American Journal of Pathology, Vol. 143, No. 1, July 1993

Murine Tissue Factor Gene Expression *In Vivo*

Tissue and Cell Specificity and Regulation by Lipopolysaccharide

Nigel Mackman,* Michael S. Sawdey,† Mark R. Keeton,† and David J. Loskutoff†

From the Department of Immunology and the Committee on Vascular Biology,† The Scripps Research Institute, La Jolla, California*

Regulation of tissue factor (TF) gene expression was studied* in vivo *employing a murine model system. In untreated mice, TF mRNA was detected in brain, lung, kidney, and heart by Northern blot analysis. After administration of lipopolysaccharide, steady-state levels of TF mRNA were unchanged in brain, decreased in heart, and increased in both kidney and lung. In the brain, Bergmann glia within the Purkinje cell layer of the cerebellum and neuroglia within the cerebral cortex expressed TF mRNA by* in situ *hybridization. Epidermal cells of the skin and tongue also expressed TF mRNA. At present, we have not identified the cell type(s) in the kidney and lung responsible for increased TF gene expression. These results demonstrate tissue- and cell-specific TF gene expression* in vivo. *Lipopolysaccharide-mediated increases in TF expression in the kidney and lung may promote fibrin deposition in these organs during Gram-negative sepsis. (Am J Pathol 1993, 143:76–84)

Tissue factor (TF)[1] is the major cellular initiator of the coagulation protease cascades[1,2] and serves as a cell-surface receptor and specific cofactor for plasma factors VII/VIIa.[3] Activation of the coagulation protease cascades by aberrant expression of TF may be responsible for thrombotic episodes in patients with a variety of clinical disorders, including Gram-negative sepsis,[4] atherosclerosis,[5] adult respiratory distress syndrome,[6] systemic lupus erythematosus,[7] Crohn's disease,[8] rheumatoid arthritis,[9] and various forms of cancer.[10,11] Gram-negative sepsis frequently results in septic shock syndrome, characterized by cardiovascular collapse, multiple organ failure, and high mortality. Recently, TF expression was implicated in *Escherichia coli*-induced septic shock in a baboon model. Inhibition of TF activity in this model using a specific monoclonal antibody was found to attenuate the coagulopathy associated with septic shock and protect against lethality.[12] In atherosclerosis, the presence of TF protein in the matrix of the necrotic core of human plaques may contribute to the hyperthrombotic state of atherosclerotic vessels, and possibly to the progression of the disease. Wilcox et al[5] found that macrophage-derived foam cells and monocytes adjacent to the cholesterol clefts in human atheroma contained TF mRNA and protein.

To date, studies on the regulation of TF expression have been performed almost exclusively *in vitro*. In cultured cells, the TF gene is positively regulated by many agents, including lipopolysaccharide (LPS), the toxic principal of Gram-negative bacteria; the inflammatory cytokines interleukin-1 and tumor necrosis factor-α; growth factors such as platelet-derived growth factor, fibroblast growth factor, and transforming growth factor-β; vascular permeability factor; oxidized low-density lipoprotein; and hormones, such as estrogen.[2,13–18] Recently, we identified a 56-bp DNA element within the 5′ flanking region of the human TF gene that confers responsiveness to LPS in cultured THP-1 monocytic cells.[19] Regulation of the TF gene by this large and diverse group of molecules suggests that TF may participate in biological processes other than hemostasis, including cell proliferation and inflammatory responses.

In vivo, TF protein was found to be present in the vascular adventitia, organ capsules, epidermis, and mucosal epithelium of normal human tissues.[20] In ad-

Supported by NIH grant PO1-HL16411 and P50-MH47680 (to NM) and NIH grant HL47819 (to DLJ).

Accepted for publication January 14, 1993.

Address reprint requests to Dr. Nigel Mackman, Department of Immunology, IMM-17, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

dition, tissues known to possess large amounts of TF procoagulant activity, such as brain, lung, and placenta,[21,22] all stained strongly for TF protein.[20,23,24] This cellular distribution suggests that TF represents a hemostatic "envelope," poised to activate the coagulation system when vascular integrity is disrupted.

In spite of these reports, few studies have investigated the regulation of TF biosynthesis *in vivo*. In this paper, we employ a murine model system to demonstrate the presence of relatively high concentrations of TF mRNA in the lung and brain, with intermediate levels in the kidney and heart. Furthermore, we show that experimental administration of LPS increased the steady-state levels of TF mRNA in the kidney and lung. At present, we have not identified the cell type(s) responsible for increased expression of TF mRNA in the kidney and lung following administration of LPS. In the brain, Bergmann glia in the Purkinje cell layer of the cerebellum and neuroglia in the cerebral cortex express TF mRNA. In addition, cell-specific expression of TF mRNA was observed in epidermal cells of the skin and tongue.

Materials and Methods

Experimental Protocols

Adult male CB6 mice (BALB/c/ByJ × C57B16/J; Scripps Clinic Rodent Breeding Colony, La Jolla, CA), weighing 25 to 30 g, were used for all experiments. LPS (*E. coli* serotype O111:B4; Sigma Chemical Co., St. Louis, MO) was diluted to the appropriate concentration in 300 μl sterile saline (Baxter, Deerfield, IL) and injected intraperitoneally into mice anesthetized by inhalation of Metofane (methoxyflurane; Pitman-Moore, Mundelein, IL). Control mice were anesthetized and injected with an equivalent volume of saline alone. At the conclusion of experiments, mice were anesthetized by Metofane inhalation and killed by cervical dislocation. Tissues were rapidly removed by standard dissection techniques, minced, and immediately frozen in liquid nitrogen before preparation of total RNA.

