Transcriptional Regulation of Interleukin-1β Gene by Interleukin-1β Itself Is Mediated in Part by Oct-1 in Thymic Stromal Cells*

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Interleukin (IL)-1 is involved in many processes, including thymic development. However, control of IL-1 expression in thymic-derived stromal cells (TSC) has not been reported. We found that IL-1β increased steady-state mRNA levels for IL-1α and IL-1β in TSC-936 and TSC-2C4 cells; stability was not a major determinant of this effect. To study transcriptional regulation of IL-1β, we functionally characterized 4 kilobase pairs of the 5′-flanking region and first intron of the bovine IL-1β gene. The −470/+14 fragment was sufficient to confer maximal responsiveness to IL-1β upon transfection into these cell lines. Progressive 5′ deletions identified several IL-1β-responsive regions, including −308 to −226, which we further characterized. Electrophoretic mobility shift and supershift analyses showed that IL-1β induced the ability to form multiple protein complexes with −261/−226 and that one of these contained nuclear factor Oct-1. A competitor containing a mutated Oct consensus site failed to compete not only for this complex but others as well, suggesting that this sequence regulates binding of other proteins to this region. Functional analysis confirmed that this element was essential for maximal induction of transcription. These findings document a heretofore undescribed mechanism utilized by TSC for regulation of IL-1β transcription by IL-1β itself.

The cytokine interleukin 1 (IL-1) mediates a wide variety of inflammatory and hematopoietic processes. Although it is primarily recognized as an inflammatory agent, it exerts diverse effects on numerous cell types (for reviews, see Refs. 1–5). Two distinct agonists, IL-1α and IL-1β, are encoded by distinct but closely related genes, and are produced by several cell types. Monocytes are the major sources of IL-1 in response to components derived from infectious agents such as lipopolysaccharide (LPS), and much of our knowledge about the control of IL-1 expression has been derived from these cells. In addition to its well known function in inflammation, IL-1 also plays an important role in T-cell development (for reviews, see Refs. 6 and 7). In the thymus, thymic stromal cells (TSC) produced a number of cytokines, including IL-1. IL-1 has been described as a co-mitogen for some populations of immature thymocytes (8–10) and prevents apoptosis (11). IL-1α and tumor necrosis factor-α were shown to be required for early thymocyte commitment and differentiation (12), and IL-1β was shown to be essential for positive selection by thymic nurse cells (13). In addition to effects on thymocytes, IL-1 also induced DNA synthesis and morphological changes in TSC (14) and stimulated thymic epithelial cells to produce other cytokines, such as IL-6, IL-8, granulocyte/macrophage-colony stimulating factor, and leukemia inhibitory factor (15–17). These studies suggest that TSC-derived IL-1 serves as an autocrine/paracrine factor to modulate cytokine production within the thymus. A number of growth factors and hormones have been shown to regulate IL-1 expression in TSC, including epidermal growth factor, transforming growth factor-α (18, 19), growth hormone, and prolactin (20). However, despite its biological significance, the regulation of IL-1 expression at the transcriptional and post-transcriptional levels in TSC remains poorly understood.

IL-1 has been shown to induce the expression of its own gene in several cells, including mononuclear cells (21), vascular smooth muscle cells (22), vascular endothelial cells (23), thymoma cells (24), and dermal fibroblasts (25). Whether it exerts a similar autocrine effect in other cells such as TSC has not been examined. Although transcriptional control has been implicated in some studies, little is known about the mechanisms(s) of the response.

The regulation of IL-1 transcription has been most extensively studied in response to LPS in monocytes (for reviews, see Refs. 1, 3, and 26). Several DNA regulatory elements and corresponding nuclear proteins have been identified, which ultimately participate in the control of IL-1β transcription. These include an upstream induction sequence, which binds transcription factors NF-IL6, cAMP-response element binding protein (27), NF-κB (28) and a STAT-like factor (24), and promoter-proximal regulatory elements containing binding sites for NF-IL6 (29, 30) and a B-cell and myeloid-specific transcription factor PU.1 (Spi-1, NF-βA; Refs. 31 and 32). In addition to transcriptional regulation, the stability of IL-1 mRNA can be selectively modulated by various stimuli (for review, see Ref. 26). Little is known about how this gene is controlled in other cell types by different stimuli. Moreover, several lines of evidence have suggested that regulation of IL-1 expression may occur at multiple levels, and the mechanisms may vary with the particular cell type and stimulus examined (18, 20, 33–37).

