Endotoxin Increases Parathyroid Hormone-related Protein mRNA Levels in Mouse Spleen

Mediation by Tumor Necrosis Factor

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Abstract

Parathyroid hormone-related protein (PTHrP) causes hypercalcemia in malignancy. However, the role and regulation of PTHrP in normal physiology is just beginning to be explored. PTHrP is found in the spleen and has several other features common to cytokines. Since endotoxin (LPS) causes many of its effects indirectly by inducing cytokines, studies were undertaken to determine whether LPS might also induce splenic PTHrP expression. LPS (100 ng/mouse) increased splenic PTHrP mRNA levels 3.6-fold in C3H/OuJ mice. This effect was maximal at 2 h and returned to baseline by 4 h. PTHrP peptide levels also increased 3.3-fold in splenic extracts in response to LPS (1 μg/mouse). Murine TNF-α and human IL-1β, cytokines that mediate many of the effects of LPS, also increased splenic PTHrP mRNA levels. LPS-resistant C3H/HeJ mice, which produce minimal amounts of TNF and IL-1 in response to LPS, were resistant to LPS induction of splenic PTHrP mRNA, while TNF-α and IL-1β readily increased PTHrP mRNA levels in C3H/HeJ mice. Anti-TNF antibody blocked LPS induction of splenic PTHrP mRNA in C3H/OuJ mice by 68%, indicating that TNF is a mediator of the LPS induction of PTHrP levels. In contrast, an IL-1 receptor antagonist (IL-1ra) was ineffective. The increase in PTHrP in the spleen during the immune response suggests that PTHrP may play an important role in immune modulation, perhaps by mediating changes in lymphocyte proliferation and/or function. (*J. Clin Invest.* 1993. 92:2546-2552). Key words: parathyroid hormone-related protein • lipopolysaccharide • endotoxin • tumor necrosis factor · interleukin-1

Introduction

Parathyroid hormone-related protein (PTHrP)[1] is a recently identified peptide that has been isolated from tumors associated with hypercalcemia (1-3). PTHrP, which is produced at high levels by these tumors, possesses parathyroid hormone (PTH)-like activity and is the humoral factor responsible for the hypercalcemia associated with these malignancies (4, 5). Structural evidence suggests that while PTHrP and PTH are derived from one ancestral gene, the PTHrP gene is more highly conserved (2). Although this evidence suggests an important role for PTHrP, the exact function of this protein in normal physiology is only beginning to be elucidated.

Studies suggest that PTHrP has many features that are common to cytokines, the hormonal mediators of the immune system. (*a*) PTHrP, which is produced in many different tissues, is thought to act locally as an autocrine or paracrine factor (2, 3); (*b*) PTHrP has a 3′ untranslated motif found in many cytokines that is thought to destabilize cytokine mRNA and thereby contribute to the transient local expression of these peptides (2, 3); (*c*) in vitro evidence suggests that PTHrP can act as an immune mediator, being produced by and in turn affecting the function of both HTLV-1-infected T cells and normal mitogen-stimulated T cells (6, 7); and (*d*) PTHrP mRNA is found in the spleen, an immunoregulatory organ that is an important site of production of cytokines (8-10).

Cytokines such as TNF and IL-1 are produced during the host's response to infections and inflammatory disorders (11, 12). These cytokines mediate many of the immune and metabolic changes associated with these disorders (11-13). The administration of endotoxin (LPS) has been used to mimic infections. Numerous studies have demonstrated that LPS administration stimulates cytokine transcription and translation (11-13). Because PTHrP shares many features common to cytokines, in this study we investigated whether LPS or cytokines induced by LPS might also stimulate PTHrP gene expression in the spleen.

Methods

Materials. Escherichia coli, strain O55:B5 endotoxin, was purchased from Difco Laboratories (Detroit, MI) and was freshly diluted in pyrogen-free 0.9% saline (Kendall McGraw Laboratories Inc., Irvine, CA). Murine TNF-α was kindly provided by Genentech, Inc. (South San Francisco, CA). Recombinant human IL-1β (112-269) was kindly provided by Dr. Charles Dinarello (Tufts New England Medical Center, Boston, MA) (14). Antibodies were generated against mTNF in rabbits by immunization of New Zealand White Rabbits by standard techniques at Caltag Laboratories (South San Francisco, CA). Serum was purified by ammonium sulfate precipitation using previously de-

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1. *Abbreviations used in this paper:* IL-1ra, interleukin-1 receptor antagonist; PTHrP, parathyroid hormone-related protein.