Northern Blot Analysis

Total RNA was prepared from frozen tissues by the acid guanidinium thiocyanate-phenol-chloroform method[25] and its concentration was determined by sample absorbance at 260 nm. Total RNA was analyzed for TF mRNA by Northern blotting as described previously.[26] Briefly, an 821-bp murine TF cDNA probe, containing nucleotides 229–1049, was isolated from plasmid pcmTF2253[27] and radiolabeled by the random primer technique[28] employing [α-^{32}P]dATP (>3000 Ci/mmol; Amersham Corp., Arlington Heights, IL). Autoradiography was performed at −80 C, employing Kodak XAR-5 film with intensifying screens.

The level of TF mRNA was quantitated by densitometric analysis of Northern blot autoradiograms employing an LKB Ultroscan XL laser densitometer (LKB, Bromma, Sweden). To assess variability in sample loading, Northern blots were rehybridized with a radiolabeled plasmid probe carrying a cDNA encoding the housekeeping gene CHO-B.[29]

Tissue Preparation for In Situ *Hybridization*

After sacrifice, mice were immediately perfused for 5 minutes through the left ventricle with 30 ml of chilled 4% (wt/vol) paraformaldehyde in Dulbecco's phosphate-buffered saline (Whittaker Bioproducts Inc., Walkersville, MD). After perfusion, the kidney, lung, heart, brain, skin, and tongue were removed, immersed in chilled 4% paraformaldehyde, and fixed at 4 C overnight. The fixed tissues were then embedded in paraffin blocks and sectioned at 2- to 5-μ thickness using a microtome. The sections were mounted onto polylysine slides and stored at room temperature pending analysis.

Riboprobe Preparation

The 821-bp murine TF cDNA fragment described above was subcloned into the vectors pGEM-3Z and pGEM-4Z (Promega, Madison, WI) and utilized as a template for in vitro transcription of radiolabeled sense (pGEM-3Z) and antisense (pGEM-4Z) TF riboprobes employing SP6 polymerase (Promega) in the presence of [^{35}S]UTP (>1200 Ci/mmol; Amersham). Templates were removed by digestion with RQ1 DNAse (Promega) for 15 minutes at 37 C, and the riboprobes were purified by phenol/chloroform extraction and ethanol precipitation.

In situ *Hybridization*

In situ hybridization was performed as described previously.[5,30,31] Briefly, paraffin-embedded tissue sections were pretreated sequentially with xylene (3 × 5 minutes), 2X SSC (300 mmol/L NaCl, 30 mmol/L sodium citrate, pH 7.0, containing 10 mmol/L 2-mercaptoethanol, 1 mmol/L EDTA) (1 × 10 minutes), paraformaldehyde (1 × 10 minutes, 4 C) and

proteinase K (1 μg/ml in 500 mmol/L NaCl, 10 mmol/L Tris-HCl, pH 8.0) (1 × 10 minutes). (Note: all incubations and washes were performed at 25 C unless specified otherwise.) Slides were prehybridized for 2 hours in 100 μl of prehybridization buffer [50% (wt/vol) formamide, 0.3 mol/L NaCl, 20 mmol/L Tris-HCl, pH 8.0, 5 mmol/L EDTA, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin, 10% (wt/vol) dextran sulfate, 10 mM dithiothreitol] at 42 C. Prehybridization buffer (20 μl) [containing 2.5 mg/ml of tRNA and 600,000 cpm of the ^{35}S-labeled riboprobe] was then added and the slides were hybridized for 18 hours at 55 C. After hybridization, slides were treated with 2X SSC containing 10 mmol/L 2-mercaptoethanol, 1 mmol/L EDTA (2 × 10 minutes), RNAse A (20 μg/ml in 500 mmol/L NaCl, 10 mmol/L Tris-HCl) (1 × 30 minutes), 2X SSC (10 mM 2-mercaptoethanol, 1 mmol/L EDTA) (2 × 10 minutes), 0.1X SSC (10 mmol/L 2-mercaptoethanol, 1 mmol/L EDTA) (1 × 2 hours, 60 C) and 0.5X SSC (2 × 10 minutes). Finally, the slides were dehydrated by immersion in a graded alcohol series containing 0.3 M NH_4Ac, dried, coated with NTB2 emulsion (Kodak; 1:2 in water), and exposed in the dark at 4 C for 8 to 14 weeks. Slides were developed for 2 minutes in D19 developer (Kodak), fixed, washed in water (3 × 5 minutes), and counterstained with hematoxylin and eosin. Consecutive sections were analyzed using a TF sense probe as a control for nonspecific hybridization. No specific signal could be detected in these control hybridizations (for example, see Figure 3, C and J).