In this study, we show that IL-1β is able to increase levels of mRNAs for both IL-1α and IL-1β in TSC, documenting a positive feedback loop for IL-1 in the thymus. We show that IL-1β regulates its own gene primarily at the level of transcription by inducing Oct-1 binding to a consensus sequence in the 5′-
flanking region. These findings describe a previously unrecognized mechanism for autocrine regulation of IL-1β in TSC and establish a model system to study IL-1 gene regulation and signal transduction.

EXPERIMENTAL PROCEDURES

Materials—Recombinant bovine IL-1β was a generous gift from Dr. Dale Shuster, American Cyanamid Company, Princeton, NJ. LPS and actinomycin D were purchased from Sigma. Antibodies, anti-IL-1 (sc-2252), and anti-STAT3b (sc-8363a) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Tissue culture plates and flasks were obtained from Fisher.

Cell Culture and Induction—Three bovine thymic stromal cell lines (TSC-936, TSC-2C4, TSC-934) and a bovine endometrial fibroblast line (End-6.2) were generated by a strategy of temperature-sensitive conditional transformation as described previously (20, 39). Previous immunocytochemical studies showed that both TSC-936 and TSC-2C4 cells stained positively only for vimentin, consistent with a mesenchymal origin, whereas TSC-934 cells exhibited both vimentin- and cytokeratin-specific staining (Ref. 20). Growth of these cells resembles tumor cells at the permissive temperature (33 °C) and reverts to a normal phenotype at the nonpermissive temperature (39 °C), which is also the normal temperature for bovine cells. These cells were routinely maintained at 33 °C in Dulbecco’s modified Eagle’s medium/F12 (Life Technologies, Inc.), 100 units/ml penicillin, 100 μg/ml streptomycin (Life Technologies, Inc.), and 5% fetal bovine serum (HyClone; Logan, UT). LB, a bovine dermal fibroblast line (39), was grown at 39 °C. All experiments were performed on cells within 20 passages. For stimulation with cytokine, cells were plated and grown at 39 °C for 3 days to 70–90% confluency. The cells were then washed with Dulbecco’s phosphate-buffered saline (DPBS, Life Technologies, Inc.) twice and serum-starved for 24 h in Dulbecco’s modified Eagle’s medium/F12 before treatment. In all experiments, cells were treated with IL-1β at 10 ng/ml (0.5 μM) unless otherwise noted. LPS was used at 10 μg/ml. For mRNA stability studies, cells were treated with 10 ng/ml IL-1β for 3 h followed by the addition of 2 μg/ml actinomycin D, and incubation was continued for varying times.

RNA Purification and Northern Analysis—Cells were plated at a density of 4×10^5/75-mm flask and grown at 39 °C for 3 days before serum starvation and cytokine treatment as described above. Total RNA was isolated and analyzed using methods described previously (20). The signals were quantified with a PhosphorImager Storm 840 (Molecular Dynamics, Sunnyvale, CA). All determinations were repeated 2–3 times.

Genomic Library Screening and DNA Sequencing—A bovine genomic EMBL3 phage library (40) was screened with an oligonucleotide probe (5’-GGCGAATGGGTTGGTGCTG-3’) made from the 5’-untranslated region of the bovine IL-1β cDNA (41). Nucleotide sequences were determined on both DNA strands and analyzed using the Genetics Computer Group programs (42).

