Interleukin-1β enhances retinoic acid-mediated expression of bone-type alkaline phosphatase in rat IEC-6 cells

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Interleukin-1β enhances retinoic acid-mediated expression of bone-type alkaline phosphatase in rat IEC-6 cells. Am J Physiol Gastrointest Liver Physiol 280: G510–G517, 2001.—We previously showed that vitamin A upregulated the expression of bone-type alkaline phosphatase (ALP) in fetal rat small intestine and rat intestinal IEC-6 cells. In this study, we examined interactions between retinoic acid (RA) and several growth factors/cytokines on the isozyme expression in IEC-6 cells. Epidermal growth factor and interleukins (ILs)-2, -4, -5, and -6 completely blocked the RA-mediated increase in ALP activity. In contrast, IL-1β markedly increased the activity, protein, and mRNA of the bone-type ALP only when RA was present. IL-1β and/or RA did not change the type 1 IL-1 receptor transcript level, whereas IL-1β enhanced the RA-induced expressions of retinoic acid receptor-β (RAR-β) and retinoid X receptor-β (RXR-β) mRNAs and RA-mediated RXR response element binding. The synergism of IL-1β and RA on ALP activity was completely blocked by protein kinase C (PKC) inhibitors. Our results suggest that IL-1β may modify the ALP isozyme expression in small intestinal epithelial cells by stimulating PKC-dependent, RAR-β- and/or RXR-β-mediated signaling pathways.

retinoid acid receptors; retinoid X receptors; protein kinase C; fetal rat small intestine; alkaline phosphatase isozymes

During gestation, rat small intestinal epithelial cells drastically change the expression of alkaline phosphatase (ALP) isozymes (12). Liver/bone/kidney (LBK) ALP is transiently expressed in the cells lining the gut and in crypt cells during the first and second gestational phases. Intestinal ALP first appears during the third phase, and the level continues to increase. At the same time, LBK ALP expression rapidly and reciprocally decreases. At present, the physiological meaning of LBK ALP expression during the early gestational period is unknown; however, mice lacking the LBK ALP gene were reported to show abnormal intestinal motility, suggesting that the transient expression of LBK ALP and rapid changes in the pattern of ALP isozyme expression during the early gestational phases are essential events processing normal development of the small intestine (19). Therefore, it is important to reveal the molecular mechanisms for regulation of each ALP isozyme expression in fetal small intestinal epithelial cells. Recently, we showed that retinoic acid (RA) specifically upregulated the bone-type ALP in rat fetal small intestinal crypt cells (IEC-6 cells), and we also found that LBK ALP expressed in the fetal rat intestine was the bone type and that its expression was markedly upregulated by administration of retinyl acetate to dams (20). Furthermore, retinoic acid receptor-β (RAR-β) mRNA is expressed in IEC-6 cells and in the fetal rat small intestine but not in adult tissue (22). Thus IEC-6 cells display features characteristic of fetal rat small intestinal epithelial cells.

In this study, we further examined the regulation of bone-type ALP expression in IEC-6 cells. The bone-type ALP-inducing action of RA shown in IEC-6 cells was not enough to account for the marked expression of the isozyme observed in dams treated with an excess amount of retinyl acetate (20), leading us to consider that RA-mediated induction of bone-type ALP may be greatly modulated by additional factor(s). In other words, an exogenous morphogen of RA may act as a cofactor for distinct endogenous factor(s) in regulation of bone-type ALP expression in the fetal tissue. On the basis of this idea, we investigated the effects of several growth factors and cytokines on ALP expression in IEC-6 cells. RA alone could increase the ALP expression about threefold. Macrophage colony-stimulating factor (M-CSF), transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α) had no effect on the action of RA, but the induction was completely canceled in the presence of epidermal growth factor (EGF) and interleukins (ILs)-2, -4, -5, and -6. Furthermore, we found a unique and specific interaction between RA and IL-1β on bone-type ALP expression; IL-1β itself had no stimulatory action on the isozyme...
induction, but in the presence of RA, it markedly induced the ALP activity >15-fold. To elucidate the regulatory mechanism for bone-type ALP expression in fetal small intestinal epithelial cells, in this study we focused on the synergistic effects between RA and IL-1β on the transcriptional activation of the bone-type ALP gene in IEC-6 cells.