Results

Tissue Distribution of Murine Tissue Factor mRNA

To examine the tissue-specific expression of the murine TF gene *in vivo*, total RNA was extracted from tissues of adult male CB6 mice and the level of TF mRNA determined by Northern blot analysis. TF mRNA levels were quantitated by scanning densitometry as described in Materials and Methods, and normalized to the expression of the housekeeping gene CHO-B.[29] Results from a representative animal are shown in Figure 1. Relatively high levels of TF mRNA were observed in lung and brain, whereas intermediate levels were detected in kidney and heart. Low levels of TF mRNA were present in liver and thymus, and no TF mRNA was detected in the spleen, gut, or skeletal muscle.

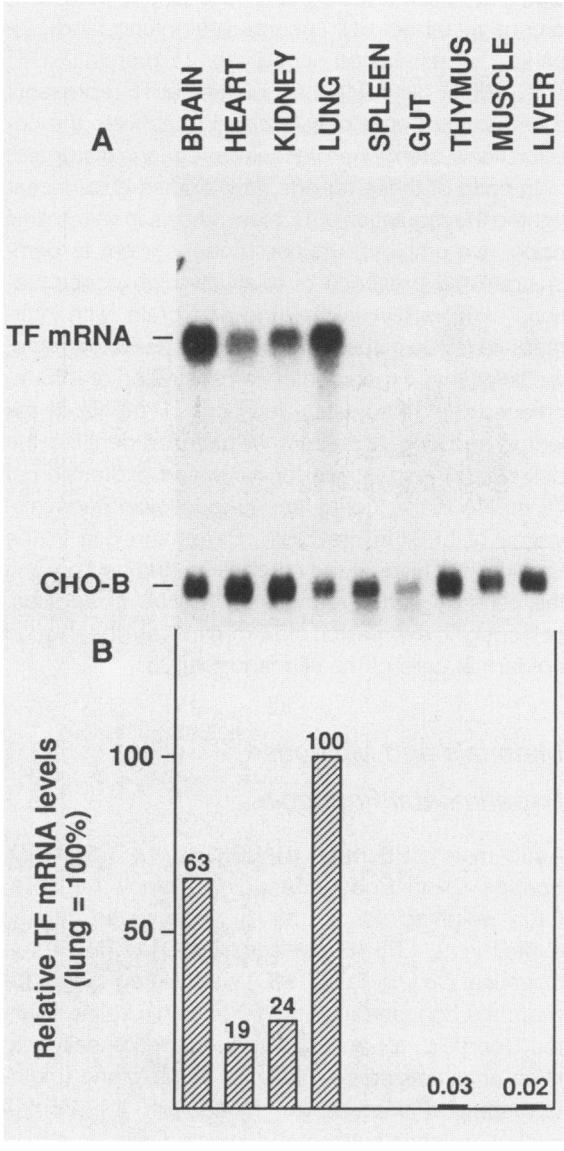

Figure 1. *Tissue specificity of murine TF gene expression.* **A:** *Total RNA was prepared from the indicated tissues and 20 μg was analyzed for TF mRNA content by Northern blot analysis using a* ^{32}P-labeled murine TF cDNA probe (see Materials and Methods). The autoradiogram was exposed for 5 days. To assess for variability in sample loading, the blot was rehybridized with a CHO-B cDNA probe, which detects a single mRNA species of 1.1 kb (shown below). **B:** *The autoradiographic signals for TF and CHO-B mRNAs were quantitated by scanning densitometry (see Materials and Methods) and the values obtained for TF mRNA normalized to those of CHO-B. The normalized data are presented in the histogram beneath each respective lane and are expressed as the percentage of expression relative to the lung.*

Regulation of Murine Tissue Factor Gene Expression by LPS

In the baboon model of septic shock, activation of coagulation has been shown to occur principally via the tissue factor pathway.[12] As a model of Gram-negative sepsis, we assessed the effect of a suble-

thal dose of LPS on murine TF gene expression *in vivo*. Mice were injected intraperitoneally with 50 μg LPS (2.0 mg/kg) or with saline vehicle. Selected tissues (kidney, lung, heart, and brain) were removed at intervals of 1, 3, 8, and 24 hours, and the level of TF mRNA determined by Northern blot analysis. As above, TF mRNA levels were quantitated by scanning densitometry and normalized to the expression of the housekeeping gene CHO-B. No change in TF mRNA levels was observed in these four tissues from mice injected with saline vehicle (data not shown). Results from one of three independent experiments are shown in Figure 2. LPS treatment increased the steady-state levels of TF mRNA in both the kidney (9.6-fold) and lung (2.7-fold), with maximal induction apparent by 8 hours. At 24 hours the level of TF mRNA in the kidney remained elevated, while the amount of TF mRNA in the lung decreased to a level similar to that present in untreated mice. In contrast to the increases observed in the kidney and lung, the level of TF mRNA in the heart decreased 4.5-fold at 8 hours in response to LPS and was still decreased 1.8-fold by 24 hours. In the brain, no significant change in TF mRNA levels was observed during the same time period (Figure 2). Similar results were obtained for all four tissues in three independent experiments. The average fold induction of TF mRNA in the kidney and the lung was 10.8 ± 5.1 (± standard deviation) and 3.1 ± 1.0, respectively.