Plasmid Constructions—The 4-kbp BamHI/BamHI fragment with or without an additional 0.6-kbp BamHI/Ncol fragment was inserted into pGL3-basic (Promega) to generate the pIL-1–luciferase constructs with or without the first intron (Fig. 4, constructs 1 and 2). Other deletion constructs were generated by digestion with appropriate restriction enzymes from these plasmids. -278/+14, -255/+14, and -234/+14 constructs (Fig. 5) were derived by polymerase chain reaction amplification using oligonucleotides corresponding to the specific regions. Site-directed mutagenesis was carried out using a mutagenic oligonucleotide (5’-GCAATGGAAGGTGGTCTGCTG-3’) made from the 5’-untranslated region of the bovine IL-1β cDNA. -226 oligonucleotides (Fig. 5D) were synthetic double-stranded DNA.

Transfections and Reporter Gene Assay—Transfections were performed by the calcium phosphate precipitation method modified from Kingston et al. (43). For transient transfections, TSC-2C4 cells were plated at 3.6×10^6/75-mm plate and grown at 39 °C for 3 days. After transfection, they were washed with Dulbecco’s phosphate-buffered saline three times, incubated in serum-free Dulbecco’s modified Eagle’s medium/F12 for 12–16 h, and then stimulated with 0.5 mM IL-1β for an additional 24 h before harvesting. Cells were lysed with 110 μl of 1× cell culture lysis reagent (Promega). Luciferase activity was measured by adding 100 μl of luciferase substrate to 50 μl of cell lysate in a Turner Designs Model 20/20 luminometer (Turner Designs, Sunnyvale, CA). Relative light units were determined by 10-s integration and normalized with the co-transfected β-galactosidase activity, as measured by the Galacto-Lite Plus kit (Tropix Inc., Bedford, MA). TSC-936 cells were stably transfected pIL-1–luciferase constructs and pcDNA3 (Invitrogen, San Diego, CA) encoding the neomycin resistance gene and selected in 3 mg/ml G418 (Life Technologies, Inc.)-containing medium. Expanded, resistant colonies were treated as above. Luciferase activity was corrected for protein, as determined by the BCA protein assay (Pierce).

Preparation of Nuclear Extracts—TSC-2C4 and TSC-936 cells were plated at a density of 1.2–2×10^6/cm2 plate and grown at 39 °C for 3 days before serum starvation and cytokine treatment as described above. Nuclear extracts were prepared by a method modified from Andrews and Faller (44). Cells from two 10-cm plates were incubated on ice for 10 min in 400 μl of buffer containing 10 mM HEPES, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, and 2 mM Na3VO4, vortexed, and then pelleted. A high salt extraction was performed by resuspending the cells in 25 μl of buffer containing 20 mM HEPES, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin and incubating on ice for 30 min. The extraction was repeated, and the supernatants were pooled and stored at −80 °C. Protein concentrations were determined by the BCA protein assay.

Electrophoretic Mobility Shift Analysis (EMSA)—Mobility shift reactions containing 5 μl of nuclear extract (5 μg of total protein), 3 μg of poly(dI:dC), 4 μl of 5× binding buffer (1× contains 10 mM HEPES, pH 7.9, 10% glycerol, 50 mM KCl, 2.5 mM MgCl2, 0.1 μg/ml bovine serum albumin, 1 mM dithiothreitol), and 1 ng of double-stranded oligonucleotide probe labeled by T4 polynucleotide kinase purified by Nick Columns (Amersham Pharmacia Biotech) in a final volume of 20 μl were incubated at room temperature for 20 min. The reactions were electrophoresed for 1.5–2 h on a 4% polyacrylamide gel with 2.5% glycerol in 0.25× Tris-bUFFERED EDTA that had been prerun for at least 1 h. The −308/−226 fragment (Fig. 5D) was generated by polymerase chain reaction amplification followed by gel purification. The −308/−274 and −261/−226 oligonucleotides (Fig. 5D) were synthetic double-stranded DNA. For the competition assays, a 100-fold excess of competitor oligonucleotides was added to the binding reaction 20 min before the addition of the radiolabeled probe. Consensus sequences (CS) used as specific competitors include Oct_CS, 5′-CGATGCCTCAAGTGACGATC-3′; GATA_CS, 5′-GACGATCCGTGACGATC-3′; PRE, 5′-AGATTTCATGAGAAACAAATG-3′; and mu−261/−226, 5′-AGGTGGTCAAGAAAAACCAATATCAGTGAAATGAC-3′ (lower-case letters indicate mutated bases). For supershift assays, extracts were preincubated with 1 μg of antibody for 45 min at room temperature before the addition of the radiolabeled probe. All experiments were repeated at least two times.

