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Endotoxin-induced Cytokine Gene Expression *In Vivo*

II. Regulation of Tumor Necrosis Factor and Interleukin-1α/β Expression and Suppression

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Tumor necrosis factor alpha (TNFα) mRNA is present in a preformed intracellular pool in the spleen, liver, and small bowel of naive rats. Endotoxin (*Salmonella typhus *lipopolysaccharide) injected intravenously induces little or no increase in whole-organ TNF mRNA levels at 15', 30', 1°, 2°, or 4°, whereas serum TNF levels are markedly elevated at 1 and 2 hours. Dexamethasone pretreatment of rats suppresses LPS-induced serum TNF concentrations, but does not suppress TNF mRNA levels in the spleen or bowel. Tachyphylaxis experiments demonstrate that a second injection of endotoxin 2 hours after an initial injection fails to induce a second peak of serum TNF, although TNF mRNA levels in the spleen and bowel remain at the levels found in naive rats. Corynebacterium parvum ***upregulates endotoxin-induced serum TNF release and intravenous injection of IL-1 induces the release of serum TNF but neither alters whole-organ TNF mRNA levels. Interleukin-1 alpha (IL-1α) mRNA was not constitutively detected in whole-organ RNA preparations of the spleen, liver, and small bowel of naive rats. Endotoxin induces IL-1α mRNA most easily appreciated in the spleen beginning at 1 hour, peaking at 2 to 4 hours, and disappearing by 6 hours. Interleukin-1 beta (IL-1β) mRNA was not constitutively detected in the organs examined or was present in small amounts. Endotoxin induces IL-1β mRNA beginning at 0.5 hours, peaking at 1 hour, and disappearing by 6 hours. Dexamethasone pretreatment prevents the LPS-induced appearance of IL-1α mRNA and suppresses but does not completely inhibit the appearance of IL-1β mRNA. C. parvum upregulates endotoxin-induced IL-1 mRNA expression. Intravenous injection of TNF or IL-1 both induce IL-1 mRNA expression. In conclusion, TNF mRNA is constitutively expressed and TNF mRNA levels as analyzed in whole-organ RNA preparations do not change in concert with serum TNF protein levels during conditions of endotoxemia, dexamethasone treatment, tachyphylaxis, priming with C. parvum, or after injection of IL-1. In contrast, IL-1 mRNA expression during endotoxemia, dexamethasone treatment, priming with C. parvum, or after injection of TNF or IL-1 shows clear increases and decreases in whole-organ RNA preparations. (Am J Pathol 1990, 137:1173–1185)***

Endotoxin, a lipopolysaccharide constituent of the cell walls of gram-negative micro-organisms, is a major cause of the local inflammation and systemic symptoms of gram-negative bacterial infection and shock.[1] Endotoxin exerts many of its biologic effects indirectly *via* the expression of a family of so called cytokines that are mediators of inflammation, immunity, and the acute-phase response.[2] Tumor necrosis factor alpha (TNFα), also known as cachectin, may be a proximal mediator in an endotoxin-initiated 'cytokine cascade.' Tumor necrosis factor serum protein levels peak 1 to 2 hours after experimental injection of endotoxin and then subside rapidly.[3] Tumor necrosis factor administered to experimental animals reproduces many of the effects of endotoxin. At low doses, TNF induces neutrophilia and lymphopenia,[4–7] at slightly higher doses it induces necrosis of the tips of intestinal villi,[7] and at much higher doses it induces fatal shock accompanied by hemorrhagic necrosis of multiple viscera.[8] Endotoxic shock is prevented by passive immunization against TNF if the antiserum is given before, but not after, the injection of endotoxin, demonstrating both the significance of TNF in the pathogenesis of endotoxic shock and the temporally

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proximal nature of endogenous TNF expression.[9] Interleukin-1 (IL-1), similarly to TNF, is a multifunctional cytokine and contributes to such diverse proinflammatory phenomena as neutrophilia, lymphopenia, fever, lymphocyte activation, and the acute-phase reaction.[10]

A knowledge of endotoxin-induced TNF and IL-1 gene expression *in vivo* is of fundamental importance to an understanding of the pathogenesis of gram-negative bacterial inflammation. The present study documents the presence of a preformed pool of TNF mRNA in the spleen, liver, and bowel and demonstrates that endotoxin, as well as a variety of other stimuli that affect serum TNF levels, do not induce a dramatic increase or decrease in whole-organ TNF mRNA expression, suggesting either that many cells within these organs constitutively express TNF mRNA and that only a minority cell population responds to endotoxemia by markedly altered TNF transcription or suggesting a post-transcriptional role in the regulation of TNF serum protein expression. The present report also documents that IL-1α and β differ from TNF in the manner and kinetics of mRNA expression *in vivo*. Interleukin-1 mRNA is not constitutively detected at high levels, increases or decreases in response to endotoxin and a variety of other stimuli, and preferentially shows different organs of origin than TNF. The peaks of endotoxin-induced IL-1α and β mRNA expression occur at different times, with IL-1β mRNA levels peaking at 1 hour and IL-1α mRNA levels peaking somewhat later. Finally the present report documents the *in vivo* induction of TNF serum protein and IL-1 mRNA expression by the intravenous injection of these cytokines themselves.

Materials and Methods

Male Lewis rats weighing approximately 250 g, either naive or at specified timepoints after the intravenous injection of *Salmonella typhosa* lipopolysaccharide (Sigma Chemical Co., St. Louis, MO), recombinant human TNFα (gift of Genentech, So. San Francisco, CA, with a specific activity of 6×10^6 U/mg), or recombinant human IL-1β (gift of Dr. Robert Newton, DuPont Glenolden Laboratory, Glenolden, PA with a specific activity of 2×10^4 U/μg), were anesthetized with ether. While the heart was still beating, the organs (spleen, liver, or small bowel) were removed and placed in petri dishes containing 10 ml of 4 mol/l (molar) guanidium isothiocyanate, 25 mmol/l (millimolar) Hepes (pH 7.0), and 0.7 ml β-mercaptoethanol. The organs in an ice bath were immediately minced, crushed, and homogenized for 30 seconds with a Tissuemizer tissue homogenizer. The whole-organ homogenate was centrifuged in a 50-ml conical tube for 10 minutes at 10,000 rpm at 12°C in a Beckman JA-13 rotor (Fullerton, CA). The RNA-containing supernatant was collected and Sarkosyl was added to a final concentration of 0.5%. The supernatant was heated at 65°C for 2 minutes and then ultracentrifuged at 25,000 rpm over a CsCl gradient for 20 hours in a Beckman SW-41 rotor. The RNA pellet was resuspended in 10 mmol/l TRIS HCl, pH 7.4, 5 mmol/l Na_2-EDTA, 1% SDS at room temperature for 1 to 2 hours, extracted with phenol/chloroform/isoamyl alcohol, and precipitated with 1/10 volume 3 mol/l sodium acetate and 2.5 volumes absolute ethanol at −30°C overnight. After drying the pellet in a vacuum desiccator, the yield and purity of RNA was quantitated by measuring the ratio of the absorbances at 260 and 280 nm. Successful isolation of undegraded RNA was monitored by mini-gel electrophoresis in the presence of ethidium bromide and examination of the sharpness of the 28S and 18S ribosomal RNA bands under ultraviolet light. Northern blotting was performed according to standard methodology[11] by electrophoretic separation of total-organ RNA (25 mg/lane) in a formaldehyde agarose gel followed by blotting of the RNA onto a nitrocellulose filter paper (Zeta-probe membrane).