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scribed precautions to avoid LPS contamination (15). IL-1 receptor antagonist (IL-1ra) was kindly provided by Dr. Robert C. Thompson (Synergen, Boulder, CO). The murine PTHrP cDNA probe, which includes the region coding for amino acids 1–114 of the mature peptide, was kindly provided by Dr. Arthur E. Broadus (Yale University School of Medicine, New Haven, CT) (8). The full-length cDNA probes for murine TNF-α and murine IL-1β were kindly provided by Dr. Bruce Beutler (University of Texas, Southwestern Medical Center, Dallas, TX) and by Dr. Arjun Singh (Genentech, Inc., South San Francisco, CA), respectively. The cDNA for actin was kindly provided by Dr. Peter Gunning (Stanford University, Stanford, CA).

^{32}P-Deoxycytidine 5'-triphosphate was purchased from New England Nuclear (Boston, MA). Multiprime DNA labeling system was obtained from Amersham Corp. (Arlington Heights, IL). X-OMAT AR film was purchased from Kodak (Rochester, NY).

Animal procedures. Male C3H/OuJ and C3H/HeJ mice, 4–5 wk old, were purchased from The Jackson Laboratory (Bar Harbor, ME). C3H/OuJ (OuJ), also designated C3H/HeOuJ, is a substrain that was separated from the C3H/HeJ (HeJ) strain before the origin of a defective LPS response in HeJ; OuJ remain sensitive to LPS (13). Animals were maintained on a normal 12-h light cycle and were fed Purina Mouse Chow (Ralston Purina Co., St. Louis, MO) and water ad libitum. On the morning of the study, after removal of food, animals were divided into groups and injected with the specified doses of LPS or cytokine or with the appropriate vehicle alone (controls). LPS was administered intraperitoneally in 0.9% saline solution. Murine TNF-α and human IL-1β were administered intramuscularly in 0.1% human serum albumin. Where indicated, animals were injected intraperitoneally with anti-murine TNF antibodies (quantity of antibody sufficient to neutralize 34 μg of mTNF) 16 h before LPS administration. IL-1ra was administered intraperitoneally in a dose of 150 μg 1 h before, immediately before, and 1 h after LPS.

Northern blot analysis. mRNA was extracted from whole mouse spleens by the method of Chomczynski and Sacchi (16). Polyadenylated mRNA was then isolated by oligo(dT) chromatography (typical yields, 5–15 μg/spleen), fractionated in 1% agarose gels containing 2.2 M formaldehyde, transferred to nylon membranes electrophoretically, and hybridized with [^{32}P]cDNA probes as previously described (13, 17). Blots were exposed to film at −70°C using Cronex intensifying screens for the time indicated in the figure legends. Autoradiograph intensity was quantitated using a densitometer from E-C Apparatus Corp. (St. Petersburg, FL).

Immunoradiometric assay of PTHrP. Splenic tissue extracts were prepared for determination of PTHrP immunoreactivity by acid urea extraction followed by an alcohol/salt precipitation as described by Stewart et al. (18, 19). PTHrP immunoreactivity was measured in splenic extracts using a commercially available two-site PTHrP immunoradiometric assay that uses antibodies directed against PTHrP (1-40) and PTHrP (60-72) (Nichols Institute, San Juan Capistrano, CA). Liver extracts, prepared as described above, were used to determine nonspecific binding in the PTHrP IRMA, as described by Thiede et al. (20) based on the assumption that nonspecific binding by liver and spleen is similar and is not affected by LPS treatment. After subtraction of counts bound to comparable dilutions of liver extract, dilutions of splenic extracts showed that the measured immunoreactive PTHrP peptide levels paralleled the PTHrP (1-86) standard curve. Levels of PTHrP immunoreactivity in splenic extracts are reported per milligram of splenic protein as measured by a standard protein assay (Bio-Rad Laboratories, Richmond, CA).

The ability of the PTHrP IRMA to detect murine PTHrP was confirmed by measurement of PTHrP in murine milk (data not shown). The values obtained were in agreement with the levels of PTHrP in murine milk that have been reported using other PTHrP assays (21).

Statistics. Values are presented as mean±SEM. Statistical significance was determined using a two-tailed Student's t test. Comparisons among more than two groups were done by analysis of variance using statistical software (Crunch, Oakland, CA).

Results

Effect of LPS on splenic PTHrP mRNA. To determine whether LPS increases PTHrP mRNA levels, LPS-sensitive OuJ mice were administered either LPS or vehicle alone, and 2 h later the spleens were removed and PTHrP mRNA levels were measured. PTHrP mRNA levels were barely detectable in the spleens of control mice administered vehicle alone (Fig. 1 *A*). LPS treatment caused a significant increase in PTHrP mRNA levels over those of controls (Fig. 1 *A*). LPS (100 ng/mouse, a dose 1,000-fold lower than the lethal dose [22]) increased PTHrP mRNA levels 3.6-fold in the spleen when averaged over multiple experiments.