RESULTS

Induction of IL-1α and IL-1β mRNAs by IL-1β in Thymic Stromal Cells—To see if there are differences in the ability of IL-1 to induce IL-1 expression among fibroblast cell lines derived from different tissues, we examined the effects of IL-1β on both IL-1 agonist mRNAs in different cell lines by Northern analysis. To eliminate the possibility that hormones or growth factors present in serum may induce the expression of IL-1, cells were cultured in serum-free media for 24 h before treatment. No IL-1 RNA was detected in any unstimulated cells (Fig. 1). IL-1β induced both IL-1α and IL-1β mRNA accumulation in all three thymic stromal cell lines (TSC-934, -936, -2C4) to a somewhat different extent. IL-1β increased only IL-1α mRNA in the endometrial stromal End-6.2 cells. However, IL-1β had no detectable effect on mRNA levels for either IL-1 agonist in the LB line, despite the fact that IL-1 receptors have been described on these cells (48). In contrast to monocytes, the thymic stromal cell lines TSC-936 and TSC-2C4 were insensitive to LPS stimulation. This also ruled out the possibility that the IL-1 responses we observed were due to endotoxin contamination in the recombinant cytokine. These data suggested that

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the ability of IL-1β to induce IL-1α and IL-1β mRNAs was cell-specific.

To further investigate the effects of IL-1β on thymic-derived stromal cells, we focused on cell lines TSC-936 and TSC-2C4. In both cell lines, IL-1α mRNA was greatly increased as early as 1 h by IL-1β, but increases in the level of IL-1β mRNA were not observed until 3 h after the addition of cytokine (Fig. 2, A and B). However, at longer times, relative transcript levels differed between these cell lines. In TSC-936 cells, both messages gradually declined with time, whereas in TSC-2C4 cells, a further rise was observed at 8 h for both IL-1α and IL-1β, and levels remained elevated at 24 h.

Regulation of IL-1α and IL-1β mRNA Stability—Expression of the IL-1α and IL-1β genes is regulated at multiple levels, including both transcriptional and post-transcriptional mechanisms. To assess the contribution of mRNA stability to the IL-1β-induced increases in steady-state levels of IL-1α and IL-1β mRNAs, we measured the rates of decay of these transcripts following IL-1β treatment after inhibiting new transcription with actinomycin D. As shown in Fig. 2, C and D, both IL-1α and IL-1β mRNA rapidly decayed in both cell lines, with an estimated half-life of less than 1 h, similar to that reported for many unstable mRNAs in different cell types (for review, see Ref. 49). These data suggested a role for transcriptional regulation in the changes in steady-state levels of mRNA that we observed.

Cloning of the Bovine IL-1β 5′-Flanking Region—As a first step toward studying the transcriptional regulation of IL-1β gene by IL-1β itself, we cloned the bovine IL-1β gene and functionally characterized the 5′-flanking region. A genomic clone with a 12-kbp SalI insert was isolated that contained about 7 kbp of 5′-flanking region plus the coding sequences. Primer extension identified a major transcript with an initiation site 45 base pairs 5′ to the first exon-intron junction; a TATA box was located 25 base pairs upstream of this site. Multiple other minor start sites were also observed in TSC-936, TSC-2C4, and peripheral mononuclear cells (data not shown), consistent with the multiple putative TATA boxes present in the 5′-flanking region of this gene (Fig. 3A). Consensus sequences for a number of putative transcription factors were present in the 5′-flanking region as well as the first intron. Comparison of the sequences of the proximal promoter region (−462 to +45) to the human genomic sequences (50) showed 76% identity, including the regions indicated in IL-1β responsiveness (Fig. 3B).