A murine TNF cDNA-containing plasmid (gift from George N. Davetelis) was transformed in our laboratory at the Department of Pathology at UC Irvine, Irvine CA into JM101 cells. The TNF cDNA is inserted in the pUC9 plasmid at the Pst I and Bam HI restriction sites. For the purpose of hybridization, however, Pst I and Eco RI were used as restriction enzymes for the purification of the insert from the vector because Eco RI cleaves off a portion of the 3′-untranslated region of the insert that contains a TTATTTATT consensus sequence that is also common to IL-1 and other cytokines[12] and might result in unwanted cross-hybridization. Murine IL-1α and β cDNA-containing plasmids (gift of Dr. Patrick Gray, Genentech, Inc., South

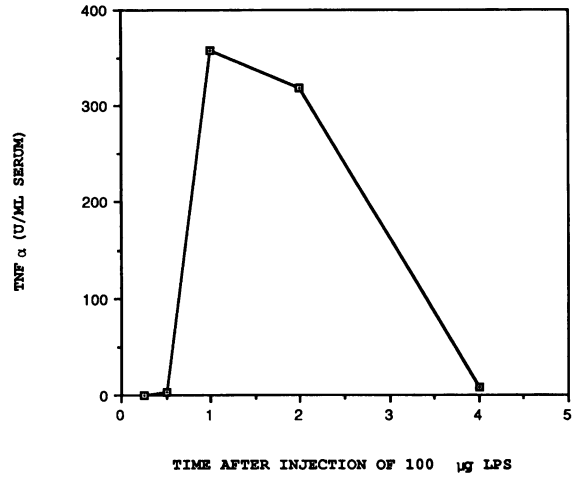

Figure 1. *Endotoxin (100 μg/rat) induces the release of a large amount of TNF into the serum 1 and 2 hours after injection, but no or very little TNF is present before or after the relatively sharp peak.*

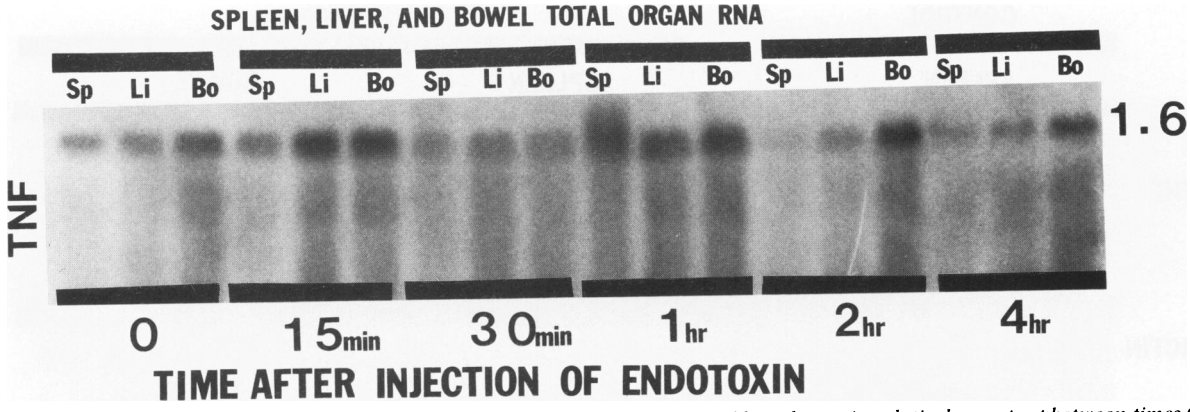

Figure 2. *TNF mRNA levels in whole-organ RNA extracts of the spleen, liver, and bowel remain relatively constant between times 0 and 4 hours after the injection of 100 μg endotoxin per rat. The bowel, however, expresses higher TNF mRNA levels than the spleen and liver. The RNA preparations at each timepoint are from the same rat whose serum TNF protein concentrations are shown in Figure 1.*

San Francisco, CA) were transformed in our laboratory into JM101 cells. Eco RI and Nsi I were used as restriction enzymes for the purification of the IL-1α insert from the vector and Eco RI and Sac I for purification of the IL-1β insert. In both instances, purification of the insert included cleavage of a portion of the 3'-untranslated region of the

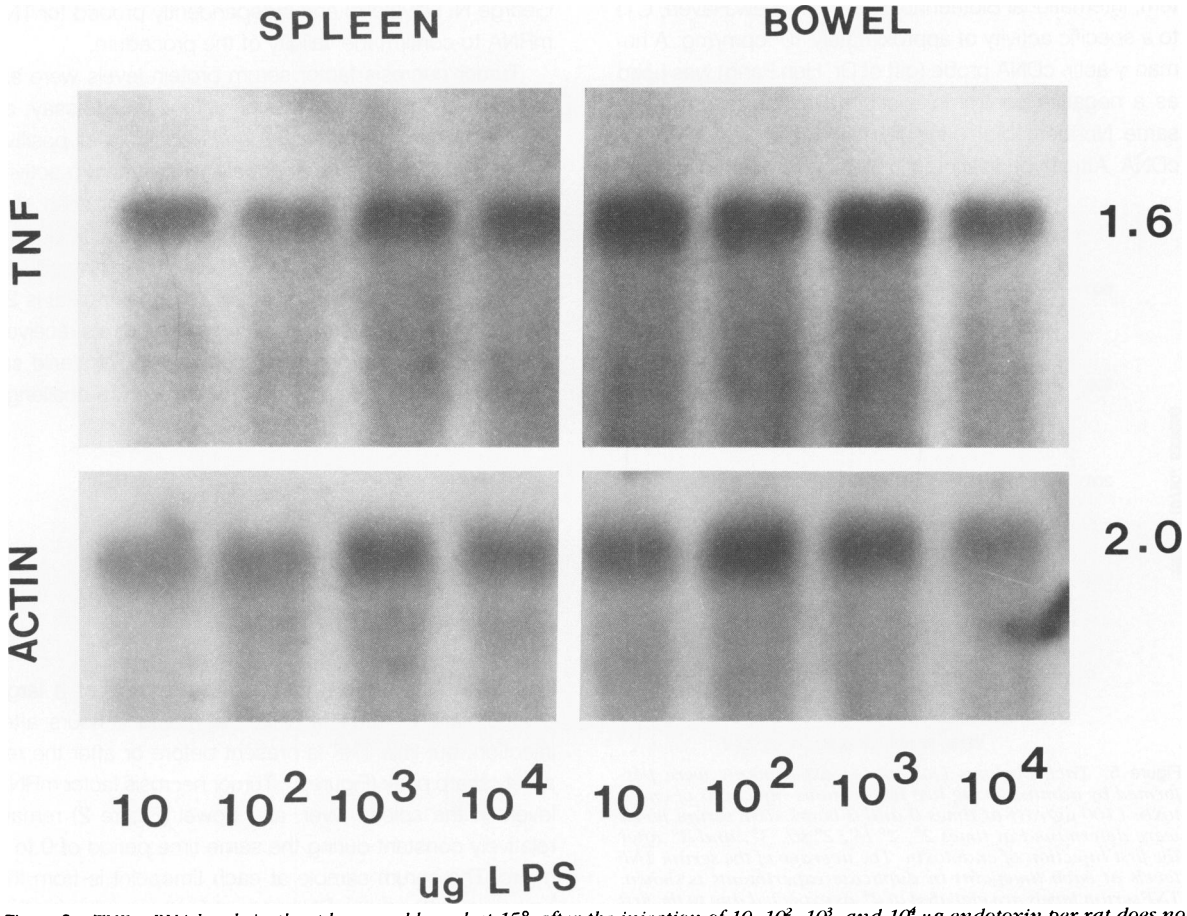

Figure 3. *TNF mRNA levels in the spleen and bowel at 15° after the injection of 10, 10^2, 10^3, and 10^4 μg endotoxin per rat does not show any evidence of a dose-response relationship.*