Cell Type-Specific Expression of Murine Tissue Factor mRNA

In situ hybridization experiments were performed to determine the cellular distribution of murine TF mRNA within various tissues derived from control and LPS-treated animals. Despite previous data indicating that glomerular interstitium, cells of the Bowman's capsule, and myocardium express human TF antigen,[20] high levels of TF mRNA were not detected in cells of the kidney or heart of control or LPS-treated mice (data not shown). In the lung of control mice, a weak hybridization signal for TF mRNA was identified in bronchiolar epithelial cells (Figure 3A), although this signal was not increased after LPS treatment (Figure 3B). Therefore, at present we have not identified the cell type(s) in the kidney and lung responsible for increased TF mRNA expression in response to LPS.

Cell-specific expression of murine TF mRNA was observed in the brain and was identical in control and LPS-treated mice. Figure 3 (D to G), shows the results employing tissue sections from an LPS-treated animal. In the cerebellum, a prominent cell-specific pattern of TF gene expression was observed in the Purkinje cell layer that was visible even at low magnification (×16) (Figure 3D). The Purkinje cell layer consists of large Purkinje neurons surrounded by smaller Bergmann glia.[32] At higher magnification, the strong hybridization signal for TF mRNA was clearly present in Bergmann glia, and no detectable signal was associated with the larger Purkinje cells (Figure 3, E and F). In the cerebral cortex, no TF mRNA was associated with the neuronal cells, whereas neuroglia were observed to express high levels of TF mRNA (Figure 3G). The morphology, distribution, and abundance of these TF mRNA-positive cells suggested that they were astrocytes (see Discussion).

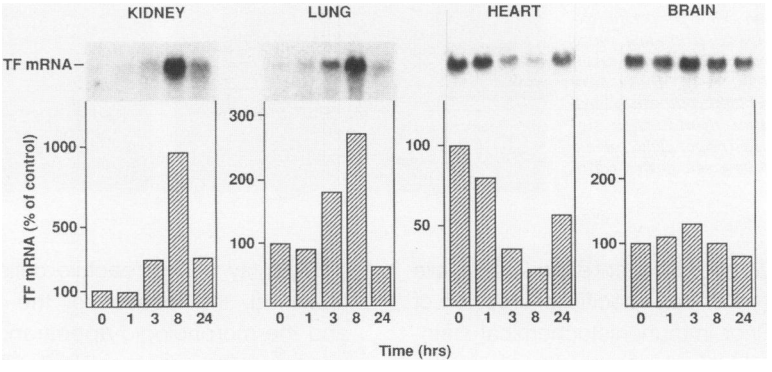

Figure 2. *Time course of changes TF mRNA levels in response to LPS. Mice were injected intraperitoneally with 50 μg LPS and the tissues removed at the indicated times. Total RNA (20 μg for lung, kidney, and heart, 10 μg for brain) was analyzed for TF mRNA by Northern blotting as described in Figure 1. The concentration of TF mRNA was determined by densitometric analysis of the blot autoradiograms. Variations in sample loading were assessed by rehybridizing the blots with a CHO-B cDNA probe as described above (data not shown). The normalized levels of TF mRNA are shown below in the histogram, and are expressed as a percent of the zero time point for each tissue. Note the use of different ordinate scales. The blots used for the kidney and lung time courses were hybridized and stripped one time before hybridization with the murine TF cDNA probe, which may explain the significantly reduced signal intensity of the time 0 samples compared with the signals observed in these tissues in Figure 1. Autoradiograms shown for each time course were exposed for 3 to 5 days to most clearly show the changes in TF mRNA levels.*