Identification of IL-1β-responsive Elements in the IL-1β 5′-Flanking Region—To determine the role of transcription in the IL-1β-induced increases in the steady-state levels of mRNA, a fragment containing 4 kbp of the 5′-flanking region plus the first intron was linked to the luciferase reporter gene, and this construct was transiently transfected into TSC-2C4 cells. Stimulation of the cells with IL-1β increased luciferase activity 3.7-fold (Fig. 4, C and D, lane 1). To further localize sequences necessary for IL-1β autoregulation, we generated a series of 5′-deletion constructs containing different amounts of the 5′-flanking region with or without the first intron (Fig. 4A) and examined their responsiveness in both TSC-2C4 and TSC-936 cells. Because TSC-936 cells were difficult to transiently transfect, we generated stably transfected cells. To normalize for different copy numbers of the foreign gene in different stable cell lines, data are presented as -fold induction by IL-1β relative to the medium control. In both stably transfected TSC-936 and transiently transfected TSC-2C4, the −470/+14 EcoRI-BamHI fragment was sufficient to confer maximal responsiveness to IL-1β (Fig. 4, B–D, lane 6). Basal levels varied with different constructs in transiently transfected TSC-2C4 (Fig. 4C), suggesting multiple positive and negative regulatory elements. The stimulatory effect of IL-1β was dose-dependent; increases in luciferase activity were observed at concentrations...
as low as 20 pM, reaching maximal levels at 0.5 nM in both cell lines (data not shown).

Because similar response patterns were observed in both cell lines, we utilized transiently transfected TSC-2C4 cells to further localize the response elements. Progressive 5'-deletions of the 2470/114 EcoRI/BamHI fragment identified several IL-1β-responsive regions (Fig. 5, A–C). Deletion of 2303 to 2234 reduced IL-1β-stimulated reporter gene activity from 6.6- to 1.9-fold. Further dissection of this region identified two smaller areas located at 2303 to 2278 and 2255 to 2234, each of which reduced IL-1β responsiveness by approximately 40%. There was no significant difference between the constructs 2278 to 2255, suggesting that 2278 to 2255 may not contain important elements for the IL-1β response. Another region, located between 278 and 255, appeared to contain elements responsible for both basal and IL-1β-induced activities. In fact, deletion of 2234 to 2180 showed a significant reduction in basal activity, and further deletion to 2100 diminished basal transcription to that of the promoterless vector control (Fig. 5B).

Characterization of Protein Complexes That Bind to the 2303/226 Region—To visualize protein-DNA interactions within the 2303/226 region, EMSA was performed. Using a probe spanning 2308 to 2226 (Fig. 5D), which included the 2303/226 fragment and some surrounding sequences, we examined complex formation in nuclear extracts prepared from TSC-2C4 cells stimulated with IL-1β for increasing times. As shown in Fig. 6A, extracts from the IL-1β-stimulated cells were able to form at least five complexes. Among these, complex 2 was not detected in the untreated cells and was strongly induced by IL-1β. Complexes 1 and 3 were present at low levels before treatment and further increased by IL-1β. These complexes were induced by IL-1β as early as 30 min and remained intact up to 9 h. Competition assays showed that all five complexes were specifically competed by excess unlabeled 2308/2226 oligonucleotide (Fig. 6B, lane 2). The oligonucleotide, PRE, containing the consensus site for transcription factor STAT5 (47) was used as a nonspecific control because it had no