MATERIALS AND METHODS

Reagents. FCS and DMEM were obtained from Flow Laboratories (McLean, VA). RA, 1-(5-isouquinolinesulfonyl)-2-methylpiperazine (H-7), and N-[2-(methyamino)ethyl]-5-isouquinolinesulfonamide (H-8) were purchased from Sigma (St. Louis, MO). Human recombinant TNF-α, IL-1α and -β, IL-2, IL-4, IL-5, IL-6, and M-CSF were provided by Otsuka Pharmaceuticals (Tokushima, Japan). EGFP from mouse submaxillary glands (receptor grade) was purchased from Collaborative Research (Bedford, MA). Human recombinant TGF-β was purchased from King Brewery (Tokyo, Japan). Dibutyryl cAMP (dbcAMP) and bisindolylmaleimide GF-109203X were purchased from Boehringer Pharmaceuticals (Mannheim, Germany). An enhanced chemiluminescence Western blotting detection system, poly(dI-dC)-poly(dI-dC), [γ-32P]ATP (2.2 × 10^4 Bq/mmol), and T4 polynucleotide kinase were purchased from Amersham (Little Chalfont, UK). [α-32P]dCTP ([γ-32P]ATP (1.1 × 10^4 Bq/mmol) was obtained from ICN Pharmaceuticals (Costa Mesa, CA). Antisera and cDNAs for rat LBK ALP and rabbit antiserum against rat LBK or intestinal ALP. Bound antibodies were detected using the enhanced chemiluminescence system.

Cell culture and analysis of ALP activity in IEC-6 cells. IEC-6 cells and F9 cells were cultured and maintained as described previously (20). When IEC-6 cells were grown to 80% of confluency, the medium was changed to DMEM containing 0.1% FCS and cells were cultured overnight.

Preparation of whole cell extracts. IEC-6 cells were suspended in lysis buffer consisting of 20 mM HEPES, pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM leupeptin, 5 mM trans-epoxyucyclinyl-1, leucylamido-(4-guanidino)butane, 0.3 mM aprotinin, 1 mM pepstatin, and 0.5 mM dithiothreitol. Whole cell extracts were obtained by homogenization in a rubber policeman, and collected in a microtube. The collected cell extracts was measured using a substrate according to the method of Hausamen et al. (6).

Immunoblot analysis for LBK ALP. The whole cell extracts from IEC-6 cells and tissue proteins from fetal rat small intestines were subjected to SDS-8% PAGE (40 μg protein/lane) and transferred to a polyvinyldene difluoride membrane. The membrane was blocked with 5% BSA and then incubated for 1 h at 25°C in PBS with a 1:1,000 dilution of rabbit antiserum against rat LBK or intestinal ALP. Bound antibodies were detected using the enhanced chemiluminescence system.

Gel mobility shift assay. Preparation of whole cell extracts and gel mobility shift assay were performed as described previously (7). Briefly, IEC-6 cells treated with RA and/or IL-1β were suspended in lysis buffer consisting of 20 mM HEPES, pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM leupeptin, 5 mM trans-epoxyucyclinyl-1, leucylamido-(4-guanidino)butane, 0.3 mM aprotinin, 1 mM pepstatin, and 0.5 mM dithiothreitol. Whole cell extracts were obtained by ultracentrifugation at 100,000 g for 5 min at 4°C were dialyzed against binding reaction buffer consisting of 10 mM Tris-HCl (pH 7.8), 50 mM NaCl, 1 mM EDTA, 8% glycerol, 1 mM dithiothreitol, 5 mM MgCl₂, and 0.5 mg/ml BSA. Synthetic double-stranded oligonucleotide (5′-AGCTAGTCGAGGCACGCTTACGTATGCGG-3′) and (5′-GGCTGTCAGCTTACGTATGCGG-3′), were used as probes for Northern blot analysis. Total RNA was isolated from IEC-6 cells with an acid guanidino thiocyanate-phenol-chloroform mixture (Nippon Gene, Tokyo, Japan). Total RNA (20 μg) was separated in a 1% agarose gel, blotted, and ultraviolet crosslinked to a Hybond N+ nylon membrane (Amerham). Prehybridization of membranes was carried out for 4 h at 42°C in buffer containing 50% formamide, 6× SSC (1× SSC consists of 0.15 M NaCl and 15 mM sodium citrate), 1% SDS, 5× Denhardt's solution (1× Denhardt's solution consists of 0.02% Ficoll, 0.02% BSA, and 0.02% polyvinylpyrrolidone), and 50 μg/ml heat-denatured, sheared salmon sperm DNA. Hybridization was performed overnight at 48°C in a mixture containing 4 vols of prehybridization solution, 1 vol of 50% dextran sulfate, and 1–5 × 10^8 cpm/ml of the appropriate cDNA probe. The isolated cDNA fragments were labeled with [α-32P]dCTP by a random primer kit (Amerham). Membranes were washed and exposed to Kodak X-ray films at −80°C for the appropriate times, and then films were developed. Autoradiography signals were quantified by densitometric analysis. Each mRNA level was standardized to that of GAPDH mRNA.
GAGTTAGCT-3') was used as a mutant RXRE competitor. Whole cell extracts (10 μg protein) were mixed with 0.1 ng of the ^32P-labeled RXRE oligonucleotide and 1 μg of poly(dI-dC)·poly(dI-dC) for 30 min at 25°C. The RXRE-containing complexes were separated in 5% nondenaturing polyacrylamide gels at 180 V for 2 h at 4°C.