The time course of splenic PTHrP mRNA induction by LPS was next determined (Fig. 1 *B*). PTHrP mRNA levels in the spleen began to increase 1 h after LPS administration, reached a peak at 2 h, and returned to baseline by 4 h. Thus,

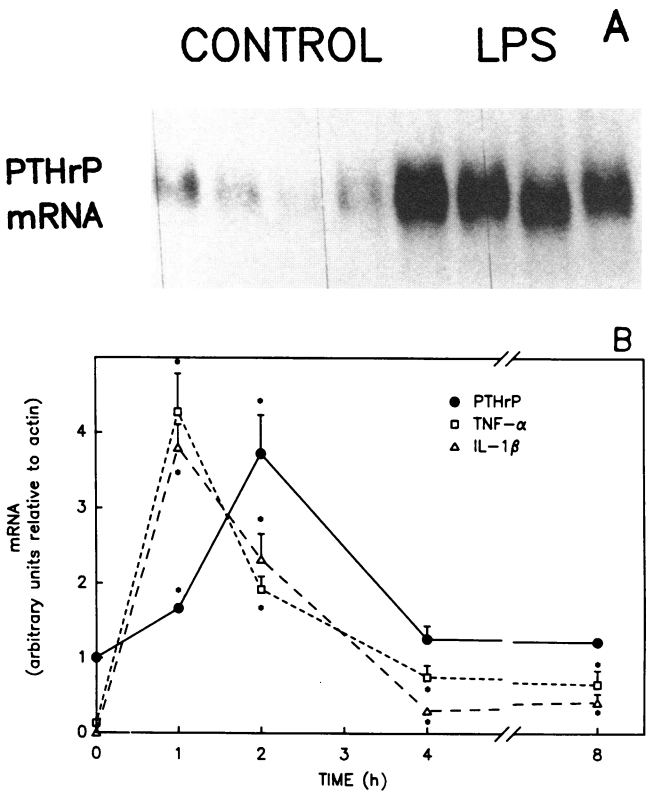

Figure 1. (*A*) Effect of LPS on splenic PTHrP levels. OuJ mice were injected intraperitoneally with LPS (100 ng/mouse) or vehicle alone (control). At 2 h, spleens were removed and polyadenylated mRNA was isolated for Northern blot analysis as described in Methods. The blot was probed for PTHrP and exposed to film for 48 h. Results shown are representative of six replicate experiments. (*B*) Time course of PTHrP, TNF-α, and IL-1β mRNA induction by LPS. OuJ mice were injected intraperitoneally with LPS (100 ng/mouse). At the indicated times, spleens were removed. Polyadenylated mRNA was isolated for Northern blot analysis. The blot was probed for PTHrP, TNF-α, IL-1β, and actin, and exposed to film for 72, 24, 24, and 1 h, respectively. PTHrP (*filled circles*), TNF (*open squares*), and IL-1 (*open triangles*) mRNA, in arbitrary densitometry units normalized to actin, are reported as mean±SEM with $n = 3$. For data points where standard error bars are not visible, the bars fall within the dot. $^*P < 0.01$ vs. $t = 0$.

LPS caused a striking yet transient increase in splenic PTHrP mRNA levels.

Effect of LPS on immunoreactive PTHrP peptide levels in spleen and plasma. To determine if the LPS induction of PTHrP mRNA levels within the spleen also is associated with an increase in PTHrP immunoreactive peptide, the effect of LPS on splenic and plasma levels of PTHrP was determined. The amount of immunoreactive PTHrP peptide present in the spleens of LPS-treated OuJ mice was measured 2.5 h after LPS administration (i.e., 30 min after the peak induction of splenic PTHrP mRNA levels) and compared with control animals. As can be seen in Fig. 2, spleens from control mice had detectable levels of PTHrP immunoreactive peptide. Moreover, LPS caused a 3.3-fold increase in splenic PTHrP peptide when compared with control animals. In contrast, levels of plasma PTHrP were below or at the limit of detection in control mice and were not increased between 0 and 4.5 h after LPS treatment (data not shown).

Thus, sublethal doses of LPS, in addition to inducing splenic levels of PTHrP mRNA, also caused a local increase in NH_2-terminal-containing PTHrP peptide within the spleen, but did not cause an elevation in plasma levels of PTHrP.

Effect of LPS on splenic PTHrP mRNA in LPS-resistant and LPS-sensitive mice. Many of the effects of LPS are mediated by TNF and IL-1. TNF-α and IL-1β mRNA levels were rapidly induced by LPS in the spleen, with maximal levels being achieved 1 h before the peak of PTHrP mRNA induction (Fig. 1 *B*). These data suggest that TNF and/or IL-1 could also mediate the LPS-induced increase in splenic PTHrP mRNA levels.