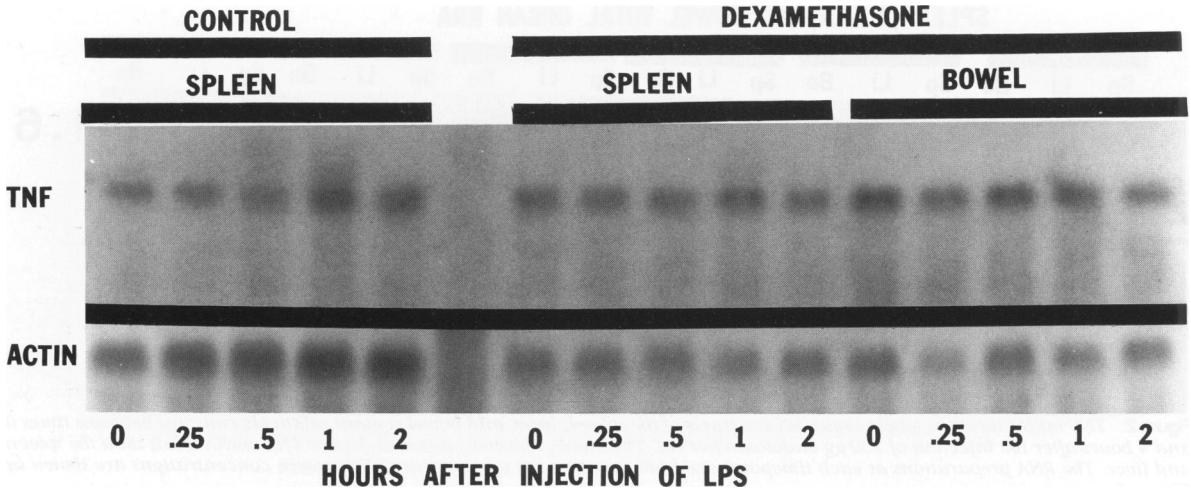

Figure 4. *Dexamethasone pretreatment followed by injection of 100 μg endotoxin does not suppress TNF mRNA levels in the spleen or bowel, although serum TNF protein levels were suppressed in the same rats. TNF mRNA levels in the spleen at corresponding times after injection of 100 μg endotoxin into a saline-pretreated rat are shown on the left-hand side of the Northern blot as a control against which to demonstrate the lack of effect of dexamethasone.*

insert that contains the TTATTTATT consensus sequence. The purified inserts wee labeled with -α^{32}P by random sequence hexanucleotide priming (prime time 'C' biosystem, International Biotechnologies, Inc., New Haven, CT) to a specific activity of approximately 10^9 cpm/mg. A human γ-actin cDNA probe (gift of Dr. Hun Fang) was used as a negative control in a second hybridization of the same Northern blot originally hybridized with the TNF cDNA. After hybridization, the blots were washed at 37°C for 15 minutes, twice with 2X SSC and 0.1% SDS and twice with 0.2X SSC and 0.1% SDS. Representative blots were sent to Rockefeller University (to the laboratory of George N. Davatelis) and independently probed for TNF mRNA to confirm the validity of the procedure.

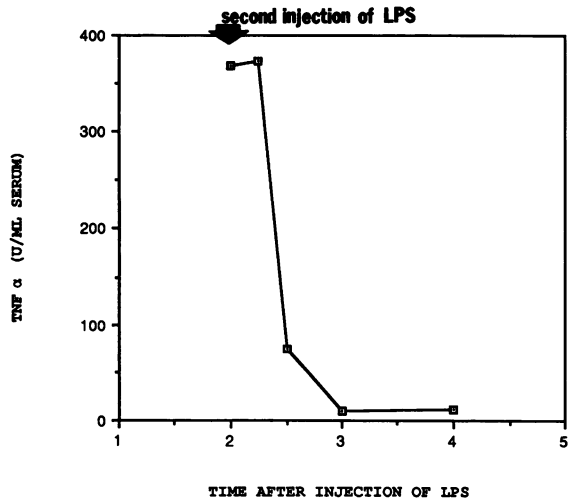

Figure 5. *Tachyphylaxis (tolerance) experiments were performed by administering two intravenous injections of endotoxin (100 μg/rat) at times 0 and 2 hours. TNF serum levels were determined at times 2°, 2° 15', 2° 30', 3°, and 4° after the first injection of endotoxin. The average of the serum TNF levels at each timepoint in duplicate experiments is shown. TNF serum levels are elevated at 2° as expected due to the first injection of endotoxin, but TNF levels are not elevated at 1° or 2° after the second injection of endotoxin.*

Tumor necrosis factor serum protein levels were assayed in a double-blind fashion with a WEHI assay, as previously described,[13] and the specificity of all positive results was confirmed by neutralization of serum activity with an anti-TNF serum obtained by immunization of a rabbit with human recombinant TNF. The specific activity of TNF units in the circulation (as determined by adding known amounts of TNF to control serum samples) is 22 U/ng TNF. *Corynebacterium parvum*-primed rats received 1.5 mg whole cells per rat dissolved in 1 ml sterile saline and injected intravenously 1 week before challenge with LPS.

Results

Effect of Endotoxin on TNF mRNA and Serum Protein Expression

Endotoxin (100 μg/rat) induces the release of a large amount of TNF into the serum at 1 and 2 hours after injection, but little TNF is present before or after the relatively sharp peak (Figure 1). Tumor necrosis factor mRNA levels in the spleen, liver, and bowel (Figure 2) remain relatively constant during the same time period of 0 to 4 hours. The serum sample at each timepoint is from the same rat as the organ RNA extract for the corresponding timepoint. The constancy of TNF mRNA levels was con-