Fig. 3. Bovine IL-1β 5'-flanking DNA sequences. A, nucleotide sequence of the bovine IL-1β gene from −1210 to +685 (GenBank/EMBL accession number AF026543). Base pairs are numbered according to the transcription start site (arrow). Relevant restriction enzyme sites are circled. Sequences with a perfect match to a consensus transcription factor binding site are underlined. Putative TATA boxes with either zero or one mismatch from published consensus sequences are shown as boxes. Exons are shown in capital letters. The translation initiation site (ATG) is shown in bold. B, comparison of bovine (top) and human (bottom; GenBank/EMBL accession number M15840) IL-1β promoter proximal region sequences. Sequences extending from −308 to −226 of the bovine IL-1β 5'-flanking region are compared with the corresponding human IL-1β genomic sequences. Vertical lines denote conserved nucleotides. Putative transcription factor sites are boxed.
sequence homology to the −308/−226 fragment. Only complex 4 was competed by PRE, suggesting that this complex is non-specific (Fig. 6B, lane 5). Nuclear extracts from IL-1β-treated TSC-936 cells yielded a very similar pattern (data not shown). To determine which region of the DNA was responsible for the specific binding, oligonucleotides −308/−274 and −261/−226, containing the two IL-1β-inducible regions described in Fig. 5, were used as competitors. We found that oligonucleotide −261/−226 effectively competed for binding in complexes 1, 2, and 3 (Fig. 6B, lane 4), whereas a 100-fold excess of the −308/−274 fragment only weakly competed for binding in complexes 1 and 3 (Fig. 6B, lane 3). Based on these results, we pursued identification of the proteins binding to the −261/−226 region.

IL-1β-induced Nuclear Factor Oct-1 Binding to the −261/−226 Region—EMSA with the −261/−226 oligonucleotide probe identified multiple complexes formed after IL-1β treatment (Fig. 7). Similar to the pattern of complexes observed with the −308/−226 probe, binding was detected after 30 min of IL-1β treatment and remained elevated after 9 h of cytokine exposure (data not shown). IL-1β specifically induced the ability to form complexes 1, 2, 3, and 5 at all the time points we examined. A faint band of lower mobility was detectable in some experiments. Complex 5 may contain some nonspecific proteins because a 100-fold excess of the −261/−226 oligonucleotide itself was not able to completely abolish the signal (Fig. 7A, lane 3). This may also be due to phosphatases present in the nuclear extracts that may interact only with phosphorylated probes but not cold competitors (51). Complex 4 appeared to be nonspecific because it was competed by PRE (Fig. 7A, lane 7).

Sequence analysis of the −261/−226 region revealed a number of potential regulatory elements including a perfect Oct consensus site (52, 53) and two GATA-like elements with one mismatch from published consensus sequences (Ref. 54 and Fig. 5D). To determine whether these sequence elements were involved in the formation of IL-1β-induced complexes, oligonucleotides containing the consensus sites for Oct (45) or GATA (46) factors were used as competitors in EMSA. As seen in Fig. 7A, Oct consensus oligonucleotides effectively blocked formation of complex 1 and slightly reduced the intensity of complexes 2 and 3 (Fig. 7A, lane 5). The identity of the Oct factor in complex 1 was confirmed by supershift analysis. Anti-Oct-1 specifically supershifted complex 1 (Fig. 7B, lane 2), whereas the control anti-STAT5 failed to shift the mobility of any complex (Fig. 7B, lane 3). To further explore the role of the Oct consensus sequence in protein binding to the −261/−226 fragment, an oligonucleotide (mu−261/−226) containing an AT to GC mutation (55) in the Oct consensus site of the −261/−226 fragment was used in the competition assay. The mu−261/−226 oligonucleotide not only failed to compete in the formation of complex 1 but also failed to compete completely for binding in the other specific complexes (Fig. 7C, lane 3, compared with the competition with the original −261/−226 oligonucleotide, lane 2). In fact, mu−261/−226 only slightly reduced the intensity of complex 3.