To further test this hypothesis, the response of LPS-resistant HeJ mice to LPS was next compared with that of OuJ mice. Previous studies have demonstrated that HeJ mice have a decreased responsiveness to LPS, including a decrease in the ability of LPS to increase splenic TNF and IL-1 mRNA levels and a decrease in the ability of LPS to induce production of TNF and IL-1 by their macrophages (13, 23, 24). Comparison of LPS dose responses in HeJ and OuJ mice showed that HeJ mice were also resistant to LPS induction of splenic PTHrP mRNA levels (Fig. 3). In LPS-sensitive OuJ mice, a relatively low dose of LPS (10 ng/mouse) produced a significant increase in PTHrP mRNA. In contrast, in LPS-resistant HeJ mice, even 1,000–10,000-fold higher doses of LPS did not cause a statistically significant increase in splenic PTHrP mRNA levels. Therefore, HeJ mice are markedly resistant to LPS induction of splenic PTHrP mRNA levels.

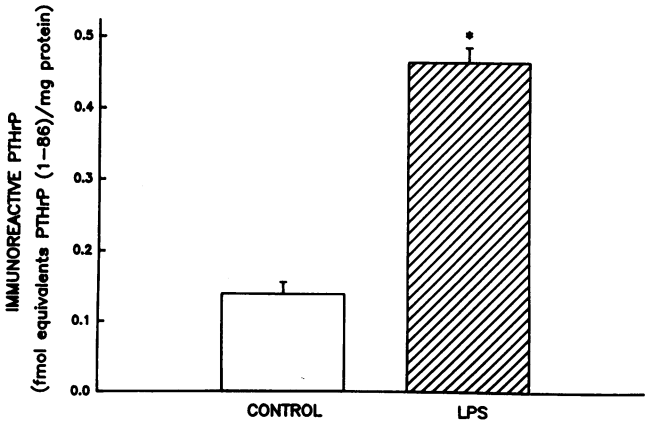

Figure 2. Effect of LPS on immunoreactive PTHrP peptide levels in the spleen. OuJ mice were injected intraperitoneally with LPS (1 μg/mouse) or vehicle alone (control). At 2.5 h, spleens were removed and splenic extracts were prepared as described in Methods using three spleens/sample. Levels of PTHrP peptide in the splenic extracts, corrected for nonspecific binding using liver extracts, were determined by IRMA using PTHrP (1-86) as a standard. Results, expressed per milligram of splenic protein, are reported as mean±SEM with $n = 5$. $*P < 0.001$.

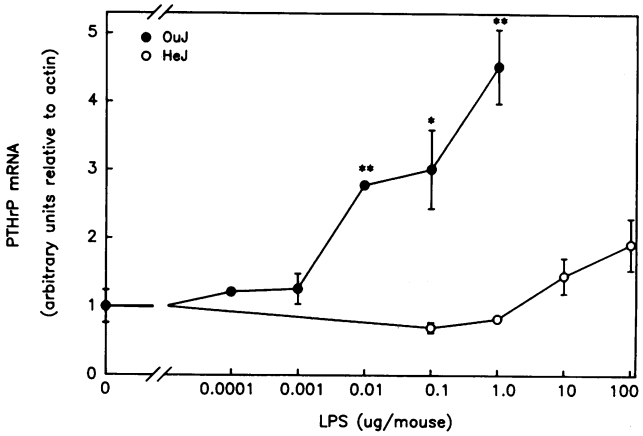

Figure 3. Comparison of the effect of LPS on splenic PTHrP mRNA levels in LPS-sensitive OuJ and LPS-resistant HeJ mice. Mice were injected intraperitoneally with the indicated dose of LPS or vehicle. At 2 h, spleens were removed and polyadenylated mRNA was isolated for Northern blot analysis. The blots were probed for PTHrP and actin, and exposed to film for 24 and 2 h, respectively. Results shown are representative of two experiments. PTHrP mRNA for OuJ (*filled circles*) and HeJ (*open circles*) mice, in arbitrary densitometry units normalized to actin, is reported as mean±SEM with $n = 3$. For data points where standard error bars are not visible, the bars fall within the dot. $*P < 0.05$ vs. control. $**P < 0.005$ vs. control.

Effect of cytokines on splenic PTHrP mRNA levels. The sequential induction by LPS of splenic cytokine mRNA levels followed by PTHrP mRNA, and the resistance of HeJ mice to LPS induction of PTHrP mRNA levels, suggest that TNF and/or IL-1 may mediate the effect of LPS on splenic PTHrP mRNA. Therefore, the ability of TNF and IL-1 to stimulate PTHrP mRNA was examined next. OuJ mice were given doses of murine TNF-α and human IL-1β that have been shown to induce fever, the acute phase response, and changes in lipid synthesis (14, 25–27). As shown in Fig. 4 *A*, both IL-1β and TNF-α increased PTHrP mRNA levels in OuJ spleens. IL-1 increased PTHrP mRNA levels 2.8-fold, while TNF induced a 3.4-fold increase. In contrast to their decreased response to LPS, HeJ mice responded to relatively low doses of IL-1β and TNF-α with an increase in PTHrP mRNA levels (Fig. 4 *B*).