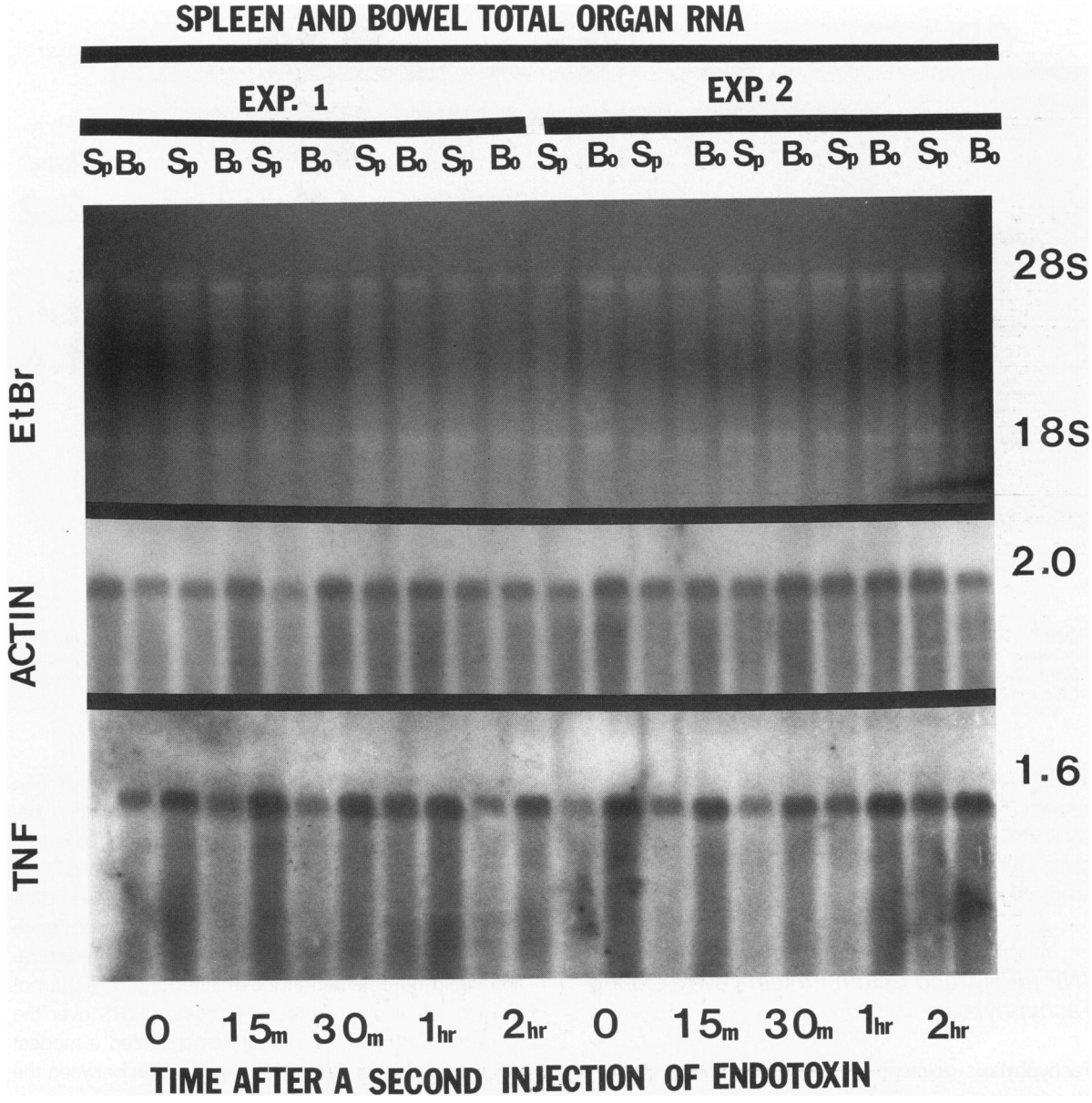

Figure 6. *TNF mRNA levels were studied in the spleen (Sp) and bowel (Bo) of the rats of the tachyphylaxis experiments shown in Figure 5. TNF mRNA levels remain constant at 0, 15′, 30′, 1°, and 2° after the second injection of endotoxin (corresponding to 2°, 2° 15′, 2° 30′, 3°, and 4° after the first injection of endotoxin).*

firmed in spleen and bowel RNA preparations from a second group of rats killed at times 0, 15′, 30′, 1°, and 2° (data not shown). The bowel (Figures 2, 3, and 6) almost always expresses higher TNF levels than the spleen or liver, an interesting observation in light of the sensitivity of the bowel to mucosal necrosis[7] and hemorrhagic infarction[8] during endotoxemia. Tumor necrosis factor mRNA levels in the spleen and bowel at 15° after the injection of 10, 10^2, 10^3, or 10^4 μg endotoxin (Figure 3) were examined to determine the presence or absence of a dose–response relationship between dose of endotoxin and level of whole-organ TNF mRNA. No dose–response relationship is present.

Effect of Dexamethasone on TNF mRNA and Serum Protein Expression

Dexamethasone (50 mg/kg, Organon, West Orange, NJ) injected intravenously 5 hours before the injection of endotoxin (100 μg) strongly suppressed serum TNF expression, as reported by previous investigators.[3,16] Tumor necrosis factor serum levels in dexamethasone-pretreated rats at times 1° and 2° after the injection of endotoxin were 153 and 7.3 U/ml, respectively, compared to averages of 359 and 351 U/ml at 1° and 2° after injection of the same amount of endotoxin in unpretreated rats. Dexamethasone pretreatment did not, however, suppress TNF

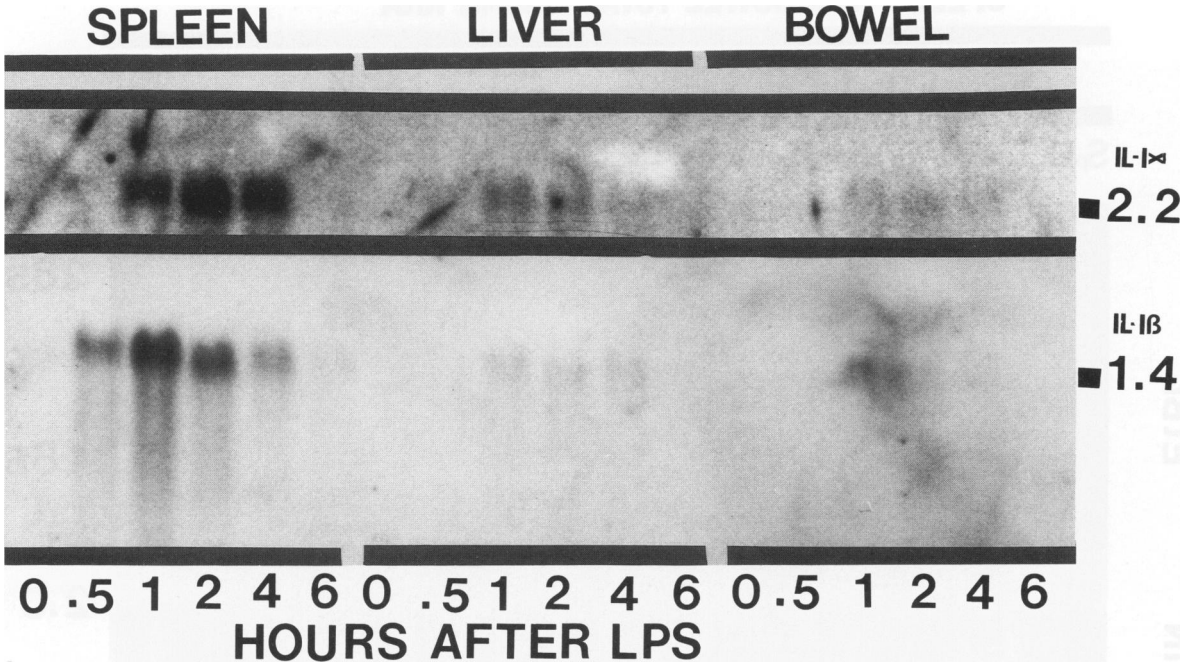

Figure 7. *IL-1 α/β mRNA are not present in the spleen, liver, or bowel of naive rats in this preparation, although IL-1β mRNA is sometimes constitutively detected in the spleen of naive rats. IL-1α mRNA expression peaks at 4 hours after injection of endotoxin (100 μg/rat). IL-1β mRNA expression peaks earlier at 1 hour after injection of endotoxin. IL-1 α/β mRNA are much more prominent in the spleen than in the liver or bowel, unlike TNF mRNA, which is generally most prominent in the bowel.*

mRNA levels in duplicate experiments (one experiment shown in Figure 4). The first five lanes of the Northern blot show TNF mRNA levels in the spleen of saline-pretreated control rats that did not receive dexamethasone before an injection of the same dose of endotoxin.

TNF mRNA and Serum Protein Levels During Tachyphylaxis

Tachyphylaxis (tolerance) experiments were performed by administering two intravenous injections of endotoxin (100 μg) at times 0 and 2 hours. Tumor necrosis factor serum and organ mRNA levels were determined at times 2 hours, 2° 15′, 2° 30′, 3°, and 4°. Tumor necrosis factor serum levels were elevated at 2°, as expected due to the first injection of endotoxin, but TNF levels were not elevated at 1° and 2° after the second injection of endotoxin (Figure 5), indicating the development of tolerance in this parameter. Tumor necrosis factor mRNA levels, however, remained constant (Figure 6).