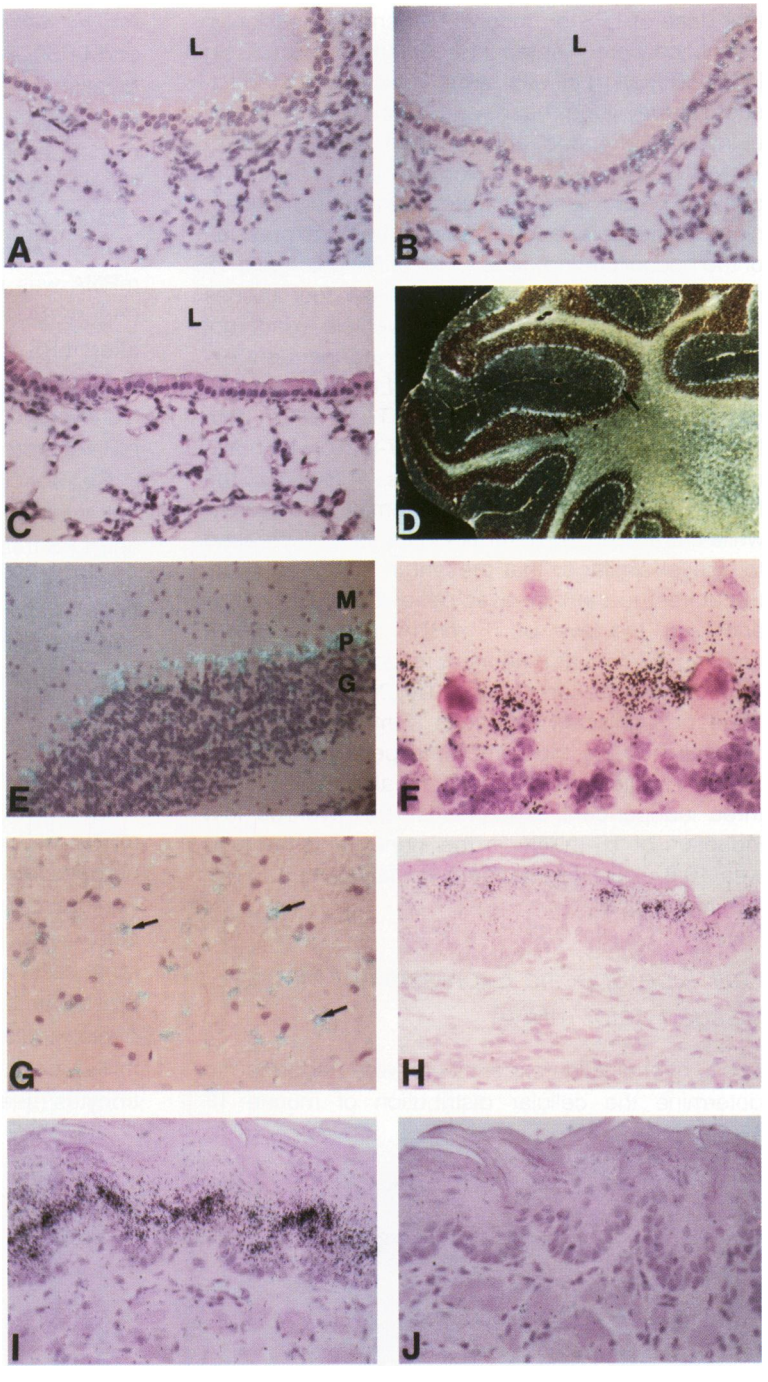

Figure 3. *Localization of TF mRNA in cells of the lung, brain, skin, and tongue. Sections of lung, brain, skin, and tongue were analyzed for TF mRNA by* in situ *hybridization as described in Materials and Methods.* A *and* B: *×400 magnification of lung sections from an untreated and LPS-treated (8 hours) mouse, respectively. Epithelial cells are weakly positive in both panels.* C: *Control experiment showing a lung section from an LPS-treated mouse hybridized with a TF sense probe (×400). In* A *to* C *(L, lumen of a bronchiole)* D: *Low magnification (×16) view of the cerebellum. Arrows indicate the positive signal for TF mRNA in the Purkinje cell layer between the molecular (green) and granular (brown) layers of the cerebellum.* E *and* F; *×200 and ×1000 magnification, respectively, of the Purkinje cell layer. Bergmann glia exhibit a strong signal (M, molecular layer; P, Purkinje cell layer; G, granular layer).* G: *Cerebral cortex of the brain. Arrows indicate the TF mRNA-positive signal expressed by neuroglia (×400).* H: *Skin section from the tail of an untreated animal (×400). The epidermis exhibits an intense hybridization signal in the stratum granulosum.* I *and* J: *Consecutive sections from the tongue of an untreated mouse hybridized with antisense* (I) *or sense* (J) *TF riboprobes, respectively (×400). The stratified squamous epithelium below the cornified dorsal surface is strongly positive in* I. *Unless otherwise stated, all slides were hybridized with a TF antisense probe. Slides were exposed for 8 weeks at 4 C, except for* H *(12 weeks) and* C *(14 weeks).* D *to* G *used tissue sections derived from an animal exposed to LPS for 3 hours. The same cell type-specific expression was observed using untreated mice (data not shown). Photomicrographs were taken either under bright field* (F, H-J), *under dark field* (D) *or using polarized light epiluminescence (remaining panels). Positive hybridization signals appear as dark grains (bright field), white grains (dark field), or white-green grains (dark field and epiluminescence).*

In separate *in situ* hybridization experiments, we extended our analysis of cell-specific expression of murine TF mRNA. Prior immunohistochemical staining for human TF identified intense signals in the epidermis of the skin and in squamous epithelium of the tongue.[20] In murine skin, relatively high levels of TF mRNA were observed in the upper stratum granulosum layer of the epidermis immediately below the stratum corneum (Figure 3H). Although the exact identity of the reactive cells remains to be established, the location of the hybridization signal and the morphologic appearance of the TF mRNA-positive cells are consistent with TF gene expression by keratinocytes. In addition, the distinct polyhedral cells of the stratum spinosum expressed high levels of TF mRNA (data not shown). In murine tongue, we observed a strong positive signal for TF mRNA that was exclusively localized to stratified

squamous epithelium below the cornified dorsal surface (Figure 3I).

As a control for the specificity of the ^{35}S-labeled TF antisense riboprobe, tissue sections derived from lung and brain were hybridized with a ^{35}S-labeled sense riboprobe. No specific hybridization was observed in any of these sections (Figure 3C). Further confirmation of the signal specificity was demonstrated by hybridizing consecutive sections from the tongue with either antisense (Figure 3I) or sense (Figure 3J) TF riboprobes.