The time course of TNF stimulation of PTHrP mRNA in the spleens of LPS-sensitive OuJ mice is shown in Fig. 5. PTHrP mRNA levels were elevated as early as 30 min after administration of TNF, reached a maximum by 1 h, remained elevated at 2 h, and returned to baseline by 3–4 h. The induction of PTHrP mRNA by TNF is more rapid than the induction seen with LPS, as peak levels of PTHrP mRNA were not seen until 2 h after LPS administration (Fig. 1 *B*).

Role of TNF and IL-1 in LPS stimulation of splenic PTHrP mRNA. Next, experiments were conducted to determine whether blockade of TNF and/or IL-1 could inhibit the induction of PTHrP mRNA by LPS. An antibody developed against TNF in our laboratory that has been shown to neutralize TNF cytotoxicity in vitro in WEHI cells, to block other metabolic

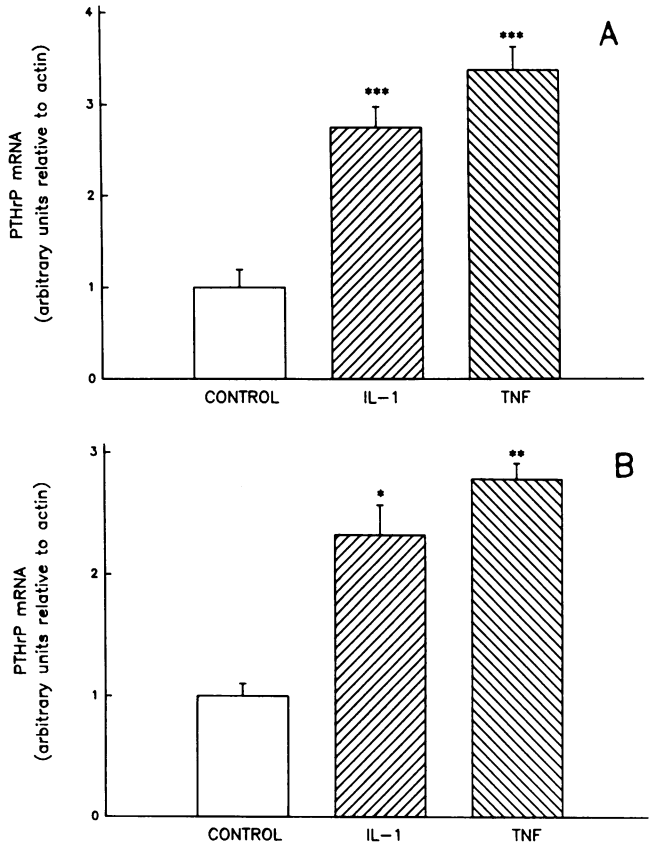

Figure 4. Stimulation of splenic PTHrP mRNA by cytokines in OuJ (*A*) and LPS-resistant HeJ (*B*) mice. (*A*) OuJ mice. Human IL-1β (80 ng/mouse), murine TNF-α (1 μg/mouse), or vehicle alone were injected intramuscularly and spleens were removed at 2 h. (*B*) HeJ mice. Human IL-1β (20 ng/mouse), murine TNF-α (50 ng/mouse), or vehicle alone were injected intramuscularly and spleens were removed at 90 min. Polyadenylated mRNA was isolated for Northern blot analysis. The blots were probed for PTHrP and for actin and exposed to film for 1–4 d and 1–3 h, respectively. PTHrP mRNA, in arbitrary densitometry units normalized to actin, is reported as mean±SEM with n = 3–5. *$P < 0.01$ vs. control; **$P < 0.002$ vs. control; ***$P < 0.001$ vs. control.

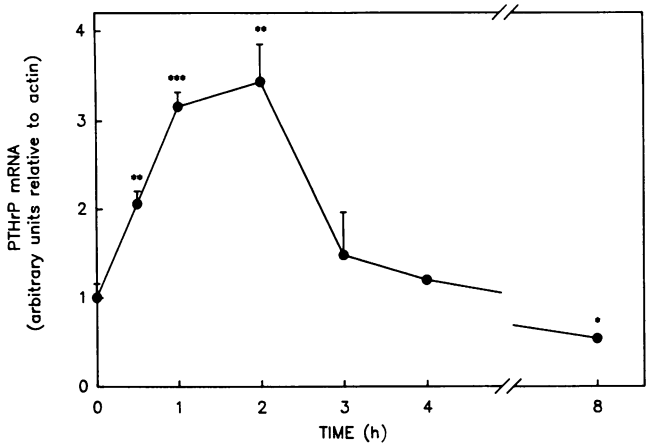

Figure 5. Time course of PTHrP mRNA induction by mTNF-α. OuJ mice were injected intramuscularly with murine TNF-α (1 μg/mouse). At the indicated times, spleens were removed, and polyadenylated mRNA was isolated for Northern blot analysis. The blot was probed for PTHrP and for actin, and exposed to film for 24 and 1 h, respectively. PTHrP mRNA, in arbitrary densitometry units normalized to actin, is reported as mean±SEM with n = 2–3. For data points where standard error bars are not visible, the bars fall within the dot. *$P < 0.05$ vs. $t = 0$. **$P < 0.005$ vs. $t = 0$. ***$P < 0.001$ vs. $t = 0$.

