithelial cells is maximal, may serve to inhibit responses to LPS.

Although it is assumed that sCD14 in plasma originates from myeloid cells (44), this hypothesis has not yet been proven. Our findings that CD14 mRNA is expressed in a variety of cells (9) raises the possibility that sCD14 may also originate from nonmyeloid cells. It is of interest that substantial induction of plasma CD14 was evident at times when expression in epithelial cells was maximal. In addition, the relative contribution of epithelial cells to the pool of CD14 in plasma can be inferred from the results obtained with pretreatment with the anti-TNF antiserum, since the effect of the antiserum appeared to be relatively specific for the tubular epithelium and hepatocytes without significantly reducing expression of the CD14 gene in myeloid cells in all organs examined. In these experiments, plasma CD14 levels were reduced by 40%, suggesting that the tubular epithelium and hepatocytes may contribute to the pool of sCD14 in plasma after stimulation.

In summary, the effects of LPS on murine CD14 gene expression appear to be both direct and indirect. The direct effects appear to be early and self-limiting, while the indirect effects result in the sustained, elevated production of CD14 by epithelial cells and appear to be mediated in large part by TNF-α.

ACKNOWLEDGMENTS

We thank T. Thinnes for her expert technical assistance.

This work was supported by National Institutes of Health grants GM53011 (C. Fearns) and HL47819 (D. J. Loskutoff).

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Editor: R. E. McCallum