Discussion

Thrombotic episodes in patients with a variety of clinical disorders are often correlated with increased expression of TF.[33] Moreover, recent studies using a baboon model revealed that inhibition of TF activity attenuated the coagulopathy associated with septic shock and protected against lethality.[12] However, few studies have investigated the regulation of TF gene expression *in vivo*. In the current study, a murine model system was employed to analyze TF gene expression in control and LPS-treated animals. In control mice, Northern blot analysis of total RNA revealed relatively high levels of TF mRNA in the lung and brain, while intermediate levels were detected in the kidney and heart (Figure 1). A similar tissue distribution of murine TF mRNA was described by Hartzell et al.[13] The distribution of TF mRNA observed in these studies directly correlate with the amounts of procoagulant activity previously reported for the corresponding human tissues.[20–22] Therefore, it appears that expression of murine TF mRNA can be used as an indicator of TF activity in various tissues.

As a model of Gram-negative sepsis, we investigated the regulation of murine TF gene expression *in vivo* in response to LPS. In the heart, we observed decreased expression of TF mRNA in three independent experiments. In contrast, steady-state levels of TF mRNA were increased in the kidney (9.6-fold) and lung (2.7-fold) after treatment with a sublethal dose of LPS (Figure 2). In spite of these changes in TF mRNA levels, at present *in situ* hybridization experiments have not identified specific cells in the kidney and lung responsible for the increased expression of TF mRNA in response to LPS. The most likely explanation for this apparent discrepancy is that in these tissues the TF mRNA may be widely distributed throughout the tissues and thus diluted below the detection threshold of the *in situ* technique. Further studies may allow identification of the specific cell type(s) in the lung and kidney that respond to LPS injection by increasing TF gene expression *in vivo*. In this regard it should be noted that, despite previous reports documenting increased TF expression in cultured human umbilical vein endothelial cells exposed to endotoxin,[16,34] we found no evidence for TF mRNA expression by endothelial cells in lung, kidney, and heart of LPS-treated mice by *in situ* hybridization, consistent with the results of Wilcox et al[35] using tissues from endotoxin-treated rats. The question of whether endothelial cells express TF *in vivo* remains controversial. TF activity was not induced in intact human saphenous veins by endotoxin.[36] In contrast, in a baboon model for lethal *E. coli* sepsis, TF expression was observed in endothelial cells of the spleen, although no expression was observed in endothelial cells of the lung.[37] These data suggest that endothelial cells *in vivo* may behave differently from isolated endothelial cells stimulated in cell cultures. In addition, endothelial cells present in different tissues may be exposed to distinct regulatory factors present in the local environment that influence their ability to express TF.

In a recent study in baboons, TF antigen was observed to increase in lung alveolar epithelial cells and in epithelial cells of the renal glomeruli of septic animals.[37] Therefore, in this murine model, it appears likely that these same cells of the lung and kidney increase TF mRNA expression after administration of LPS. In fact, we have observed induced expression of TF mRNA in alveolar epithelial cells adjacent to the fibrotic lesions in a murine model of bleomycin-induced pulmonary fibrosis.[38] The reason for increased expression of TF mRNA in the lung and kidney of LPS-treated mice is unclear, although these tissues of experimental animals have been found to accumulate high levels of LPS after injection.[39,40] We speculate that LPS-induced increases in TF gene expression in the kidney and lung observed in this study may promote fibrin deposition and contribute to the failure of these organs during Gram-negative sepsis. Thrombotic lesions can be found in the kidneys and lungs of patients exhibiting disseminated intravascular coagulation resulting from exposure to endotoxin.[33]

In contrast to the results with the kidney and lung, a strong and cell-specific *in situ* hybridization signal for TF mRNA was detected in the brain. No change in TF mRNA levels was observed in Northern blots in response to LPS, possibly due to the relatively low concentration of LPS found in this organ after injection.[39,40] In addition, the pattern of cell specific TF mRNA expression revealed by *in situ* hybridiza-

tion was identical in control and LPS-treated mice. In the cerebellum, a prominent hybridization signal was detected in Bergmann glia, a specialized astrocyte in the Purkinje cell layer. In the cerebral cortex and throughout the brain, neuroglia were also observed to express high levels of TF mRNA. The distribution and abundance of the TF mRNA-positive cortical cells suggested that they may be astrocytes. This was confirmed[41] using immunohistochemistry with glial fibrillary acidic protein, a specific marker of astrocytes in the central nervous system,[42] to double-label TF mRNA expressing cells throughout the brain. Expression of high levels of TF in the brain may be primarily required to prevent bleeding in the event of vascular injury. In the brain, astrocytes and Bergmann glia contribute to the glial limiting membrane of the perivascular space, and surround larger blood vessels after they have penetrated the brain substance.[32] In addition, astrocytes throughout the brain are intimately involved in the formation of the tight junctions between endothelial cells that comprise the blood-brain barrier.[43] The expression of TF at these interfaces is thus consistent with its essential role in hemostasis and suggests that TF may play an important role in the maintenance of the blood-brain barrier.

Recently, it has been suggested that components of the coagulation cascade, such as thrombin, may also regulate normal brain development and defend the brain against damage caused by stroke, trauma, and other injuries.[44] TF expression in the brain may be involved in these processes by generating thrombin. An imbalance between thrombin and its primary inhibitors in the brain, namely protease nexin-1 and -2, may contribute to the nerve-cell damage seen in neurodegenerative diseases such as Alzheimer's. This may explain the recent observation of enhanced TF expression in senile plaques of Alzheimer's patients.[45] Furthermore, Purkinje cells have been reported to express the thrombin receptor (S. Coughlin, personal communication). The juxtaposition of TF and thrombin receptor in Bergmann glia and Purkinje cells, respectively, is very provocative and may indicate that TF is involved in cell-cell communication in the cerebellum.