Statistical analysis. All data are expressed as means ± SD for three to eight individual samples per group and were analyzed by one-way analysis of variance using SPSS (release 6.1, SPSS Japan, Tokyo). Differences between means were tested by Scheffé's test. A P value of <0.01 was considered to be statistically significant.

RESULTS

Stimulatory effect of IL-1 on RA-mediated increase of ALP activity in IEC-6 cells. Treatment of IEC-6 cells with 1 μM RA for 5 days increased ALP activity about threefold (Fig. 1A). When growth factors (EGF, M-CSF, TGF-β) and cytokines (TNF-α, ILs-1, -2, -4, -5, and -6) at the indicated concentrations were added simultaneously with 1 μM RA to IEC-6 cells, M-CSF, TGF-β, and TNF-α did not affect the RA-mediated increase in ALP activity, whereas EGF, IL-2, IL-4, IL-5, and IL-6 completely canceled the RA-mediated ALP activation. On the other hand, the RA-induced ALP activation was additionally enhanced with IL-1α (10 ng/ml) and IL-1β (1 ng/ml); the activity increased 9- and 16-fold, respectively, compared with that of vehicle-treated control cells. In the absence of RA, none of these cytokines or growth factors changed the ALP activity in IEC-6 cells (data not shown).

In the presence of 1 μM RA, the maximum synergistic effects with IL-1α and IL-1β were achieved at 10 and 1 ng/ml, respectively (Fig. 1B). In the presence of 10 ng/ml IL-1α or 1 ng/ml IL-1β, RA at 1 μM was required to exhibit the maximum effect on ALP activity (data not shown). IL-1β markedly accelerated the time-dependent increase in ALP activity by RA, and the maximum activation was observed on day 3 (Fig. 1C).

Effect of RA and IL-1β on growth of IEC-6 cells. When IEC-6 cells were cultured in 5% FCS-containing DMEM, the number of IEC-6 cells linearly increased over 5 days, and the treatment with 1 μM RA significantly inhibited the cell growth by ~25%. The simultaneous treatment with 1 ng/ml IL-1β and 1 μM RA suppressed the cell growth to ~50% of that of control cells, although IL-1β (1 ng/ml) did not affect the cell growth alone.
growth of IEC-6 cells cultured in 5% FCS-containing DMEM (Fig. 1D). Hematoxylin-eosin staining showed that RA- and IL-1β-treated cells became spindle shaped (data not shown). Therefore, the morphological changes were further examined by transmission electron microscopy. Treatment with RA and IL-1β appeared to stimulate the development of rough endoplasmic reticulum; however, the cells treated with RA and IL-1β did not exhibit morphological features characteristic of absorptive cells such as microvilli formation (data not shown).

**Stimulatory effect of IL-1β on RA-mediated LBK ALP expression in IEC-6 cells.** Western blot analysis showed that RA-treated IEC-6 cells contained a 75-kDa protein corresponding to rat LBK ALP (Fig. 2A). IL-1β further enhanced the RA-mediated increase in LBK ALP protein, whereas the IL-1β itself did not induce LBK ALP protein in IEC-6 cells (Fig. 2A). Intestinal ALP protein was not detected in IEC-6 cells even after simultaneous treatment with RA and IL-1β (data not shown).

Lower levels of the LBK ALP transcript were detected in untreated IEC-6 cells or cells treated with vehicle and IL-1β (Fig. 2B). Treatment with RA time-dependently increased the concentration of LBK ALP mRNA on days 1–5 (Fig. 2B). IL-1β further enhanced this RA-mediated expression of LBK ALP transcript (Fig. 2B).

RT-PCR analysis with the primer set specific for exon 1 or 2 revealed that only the bone-type transcript was amplified in IEC-6 cells and that RA and RA plus IL-1β increased the level of the amplified bone-type transcript (Fig. 2C).