Effect of Endotoxin and Dexamethasone on IL-1α mRNA Expression

Interleukin-1α mRNA was not detected in the spleen, liver, or bowel of naive rats (ie, at time 0) nor at 30 minutes after injection of 100 μg LPS (Figure 7). Interleukin-1α mRNA expression was most prominent in the spleen, beginning at 1 hour and peaking at 2 to 4 hours. Interleukin-1α mRNA expression returned to baseline again at 6 hours after the injection of LPS. Lower levels of IL-1α mRNA than in the spleen were seen in the liver and bowel (Figure 7), although in some endotoxin-treated rats no hybridization could be detected in these latter organs (data not shown). Injection of increasing doses of LPS over the range of 1, 10, 10^2, to 10^3 μg demonstrated a modest increase in splenic IL-1α mRNA expression between the lower and higher doses of LPS at 2 hours (data not shown). Dexamethasone pretreatment (50 mg/kg dexamethasone injected intravenously immediately before the injection of 100 μg LPS) inhibited the appearance of splenic IL-1α mRNA at all timepoints (Figure 8).

Effect of Endotoxin and Dexamethasone on IL-1β mRNA Expression

Interleukin-1β mRNA was constitutively detected either only in small amounts or not at all in the spleens of naive rats. Interleukin-1β mRNA was not constitutively detected in the liver and bowel of naive rats. Lipopolysaccharide induced an increase in splenic IL-1β mRNA by 0.5 hours, with a peak of mRNA expression at 1 hour and a gradual return to near-baseline levels of mRNA by 6 hours (Figure

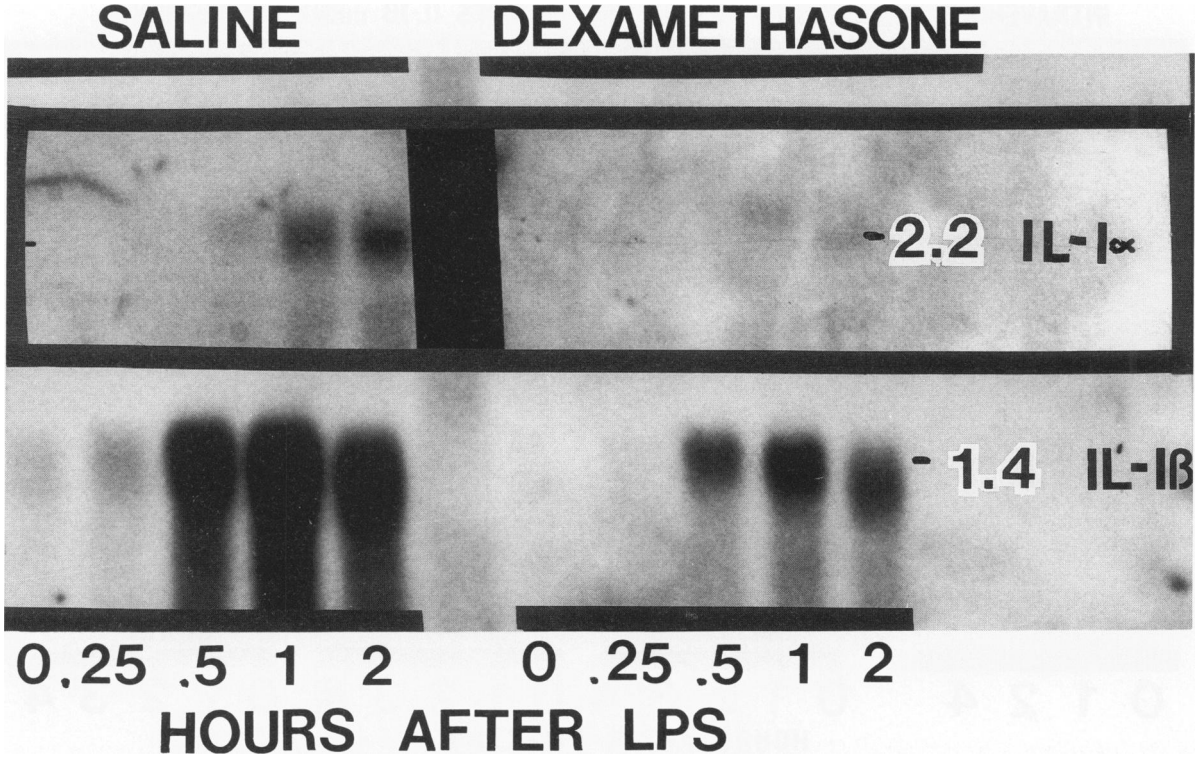

Figure 8. *Dexamethasone pretreatment before the injection of 100 μg endotoxin abrogates the endotoxin-induced expression of IL-1α in the spleen and suppresses endotoxin-induced IL-1β expression. Saline pretreatment serves as a control to illustrate endotoxin-induced IL-1 α/β mRNA expression in the absence of dexamethasone.*

7). Lipopolysaccharide induced a lesser increase in IL-1β mRNA expression in the liver and little or no IL-1β mRNA in the bowel (Figure 7). Injection of increasing doses of LPS over the range of 1 to 10^3 μg caused a similar level of IL-1β mRNA expression in the spleen at 2 hours (data not shown). Dexamethasone pretreatment decreased but did not completely abrogate IL-1β mRNA expression (Figure 8).

Effect of Intravenous Injection of TNF on IL-1 mRNA and TNF mRNA Expression

Tumor necrosis factor (10,000 U recombinant human TNFα) injected intravenously induces IL-1β mRNA expression in the spleen (Figure 9) peaking at 2 to 3 hours after injection. Interleukin-1β mRNA is also constitutively present at lower levels in the spleen at time 0 (Figure 9). Little or no IL-1β mRNA is detected in the liver or bowel either constitutively or after the injection of TNF (Figure 9). The amount of IL-1β mRNA induced by TNF is substantially less than that induced by 100 μg LPS (Figure 9). Interleukin-1α mRNA was not constitutively detected nor did TNF induce detectable expression of IL-1α mRNA. Tumor necrosis factor mRNA was constitutively present in substantial amounts in both spleen and liver. No increase in TNF mRNA levels were noted after injection of TNF (data not shown). Actin mRNA levels and the ethidium bromide-stained gel confirmed that the observed levels of cytokine mRNA were not due to significant over- or underloading of RNA.

Effect of Intravenous Injection of IL-1 on IL-1 mRNA, TNF mRNA and TNF Serum Protein Expression

Interleukin-1 (10 μg recombinant human IL-1β) induced IL-1β mRNA expression (Figure 10) in the spleen, peaking at 1 to 2 hours after intravenous injection. Interleukin-1β mRNA is constitutively present in the spleen in a small amount (Figure 10). Interleukin-1β mRNA was not detected in the liver constitutively or at any timepoint after the injection of IL-1 (Figure 10). Interleukin-1α mRNA was not detected in the spleen or liver either constitutively or after the injection of IL-1 (data not shown). Tumor necrosis factor mRNA was present constitutively in both the spleen and liver (Figure 11). No increase in TNF mRNA was noted in the spleen after the injection of IL-1β, but a slight increase in TNF mRNA may have occurred in the liver at 1