effects of LPS in mice, and to prevent LPS-induced death in rats was given to OuJ mice 16 h before murine TNF-α (1 μg/mouse) administration (28, 29). The administration of TNF neutralizing antibody alone to control mice caused a small but statistically significant decrease in basal PTHrP mRNA levels in the spleen as compared with mice that received control antibody (Fig. 6 *A, first* and *third columns*). Moreover, the administration of TNF neutralizing antibody to TNF-treated animals completely inhibited the induction of PTHrP mRNA by TNF (Fig. 6 *A, second* and *fourth columns*).

Having shown that the TNF neutralizing antibody is capable of blocking TNF induction of splenic PTHrP mRNA levels, we next administered the same dose of TNF neutralizing antibody to animals that were subsequently injected with 100 ng LPS/mouse. As was seen before, TNF antibody decreased basal levels of splenic PTHrP in control animals when compared with control mice receiving control antibody, although this decrease did not reach statistical significance (Fig. 6 *B, first* and *third columns*). More importantly, pretreatment with TNF neutralizing antibody significantly blocked the induction of PTHrP mRNA in the spleen by LPS (Fig. 6 *B, second* and *fourth columns*). TNF neutralizing antibody inhibited the increase in PTHrP mRNA levels in LPS-treated animals by 68±10%.

IL-1ra has been shown to block many effects of IL-1 and to prevent LPS-induced death (12). OuJ mice were administered a dose of IL-1ra that has been shown in mice to block changes in lipid and glucose metabolism caused by LPS (29, 30). Administration of IL-1ra alone had no effect on splenic PTHrP mRNA levels (data not shown). Stimulation of splenic PTHrP mRNA levels by human IL-1β (80 ng/mouse) was completely blocked by IL-1ra (Fig. 7 *A*). In contrast, IL-1ra had no effect on the increase in splenic PTHrP mRNA caused by a sublethal dose of LPS (100 ng/mouse) (Fig. 6 *B*). Even when a fivefold higher dose of IL-1ra was given, a dose that has been shown to prevent hypotension and death caused by lethal doses of LPS (31), the stimulation of splenic PTHrP mRNA levels by 100 ng LPS/mouse was not blocked (data not shown).

Discussion

While PTHrP has been shown to produce hypercalcemia during malignancy, its normal physiologic role and regulation is not fully understood. PTHrP mRNA or protein has been detected in a wide variety of normal tissues, including skin, smooth muscle, heart, lung, kidney, spleen, lymph node, and thymus (2, 3, 32). Evidence to date suggests that PTHrP may act locally in a paracrine or autocrine fashion and that its effects may broadly include: (*a*) stimulation or inhibition of cell differentiation and proliferation (6, 7, 33); (*b*) induction of smooth muscle relaxation (3, 34); and (*c*) regulation of calcium balance, such as placental calcium transport (2).

In vitro studies have shown that various factors, such as epidermal growth factor, transforming growth factor-β, phorbol esters, calcitonin, endothelin, 1,25-dihydroxy vitamin D_3, and dexamethasone, can regulate PTHrP mRNA and protein levels (3, 35, 36). However, the only agents known to induce PTHrP in vivo are estradiol and prolactin, hormones that stim-