To determine the contribution of the Oct element in autocrine regulation of IL-1β transcription, functional analyses were performed using mutated promoters, −303 to +14 and −255 to +14. A significant reduction in reporter gene activity was seen in cells transfected with either construct carrying an AT to GC mutation in the Oct consensus site compared with the wild-type construct (Fig. 8). However, basal levels of transcription were not affected by the mutation. Taken together, these
The data suggest a critical role for Oct-1 in IL-1β transcriptional regulation of the IL-1β gene. A GATA consensus oligonucleotide competed for some, but not all, of the binding activity in complexes 3 and 5 (Fig. 7A, lane 6), suggesting that GATA factors may be involved in the IL-1β response. In addition, we found that oligonucleotide −308/−274 was able to compete for binding to complex 3 and part of complex 5 (Fig. 7A, lane 4), suggesting that −261/−226 and −308/−274 share some common binding factors.

**DISCUSSION**

We have demonstrated that IL-1β increases mRNAs for both IL-1 agonists in TSC, providing a potential autocrine-paracrine positive feedback loop for IL-1 activity in the thymic microenvironment. This effect appears to be cell-specific, since the expression patterns we observed differed in stromal cell lines derived from different tissues as well as from those described in other cell types. The mechanism by which IL-1 exerts this effect appears to be primarily at the level of transcription, despite the major role for modification of mRNA stability in control of gene expression for these cytokines in other cells (26, 49). Our investigation of the IL-1-induced increase in transcription of the IL-1β gene in TSC revealed a different mechanism than has been described for IL-1 action on other target genes in other cell types and required distinct sequences in the 5′-flanking region of the IL-1β gene than have been described in the response to other agents. In TSC, this involves a member of the pit, oct, unc (POU) domain family of transcription factors, Oct-1, binding to an Oct consensus element positionally conserved in the IL-1β gene across species.

Oct-1 is a widely expressed member of the POU domain family of transcription factors. POU factors contain two highly conserved domains, a POU-specific domain and a POU homeodomain, which mediate binding to DNA as well as interactions with other proteins that contribute to regulation of transcription (for reviews, see Refs. 52 and 53). Oct-1 is a member of the pit, oct, unc (POU) domain family of transcription factors, which mediate binding to DNA as well as interactions with other proteins that contribute to regulation of transcription.
transcription of these target genes; accumulating evidence indicates this is dependent on sequence context, allowing interactions with other transcription factors or regulators.

In TSC, we found that the binding activity of Oct-1 to sequences in the 5′-flanking region of the IL-1β gene was specifically induced by IL-1β, and our functional analysis confirmed an essential role of this mechanism in mediating the response. Oct-1 did not appear to play a role in determining basal transcription of IL-1β in TSC, in contrast to the role of Oct-1 in regulation of some of the cytokine genes noted above (59, 60). The role of Oct-1 in IL-1β autocrine regulation and other IL-1 actions in other cell types remains to be determined. Factors not implicated in the present studies appear to be involved in IL-1-stimulated IL-1β gene transcription in some cells. Tsukada et al. (24) have shown that in the murine EL4 thymoma cell line, IL-1β-induced binding of a STAT-like factor to the LPS and IL-1-responsive element located at position −2863 to −2841 of the human IL-1β gene. The −4- to −0.5-kbp region was not implicated in the IL-1 response in our cells, nor were we able to detect any IL-1β-induced complex formation in TSC using EMSA with consensus sequences for STAT 1, 3, or 5.2 In addition, IL-1 does not stimulate Oct-1 activity in all systems. It has been reported that IL-1β in combination with cycloheximide or actinomycin D could not superinduce the binding activity of Oct-1 in A549 cells (61), and in Caco-2 and HepG2 epithelial cell lines, IL-1β treatment resulted in removal of Oct-1 from the IL-8 promoter, where it functions as a repressor (60). Taken together, our findings and these reports are consistent with different mediators of IL-1 action depending on the target cell as well as complex regulation of the activity of Oct-1 by IL-1β, which is dependent on promoter sequence and cell context.