Cell-specific expression of murine TF mRNA also was detected in cells morphologically resembling keratinocytes in the epidermis of the skin and in stratified squamous epithelium of the tongue. The distinct pattern of TF mRNA expression in these tissues illustrates the dramatic differentiation-dependent induction of the TF gene. In addition, these data are consistent with the expression of human TF protein noted previously in the epidermis of the skin and in squamous epithelium of the tongue.[20] Expression of TF at these surfaces would be expected to initiate coagulation after injury, supporting the hypothesis that TF forms a hemostatic "envelope" to limit bleeding.

In summary, these results demonstrate that the TF gene is expressed and regulated in a tissue- and cell-specific manner *in vivo*. Future analysis of TF expression during normal mouse development and in mouse models of important human diseases, such as septic shock and atherosclerosis, should provide insight into the role of TF in hemostasis, development, and other biological processes.

Note Added in Proof

Since the submission of the manuscript, we have identified TF mRNA-positive cells by *in situ* hybridization in lungs of mice treated with LPS for 8 hours. The morphology, abundance, and location at branch points of the interalveolar septa strongly suggested that these cells were alveolar type II cells. These cells did not express detectable levels of TF mRNA in lungs of control mice.

Acknowledgments

We thank many colleagues for helpful discussions, including M. Eddleston and F. Bloom; T. Drake for providing data before publication; the photographic assistance of E. Battenberg; the excellent technical assistance of T. Thinnes, B. Fowler, and R. Fletcher; and the preparation of the manuscript by R. Farrell. This is manuscript number 7424-IMM from the Department of Immunology and the Committee for Vascular Biology.

References

1. Bach RR: Initiation of coagulation by tissue factor. CRC Crit Rev Biochem 1988, 23:339–368
2. Edgington TS, Mackman N, Brand K, Ruf W: The structural biology of expression and function of tissue factor. Thromb Haemost 1991, 66:67–79
3. Broze GJ Jr: Binding of human factor VII and VIIa to monocytes. J Clin Invest 1982, 70:526–535
4. Osterud B, Flaegstad T: Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection related to an unfavourable prognosis. Thromb Haemost 1983, 49:5–7
5. Wilcox JN, Smith KM, Schwartz SM, Gordon D: Localization of tissue factor in the normal vessel wall and in