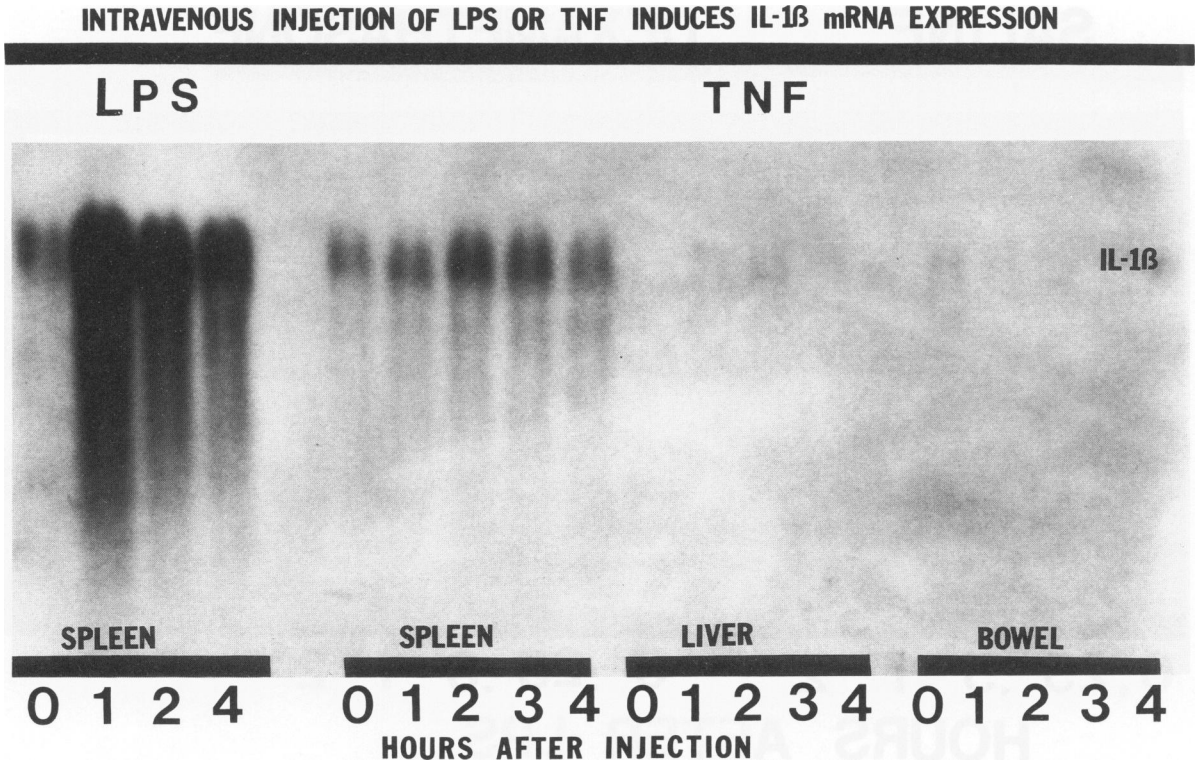

Figure 9. *TNF (10^3 U) injected intravenously induces an increase in IL-1β mRNA expression at 2 to 3 hours in the spleen, but not in the liver or bowel. LPS (100 μg) injected intravenously induces a substantially greater amount of IL-1β mRNA. Note that a small amount of IL-1β mRNA is constitutively present in the spleen at time zero.*

to 2 hours (Figure 11). Serum TNF levels increased very slightly from 0.03 U/ml at time 0 to 1.1 U/ml 2 hours after injection of IL-1β. Actin mRNA levels and the ethidium bromide-stained gel confirmed that the levels of cytokine mRNA expression were not due to significant over- or underloading of RNA.

Endotoxin-induced IL-1 mRNA, TNF mRNA, and TNF Serum Protein Expression in C. Parvum*-primed Rats*

Corynebacterium parvum-primed and saline-primed rats showed equivalent constitutive and post-LPS TNF mRNA levels (Figures 12 and 13), although *C. parvum*-primed rats expressed much greater amounts of serum TNF protein after injection with LPS. *C. parvum*-primed rats contained more than 1000 U TNF/ml in their serum 1 and 2 hours after injection of 100 μg LPS, while saline 'primed' control rats contained 20 and 160 U TNF/ml 1 and 2 hours, respectively, after the injection of LPS. Interleukin-1α mRNA was not detected constitutively in the spleens or livers of either saline- or *C. parvum*-primed rats. Interleukin-1β mRNA was detected constitutively in small amounts in the spleens (Figure 12) but not in the livers (data not shown) of both naive and *C. parvum*-primed rats (Figure 12). Lipopolysaccharide induced IL-1 α/β mRNA expression in the spleens (Figure 12) but not in the livers (data not shown) of both saline- and *C. parvum*-primed rats. Lipopolysaccharide-induced IL-1β mRNA expression was greater in *C. parvum*-primed than in saline-primed control rats as quantitatively demonstrated by standardizing the absorbance (measured by laser densitometry) of the IL-1β hybridization bands against the absorbance of actin hybridization bands (Figure 13).

Discussion

Tumor necrosis factor mRNA is constitutively present in the spleen, liver, and bowel of rats, as previously reported by our laboratory.[14] Tovey et al[15] have reported that TNF mRNA is also expressed at high levels in the spleen, liver, and leukocytes of normal humans. Tumor necrosis factor serum protein expression and suppression in the present study occurred under a variety of experimental conditions in the face of slight or no increases in TNF mRNA content in whole-organ RNA extracts of spleen, liver, or bowel. Tumor necrosis factor mRNA may be constitutively present in a large number of cells that do not respond to endo-

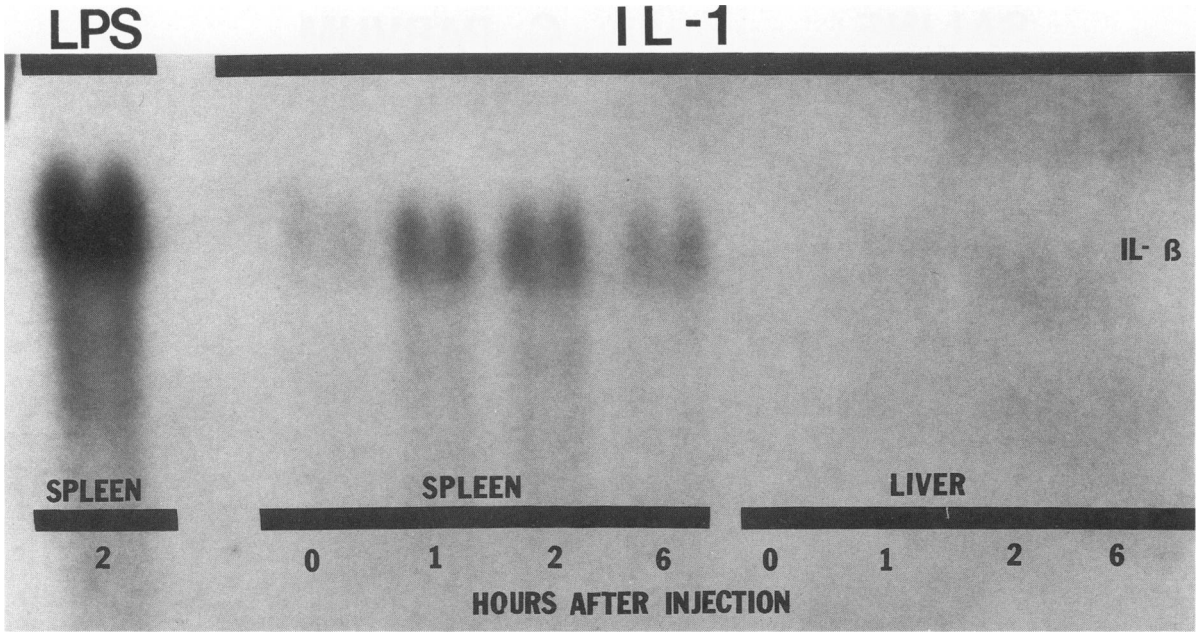

Figure 10. *IL-1β (10 μg) injected intravenously induces an increase in IL-1β mRNA levels at 1 to 2 hours in the spleen. The intensity of the hybridization band for IL-1β mRNA at 2 hours after the injection of LPS (100 μg) is shown for comparison.*

toxemia, and the serum TNF noted 1 to 2 hours after injection of endotoxin may be the product of a small number of endotoxin-responsive cells within each organ that transcribes, translates, and releases TNF. Alternatively TNF serum protein expression may be post-transcriptionally regulated. Finally a combination of transcriptional and post-transcriptional events may regulate TNF *in vivo* because previous investigators have provided evidence for both modes of regulation of TNF gene expression.[16-21]