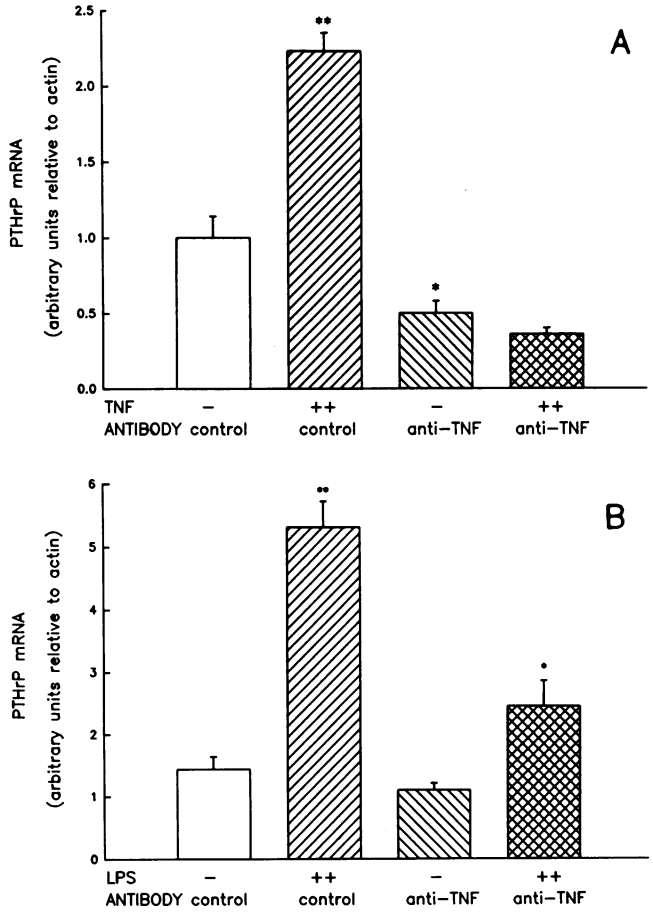

Figure 6. Effect of anti-TNF antibody on TNF (*A*) and LPS (*B*) induction of splenic PTHrP mRNA. OuJ mice were injected intraperitoneally with anti-TNF antibody (an amount capable of neutralizing 34 μg murine TNF) or control antibody. (*A*) TNF. 16 h later, mice were injected intramuscularly with murine TNF-α (1 μg/mouse) or with vehicle alone. Spleens were removed 90 min after TNF administration, and polyadenylated mRNA was isolated for Northern blot analysis. (*B*) LPS. 16 h later, mice were injected intraperitoneally with LPS (100 ng/mouse) or with vehicle alone. Spleens were removed 2 h after LPS and polyadenylated mRNA was isolated for Northern analysis. In both *A* and *B*, the blots were probed for PTHrP and actin and exposed to film for 24–48 and 2 h, respectively. PTHrP mRNA, in arbitrary densitometry units normalized to actin, is reported as mean±SEM with n = 3–4. (*A*) $*P < 0.05$ vs. control (*first column*). $**P < 0.001$ vs. control (*first column*). (*B*) $*P < 0.05$ vs. control (*third column*). $**P < 0.001$ vs. control (*first column*).

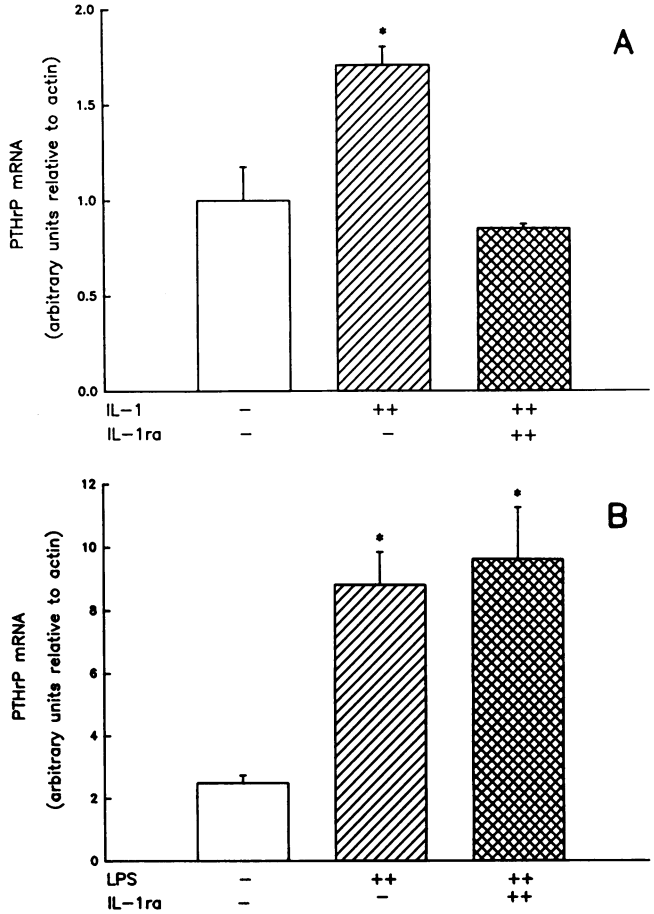

Figure 7. Effect of IL-1ra on IL-1 (*A*) and LPS (*B*) induction of splenic PTHrP mRNA. (*A*) IL-1. OuJ mice were injected intraperitoneally with 150 μg IL-1ra or vehicle 1 h before, immediately before, and 1 h after intramuscular injection of human IL-1β (80 ng/mouse) or vehicle. Spleens were isolated 2 h after IL-1 administration and polyadenylated mRNA was isolated for Northern analysis. (*B*) LPS. OuJ mice were injected intraperitoneally with 150 μg IL-1ra or vehicle 1 h before, immediately before, and 1 h after intraperitoneal injection of LPS (100 ng/mouse) or vehicle. Spleens were isolated 2 h after LPS administration and polyadenylated mRNA was isolated for Northern analysis. In both *A* and *B*, the blots were probed for PTHrP and actin and exposed to film for 48 and 2 h, respectively. PTHrP mRNA, in arbitrary densitometry units normalized to actin, is reported as mean±SEM with n = 4–5. (*A*) $*P < 0.01$ vs. control; (*B*) $*P < 0.001$ vs. control.

ulate PTHrP mRNA in the uterus and in mammary tissue, respectively (37, 38).