In the mobility shift assays, we found that the IL-1β-induced complexes were formed as early as 30 min. This rapid response suggested that IL-1β may activate the participating protein factors through a post-translational mechanism, such as phosphorylation or dephosphorylation. Phosphorylation has been shown to increase or decrease the activity of POU domain transcription factors in a site-specific manner (52, 53). Several of the multiple kinases and phosphatases that have been demonstrated to be activated by IL-1 in different systems (for reviews, see Refs. 2 and 62) have been implicated in control of the activity of Oct-1, including protein kinase A (63, 64) and protein kinase C (63). Phosphorylation of Oct-1 at Ser-385 in the POU homeodomain inhibited DNA binding to the H2B promoter (64), and inhibition of phosphatases resulted in decreased Oct-1 binding to DNA by EMSA using extracts from the B-cell Daudi cell line (65). Of note, pretreatment with the phosphatase inhibitor sodium orthovanadate blocked IL-1 induction of IL-1β transcription in TSC.2 However, our observed recruitment of Oct-1 to the DNA-protein complex in response to IL-1 may not necessarily be a result of modification of Oct-1 itself but rather an accessory protein that then facilitates Oct-1 binding. Additional studies to examine IL-1-induced modifications of Oct-1 and the IL-1 signaling cascade in TSC as well as other IL-1 target cells are necessary to clarify the mechanism and cell/target gene specificity.

The POU domain also provides an interface for interactions with other proteins. Many cellular as well as viral factors have been shown to associate with Oct-1 in response to different stimuli (52, 53). In our studies in TSC, we found that an oligonucleotide containing a mutation in the Oct consensus site not only failed to abolish formation of the Oct-1-containing complex but also failed to compete for the other complexes formed with the −261/−226 probe, suggesting that binding of Oct-1 was required for binding of additional proteins to this DNA. Involvement of the GATA family of transcription factors suggested by our data provides candidates to study these interactions. The −308/−274 region, adjacent to the region containing the Oct consensus site, contains three additional GATA-like response elements. These sequences were also able to compete for some protein binding to the −261/−226 probe,
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Acknowledgments—The authors are grateful to Drs. C. J. Czuprynski (Department of Pathobiological Sciences, University of Wisconsin, Madison, WI) and T. G. Golos (Wisconsin Regional Primate Center, University of Wisconsin, Madison, WI) for reagents and thoughtful advice. We would like to thank Dr. H.-T. Chen (currently at Cornell University Medical Center, New York, NY) for providing bovine genomic library, and Erin Klaffky for her technical assistance.

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FIG. 8. Functional analysis of the Oct consensus site in IL-1β-induced transcription. A, results are shown as luciferase activity in IL-1β-stimulated cells (black bars) and untreated medium control (white bars). B, data are presented as fold induction by IL-1β relative to the medium control for each construct. Mutation constructs were generated, and luciferase assays were performed as described under "Experimental Procedures." Values were obtained from four (constructs −303/+14 and −303/+14μOct) or three (constructs −255/+14 and −255/14μOct) independent experiments, with each treatment group represented by duplicate plates; error bars indicate the S.E. of the mean. Significance was determined relative to corresponding wild-type control by ordinary ANOVA (post test is the Student-Newman-Keuls multiple comparisons test). ***p < 0.001; *p < 0.05; RLU, relative light units.

suggesting that GATA proteins could be common factors shared by these two regions. GATA factors have been most studied for their roles in regulation of hematopoiesis (54, 65), but this growing family of transcription factors has recently been found to play tissue-specific roles in multiple other cell types (66–68). Like the POU family of transcription factors, recent evidence indicates that interactions with other cell-specific proteins may modulate their activities (69).

The demonstration in this study that IL-1β increased levels of both IL-2 and IL-1β mRNAs in TSC implicates a role for IL-1β in regulating cytokine production in the thymus, contributing to T-cell development. We used this system to study IL-1 regulation of IL-1β transcription and identified several responsive regions in the 5′-flanking region of the bovine IL-1β gene. Our data demonstrated that IL-1β stimulated transcription in part through the nuclear factor Oct-1 binding to an Oct consensus element in these cells. Identification of additional regulatory elements and factors binding to these sequences may reveal how these factors interact in IL-1β control of transcription of its own gene, increase our knowledge of IL-1 signal transduction, and improve our understanding of control of thymic development and function.
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