Several previous investigators have unequivocally pointed to the important role of transcriptional regulation in TNF expression, as demonstrated by substantial increases in TNF mRNA levels in macrophages after stimulation with endotoxin.[16-18] On the other hand, several lines of evidence also point to a significant role for post-transcriptional events in the regulation of TNF protein expression. Hofsli et al[19] reported that endotoxin-stimulated TNF release by freshly isolated human monocytes was

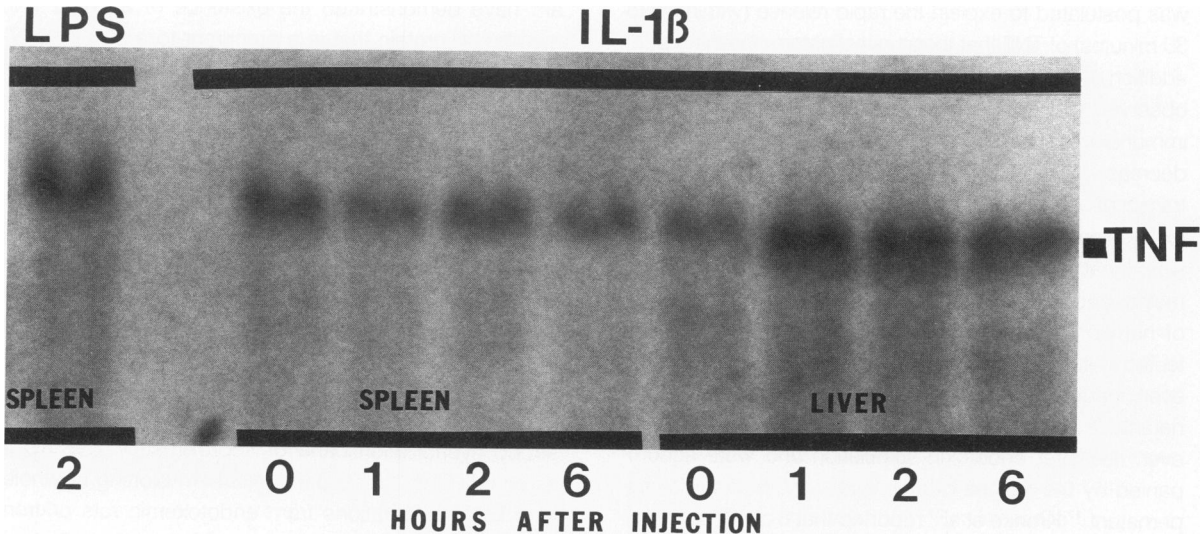

Figure 11. *TNF mRNA levels remain relatively constant after the intravenous injection of IL-1β (10 μg) as they do also after the injection of LPS.*

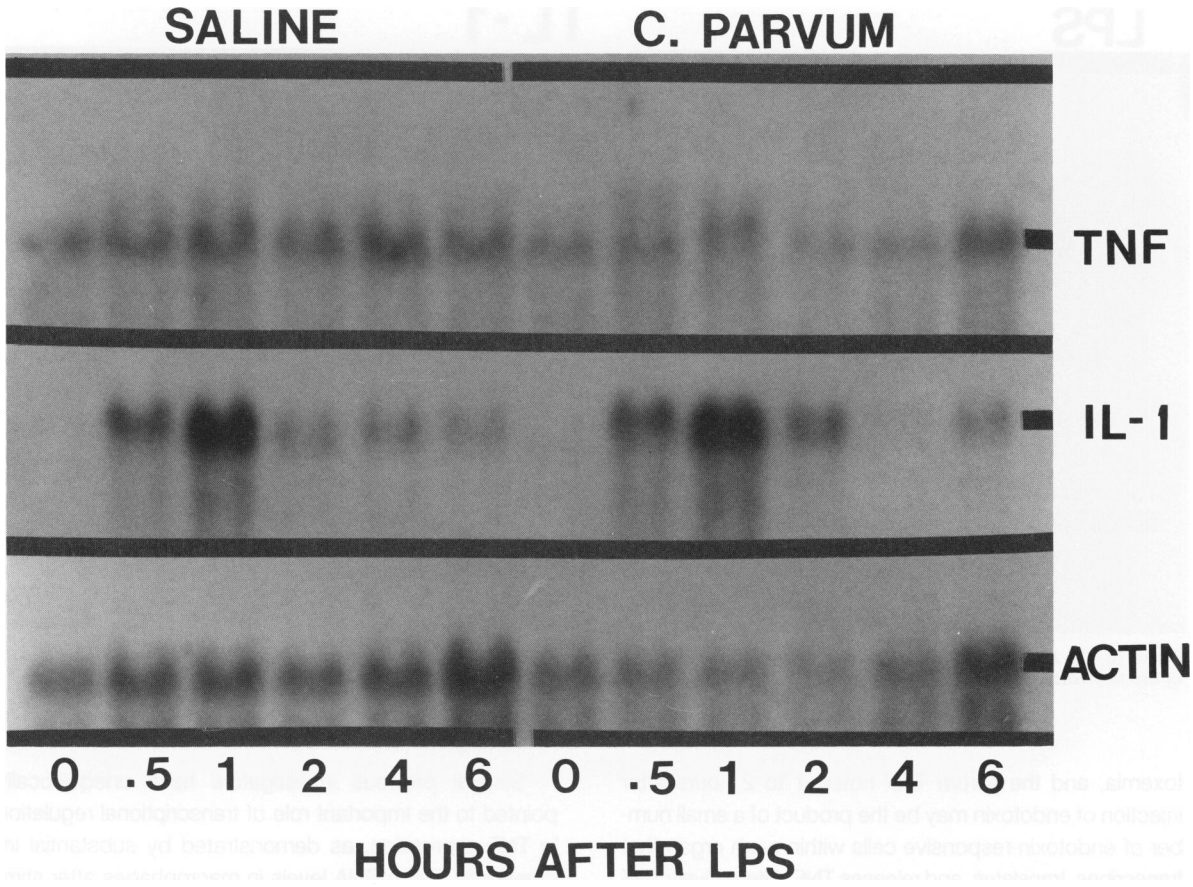

Figure 12. *TNF, IL-1β, and actin mRNA expression are shown at varying times after the intravenous injection of LPS (100 μg) in* C. parvum*-primed and saline-primed rats.*

inhibited by cycloheximide (inhibition of protein synthesis) but not by actinomycin D (inhibition of mRNA synthesis), suggesting the post-transcriptional regulation of TNF release. The possibility of the *in vivo* presence of TNF mRNA was postulated to explain the rapid release (within 20 to 30 minutes) of TNF that these investigators observed after addition of LPS into whole blood.[19] Remick et al[20] recently observed that cyclosporine A inhibits TNF production in immunologically elicited peritoneal macrophages without decreasing TNF mRNA levels, supporting a role for post-transcriptional regulation in the suppression of TNF protein expression. Beutler et al[18] also suggested that post-transcriptional regulation might, in part, be involved in dexamethasone-modulated TNF expression. In a recent study of human alveolar macrophages, TNF mRNA was detected in some abundance in unstimulated macrophages, even though no TNF was present in the culture supernatants.[17] Tumor necrosis factor mRNA levels did, however, rise after endotoxin stimulation and were accompanied by the release of high levels of TNF into the supernatant.[17] Kronke et al[21] reported that 8 of 17 tumor cell lines, including cell lines derived from carcinomas rather than hematopoietic neoplasms, constitutively expressed TNF mRNA. However most of the TNF mRNA-containing cell lines did not release TNF protein into the supernatants, suggesting that TNF protein release might be controlled at a post-transcriptional level when it occurs. Kriegler et al[22] have demonstrated the existence of a 26-kd TNF membrane protein that is a precursor to a secreted 17-kd form of TNF. If the 26-kd TNF membrane protein were constitutively present in some cells of the body, then the 1- to 2-hour peak of endotoxemia-initiated TNF release might be due to the cleavage of the 17-kd molecule from that membrane-associated pool. In this regard, TNF release by leukocytes was found by Scuderi[23] to be inhibited by a serine protease inhibitor, raising the possibility that a protease might be involved in the post-transcriptional regulation of TNF secretion. Recently our laboratory found that Northern blotting of rat alveolar macrophages incubated *in vitro* with LPS for 1 to 2 hours followed by hybridization with murine TNF cDNA demonstrates a very strong hybridization band of approximately 1.95 Kb in addition to the 1.6-Kb band. Northern blotting of whole-lung RNA preparations from endotoxemic rats or from rats injected intratracheally with LPS does not show induction of the same 1.95-Kb hybridization band. The fore-