the atherosclerotic plaque. Proc Natl Acad Sci USA 1989, 86:2839–2843
6. Tipping PG, Campbell DA, Boyce NW, Holdsworth SR: Alveolar macrophage procoagulant activity is increased in acute hyperoxic lung injury. Am J Pathol 1988, 131:206–212
7. de Prost D, Ollivier V, Ternisien C, Chollet-Martin S: Increased monocyte procoagulant activity independent of the lupus anticoagulant in patients with systemic lupus erythematosus. Thromb Haemost 1990, 64:216–221
8. Edwards RL, Levine JB, Green R, Duffy M, Mathews E, Brande W, Rickles FR: Activation of blood coagulation in Crohn's disease. Gastroenterology 1987, 92:329–337
9. Weinberg JB, Pippen AMM, Greenberg CS: Extravascular fibrin formation and dissolution in synovial tissue of patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 1991, 34:996–1005
10. Dvorak HF: Thrombosis and cancer. Hum Pathol 1987, 18:275–284
11. Bauer KA, Conway EM, Bach R, Konigsberg WH, Griffin JD, Demetri G: Tissue factor gene expression in acute myeloblastic leukemia. Thromb Res 1989, 56: 425–430
12. Taylor FB Jr, Chang A, Ruf W, Morrissey JH, Hinshaw L, Catlett R, Blick K, Edgington TS: Lethal *E. coli* septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ Shock 1991, 33:127–134
13. Hartzell S, Ryder K, Lanahan A, Lau LF, Nathans D: A growth factor-responsive gene of murine BALB/c 3T3 cells encodes a protein homologous to human tissue factor. Mol Cell Biol 1989, 9:2567–2573
14. Gregory SA, Morrissey JH, Edgington TS: Regulation of tissue factor gene expression in the monocyte procoagulant response to endotoxin. Mol Cell Biol 1989, 9:2752–2755
15. Scarpati EM, Sadler JE: Regulation of endothelial cell coagulant properties. J Biol Chem 1989, 264:20705–20713
16. Crossman DC, Carr DP, Tuddenham EGD, Pearson JD, McVey JH: The regulation of tissue factor mRNA in human endothelial cells in response to endotoxin or phorbol ester. J Biol Chem 1990, 265:9782–9787
17. Brand K, Fowler BJ, Edgington TS, Mackman N: Tissue factor mRNA in THP-1 monocytic cells is regulated at both transcriptional and posttranscriptional levels in response to lipopolysaccharide. Mol Cell Biol 1991, 11:4732–4738
18. Ranganathan G, Blatti SP, Subramaniam M, Fass DN, Maihle NJ, Getz MJ: Cloning of murine tissue factor and regulation of gene expression by transforming growth factor type β_1. J Biol Chem 1991, 266:496–501
19. Mackman N, Brand K, Edgington TS: Lipopolysaccharide-mediated transcriptional activation of the human tissue factor gene in THP-1 monocytic cells requires both activator protein 1 and nuclear factor κB binding sites. J Exp Med 1991, 174: 1517–1526
20. Drake TA, Morrissey JH, Edgington TS: Selective cellular expression of tissue factor in human tissues: implications for disorders of hemostasis and thrombosis. Am J Pathol 1989, 134:1087–1097
21. Williams WJ: The activity of lung microsomes in blood coagulation. J Biol Chem 1964, 239:933–942
22. Williams WJ: The activity of human placenta microsomes and brain particles in blood coagulation. J Biol Chem 1966, 241:1840–1846
23. Fleck RA, Rao LVM, Rapaport SI, Varki N: Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. Thromb Res 1990, 57:765–781
24. Faulk WP, Labarrere CA, Carson SD: Tissue factor: identification and characterization of cell types in human placentae. Blood 1990, 76:86–96
25. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987, 162:156–159
26. Virca GD, Northemann W, Shiels BR, Widera G, Broome S: Simplified northern blot hybridization using 5% sodium dodecyl sulfate. Biotechniques 1990, 8:370–371
27. Mackman N, Imes S, Maske WH, Taylor B, Lusis AJ, Drake TA: Structure of the murine tissue factor gene: chromosome location and conservation of regulatory elements in the promoter. Arterioscler Thromb 1992, 12:474–483
28. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specificity. Anal Biochem 1983, 132:6–13
29. Harpold MM, Evans RM, Salditt-Georgieff M, Darnell JE: Production of mRNA in Chinese hamster cells: relationship of the rate of synthesis to the cytoplasmic concentration of nine specific mRNA sequences. Cell 1979, 17:1025–1035
30. Wilcox JN, Gee CE, Roberts JL: *In situ* cDNA:mRNA hybridization: development of a technique to measure mRNA levels in individual cells. Methods Enzymol 1986, 124:510–533
31. Keeton M, Eguchi Y, Sawdey M, Ahn C, Loskutoff DJ: Cellular localization of type 1 plasminogen activator inhibitor mRNA and protein in murine renal tissue. Am J Pathol 1993, 142:59–70
32. Palay S, Chan-Palay V: The neuroglial cells of the cerebellar cortex. Cerebellar Cortex Cytology and Organization. Berlin, Springer Verlag, 1974, pp 288–321
33. Levy GA, Cole EH: The monocyte and disseminated intravascular coagulation. Human Monocytes. Edited by M Zembala and GL Asherton. San Diego, Academic Press, 1989, pp 429–438
34. Colucci M, Balconi G, Lorenzet R, Pietra A, Locati D, Donati MB, Semeraro N: Cultured human endothelial cells generate tissue factor in response to endotoxin. J Clin Invest 1983, 71:1893–1896

35. Wilcox JN, Hasko, JA, Deisher TA, Bunting S: Identification of cells expressing tissue factor mRNA be *in situ* hybridization after *in vivo* administration of endotoxin to rats. Thromb Haemost 1989, 62:1084 (abstr)
36. Solberg S, Osterud B, Larsen T, Sorlie D: Lack of ability to synthesize tissue factor by endothelial cells in intact human saphenous veins. Blood Coagul Fibrinol 1990, 1:595–600
37. Drake TA, Cheng J, Chang A, Taylor FB Jr: Expression of tissue factor, thrombomodulin, and endothelial leukocyte adhesion molecule-1 in baboons with lethal *Escherichia coli* sepsis. Am J Pathol 1993, 142:1–13
38. Olman MA, Mackman N, Moser KM, Loskutoff DJ: Induction of type 1 plasminogen activator inhibitor (PAI-1) and tissue factor (TF) mRNAs in a murine model of interstitial pulmonary fibrosis. Am Rev Respir Dis 1992, 145:A841 (abstr)
39. Musson RA, Morrison DC, Ulevitch RJ: Distribution of endotoxin (lipopolysaccharide) in the tissues of lipopolysaccharide-responsive and -unresponsive mice. Infect Immun 1978, 21:448–457
40. Mathison JC, Ulevitch RJ: The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. J Immunol 1979, 123:2133–2143
41. Eddleston MP, de la Torre JC, Oldstone MBA, Mackman N: Tissue factor expression in astrocytes *in vitro* and *in vivo*. Soc Neurosci Abstr 1992, 540:10
42. Dahl D, Björklund H, Bignami A: Immunological markers in astrocytes. Astrocytes: Cell Biology and Pathology of Astrocytes. Edited by S Fedoroff and A. Vernadakis. Orlando, FL, Academic Press, 1986, pp 1–25
43. Janzer RC, Raff MC: Astrocytes induce blood-brain barrier properties in endothelial cells. Nature 1987, 325:253–257
44. Marx J: A new link in the brain's defenses. Science 1992, 256:1278–1280
45. McComb, RD, Miller KA, Carson SD: Tissue factor antigen in senile plaques of Alzheimer's disease. Am J Pathol 1991, 139:491–494