In the experiments reported here, we have shown that splenic levels of PTHrP mRNA are increased after the administration of LPS. LPS caused a marked increase in splenic PTHrP mRNA levels, which peaked 2 h after LPS administration. In addition, splenic levels of immunoreactive, NH_2-terminal-containing PTHrP peptide are elevated 2.5 h after administration of sublethal doses of LPS. In contrast, PTHrP peptide levels in the peripheral circulation are not increased. These data suggest that PTHrP may be acting locally within the spleen in an autocrine or paracrine fashion.

Many of the effects of LPS are mediated by TNF and IL-1, cytokines that are produced by LPS-activated macrophages. We therefore postulated that induction of PTHrP by LPS may also be mediated by TNF and/or IL-1. In support of this hypothesis, we have shown that TNF and IL-1 also increased PTHrP mRNA levels in the spleen. In the case of TNF, PTHrP mRNA levels increased rapidly, reaching a maximum by 1 h. This suggests that the 2-h lag in LPS induction of PTHrP mRNA could be due to a requirement for LPS first to stimulate TNF production. Indeed, LPS induction of splenic TNF and IL-1 mRNA levels precedes the induction of PTHrP mRNA, with peak levels being achieved 1 h before maximal stimulation of PTHrP mRNA. Furthermore, LPS-resistant HeJ mice whose macrophages produce minimal TNF and IL-1 in response to LPS were markedly resistant to induction of splenic PTHrP mRNA levels by LPS; LPS doses 1,000–10,000-fold

higher than those required to stimulate PTHrP mRNA levels in OuJ mice caused a slight increase in PTHrP mRNA levels, but this increase did not reach statistical significance. In contrast to what was seen with LPS, when TNF and IL-1 were administered to HeJ mice, PTHrP mRNA levels were readily inducible. Thus, the resistance of HeJ mice to LPS-induced increases in PTHrP mRNA levels, and the ability of TNF and IL-1 to increase PTHrP mRNA levels in both HeJ and OuJ mice, suggest that TNF and/or IL-1 could mediate the increase in splenic PTHrP mRNA levels seen with LPS.

Experiments using cytokine blocking agents provide more direct evidence for the role of cytokines in LPS induction of PTHrP mRNA. The ability of TNF neutralizing antibody to reduce by 68% the LPS induction of splenic PTHrP mRNA in OuJ mice provides direct evidence that TNF is a mediator of this LPS effect. In contrast, high levels of IL-1ra that block hypotension and death caused by lethal doses of LPS had no effect on the induction of splenic PTHrP mRNA levels by sublethal doses of LPS. Thus, these data indicate that TNF is a major mediator of the LPS induction of PTHrP mRNA in the spleen, while IL-1 does not appear to play a key role. Within the limitations of inhibitor experiments, the possibility that IL-1 may play a role in the LPS induction of PTHrP mRNA cannot be totally excluded.

The experiments presented here suggest a model for the increase in splenic PTHrP seen during the immune response to LPS. First, LPS induces TNF secretion by macrophages. TNF produced locally by macrophages in the spleen or from the systemic circulation could then stimulate PTHrP gene expression within the spleen, resulting in a local induction of PTHrP peptide levels. The serial induction by LPS of TNF followed by TNF's induction of PTHrP is analogous to the pattern seen with other cytokines, such as IL-6 (39).

The localized induction of PTHrP in the spleen, an immunoregulatory organ, during the host response to LPS suggests that PTHrP may indeed play an important role in immune modulation, although its specific role can only be speculated on at this time. Studies by others suggest that T and B lymphocytes have PTHrP/PTH receptors, and that the interaction of NH_2-terminal-containing PTHrP peptides with these receptors may influence lymphocyte proliferation (6, 7, 40–42). It is possible, then, that PTHrP may act locally within the spleen to mediate changes in lymphocyte proliferation and/or function that occur with LPS administration. Indeed, in the basal state, it has been reported using immunohistochemical methods that PTHrP in the spleen is localized in dendritic cells, the cytokine-responsive, antigen-presenting cells that interdigitate with T cells in the periarteriolar lymphocyte sheaths (32, 43).

Thus, the local increase in PTHrP in the spleen in response to relatively low doses of LPS suggests that PTHrP, a hormone first discovered in the setting of malignancy, may also play an important role in the body's normal immune response to infection and inflammation. In addition, the induction of PTHrP mRNA by the cytokines TNF and IL-1 may have important implications for PTHrP regulation in malignancy as well as in infection.

Acknowledgments

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