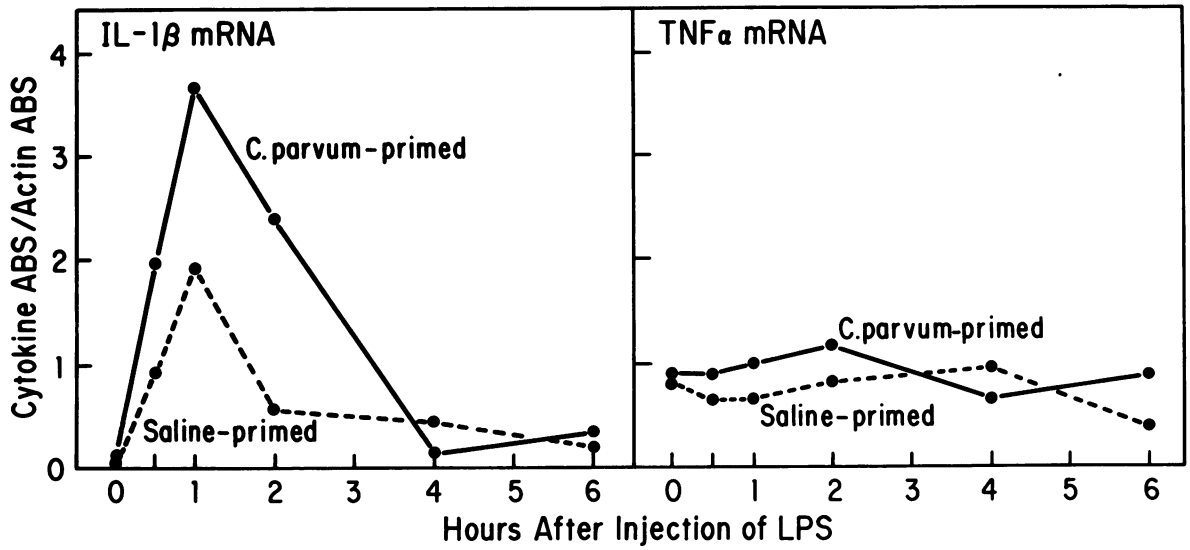

Figure 13. C. parvum-*primed rats demonstrate an increase in endotoxin-induced IL-1β mRNA, but not TNF mRNA expression, as compared to saline-primed control rats.*

going unpublished results suggest that whole-organ RNA preparations may not be sensitive enough to detect the induction of the 1.95-Kb mRNA product (the nature of which is unclear, but which presumably is a form of TNF mRNA) or that significant differences exist between endotoxin-induced TNF transcriptional events *in vitro* and *in vivo*.

The regulation of IL-1 gene expression during endotoxemia appears easier to understand than the regulation of TNF in the sense that less of a constitutive pool of IL-1 mRNA is detectable and that IL-1 mRNA expression increases dramatically in response to LPS. Endotoxin may directly trigger transcription of IL-1 mRNA, may increase mRNA levels *via* post-transcriptional mechanisms, or may, in part, indirectly increase IL-1 levels through TNF. In support of the later possibility, TNF has been well documented to induce IL-1 gene expression *in vitro* in several cell types.[24,25] Furthermore TNF *in vivo* has been shown to induce a biphasic neutrophilia[4] and biphasic febrile response,[26] the second peak of each phenomenon having been attributed to the TNF-induced release of endogenous IL-1.[4,26] Interleukin-1α and β mRNA expression were not identical. Interleukin-1β mRNA, but not IL-1α mRNA, could be constitutively detected in whole-organ RNA preparations, and IL-1β mRNA levels peaked more rapidly than IL-1α mRNA levels.

Endotoxin is the most potent known stimulus for cytokine gene expression. Cytokines themselves, however, have more recently been shown to induce their own gene expression. Tumor necrosis factor and IL-1 are among the most important of the endotoxin-induced proinflammatory cytokines. Tumor necrosis factor and IL-1 have been demonstrated by previous investigators to induce their own expression as well as that of other cytokines *in vitro*.[27-29] Dinarello et al[27] demonstrated that IL-1α induces the synthesis of IL-1β in cultures of human mononuclear cells. Biliau et al[28] and van Damme et al[29] reported that IL-1 induces granulocyte-macrophage colony-stimulating factor, interleukin-6, and interferon in fibroblasts. Tumor necrosis factor and IL-1 *in vivo* both have been demonstrated to induce IL-1–like serum activity.[26,27] The present study documents that intravenously administered recombinant human TNF and IL-1 each induce TNF and IL-1 gene expression *in vivo*, although the regulation and organ sources of TNF and IL-1 gene expression appear to differ.

Tumor necrosis factor and IL-1 both induced IL-1 mRNA expression in the spleen. Interleukin-1 mRNA was absent from the liver and bowel. The absence of IL-1 mRNA in the liver was somewhat surprising in view of the many Kupffer cells in this reticuloendothelial cell-rich organ. The possibility cannot be excluded that the large amount of hepatocyte RNA diluted the concentration of Kupffer cell RNA to a point that IL-1 mRNA might not have been detectable by our methods. The level of IL-1 mRNA expression in the spleen induced by TNF and IL-1 was less than that induced by LPS. The amount of TNF administered in the present study causes hematologic effects such as neutrophilia and lymphopenia[4-6] but is an amount much less than that required to induce shock or death.[8] The TNF-injected rats in the present study did not show any clinical signs of distress. The dose of LPS injected in the present study also did not cause any overt clinical signs of distress. The IL-1–injected rats, on the other hand, received a sufficiently high dose to exhibit a clinical appearance suggestive of general malaise, fever, and hypotension, as previously described by Okusawa et al[30] to

occur after injection of high doses of IL-1. The prolonged leukopenia preceding a subsequent neutrophilia induced by the dose of IL-1 used in the present study is similar to the hematologic effects of IL-1 as described by Okusawa et al,[30] Dinarello et al,[27] and van Damme et al.[31] The interpretation of the results of any *in vivo* study of cytokine-induced cytokine gene expression is complicated by the inability to discriminate between the direct and indirect effects of the injected cytokine. On the other hand, *in vivo* study of cytokine-induced cytokine gene expression is necessary to confirm the significance of similar *in vitro* observations.

Corynebacterium parvum-primed rats demonstrated increases in both LPS-induced TNF serum protein and IL-1 mRNA expression as compared to saline-primed negative control rats. No constitutive increase in TNF or IL-1 mRNA levels were noted in the *C. parvum*-primed animals. The increase in LPS-induced TNF expression in *C. parvum*-primed rats was documented as an increase in TNF serum protein levels and was not accompanied by any change in whole-organ TNF mRNA, again suggesting post-transcriptional regulation of TNF expression. Recently Kruys et al[32] demonstrated the importance of the 3′-octanucleotide UUAUUUAU[12] in imposing a translational blockade on cytokine mRNAs such as TNF. Regulation of TNF serum protein expression largely at the translational level in the rat would be consistent with and would help explain our unusual *in vivo* observation of a large and relatively constant pool of organ TNF mRNA in the face of a dramatic upregulation of TNF serum protein levels.

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