Changes in RAR and RXR mRNA levels in IEC-6 cells treated with RA and IL-1β. Northern blot analyses showed that the mRNAs of RAR-α, RAR-γ, and RXR-γ were expressed at higher levels in IEC-6 cells, similarly to F9 cells (Fig. 3). In contrast, IEC-6 cells contained the transcripts of RAR-β, RAR-α, and RXR-β at low concentrations. RA significantly increased the concentrations of RAR-α and RXR-α mRNAs. IL-1β also upregulated the expression of RAR-β and RXR-β mRNAs in a time-dependent manner, whereas it did not change the levels of other receptor transcripts. When IEC-6 cells were treated with both RA and IL-1β, the expressions of α- and β-isoforms transcripts of both RAR and RXR were enhanced. Among these changes, the upregulation of RAR-β and RXR-β mRNA expression most rapidly occurred within 4 h after treatment with RA and IL-1β.

**Type 1 IL-1 receptor mRNA in IEC-6 cells.** It has been reported that immature rat small intestinal epithelial cells such as IEC-18 cells expressed low levels of the type 1 IL-1 receptor mRNAs, which were detected by RT-PCR but not by Northern blot analysis (26). Therefore, we measured the levels of type 1 IL-1 receptor mRNA in IEC-6 cells by a semiquantitative RT-PCR. The type 1 IL-1 receptor mRNA was linearly amplified from 25 to 34 cycles of PCR (data not shown). Treatment with RA and/or IL-1β did not change the ratio of type 1 IL-1 receptor mRNA to β-actin mRNA, as shown in Fig. 4.

**RXRE-binding activity in IEC-6 cells.** Gel mobility shift assay showed that vehicle-treated IEC-6 cells did not have any specific RXRE-binding activity (Fig. 5).
RA produced RXRE-binding activity in IEC-6 cells on days 1 and 3 (Fig. 5). In the absence of RA, IL-1β did not produce the RXRE-binding activity on day 1, whereas it enhanced the RA-mediated RXRE-binding activity (Fig. 5). This binding activity was inhibited by addition of excess unlabeled RXRE oligonucleotide but not by the mutant RXRE oligonucleotide (Fig. 5).

**Effect of protein kinase inhibitors on ALP activity.** We examined whether protein kinases were involved in the interaction between IL-1β and RA on ALP induction. A high concentration (20 μM) of a protein kinase A (PKA) inhibitor, H-8, was required to significantly inhibit the induction of ALP activity with IL-1β plus RA (Fig. 6), and H-8 at this concentration suppressed the induction by only 13%. In contrast, protein kinase C (PKC) inhibitors H-7 and GF-109203X more effectively inhibited the increase in ALP activity. Twenty micromolar H-7 or five micromolar GF-109203X completely suppressed the synergistic activation with RA and IL-1β, and the levels returned to those of RA-treated IEC-6 cells. Cell viability assessed by trypan blue exclusion and adherence to the culture plates was maintained throughout the experiments (data not shown).

**DISCUSSION**

IEC-6 cells may be a useful model to study the regulation of LBK ALP expression in normally developing fetal small intestine because they have features characteristic of fetal small intestinal epithelial cells (20, 23). In the present study, we examined the interaction between RA and several cytokines and growth factors on ALP activity in IEC-6 cells. Among the cytokines and growth factors tested, only IL-1, especially IL-1β, synergistically increased the RA-mediated upregulation of bone-type ALP expression in IEC-6 cells, suggesting that IL-1 may be a potent endogenous regulator for the bone-type isozyme expression in fetal rat small intestine. Several investigations have suggested that IL-1 is one of the important cytokines for the regulation of gene expression in fetal tissues. For example, IL-1 released from placenta could upregulate metallothionein expression in the fetal liver (8) and regulate glucocorticoid levels by stimulating the release of adrenocorticotropic hormone from fetal pituitary gland (3). IL-1 may interact with RA, an exogenous morphogen, to upregulate the expression of bone-type ALP in the fetal rat small intestine. In contrast, EGF and ILs-2, -4, -5, and -6 completely inhibited the RA-mediated increase in ALP activity, although M-CSF, TGF-β, and TNF-α did not affect it. EGF, IL-2, and IL-4 are known to be potent mitogens for IEC-6 cells.

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**Fig. 3.** Time-dependent changes in RA receptor (RAR) and retinoid X receptor (RXR) mRNA concentrations after treatment with RA and/or IL-1β. A: IEC-6 cells were untreated (lanes 2 and 3) or treated with 1 μM RA (lanes 4–9), 1 ng/ml IL-1β (lanes 10–15), or 1 μM RA + 1 ng/ml IL-1β (lanes 16–21) for the indicated hours, and then total RNAs were extracted. Total RNA was also extracted from F9 cells treated with 1 μM RA for 1 day (lane 1). Samples of 20 μg of RNA were subjected to 1% agarose-0.6 M formaldehyde gel electrophoresis and transferred to nylon filters. The filters were hybridized with the 32P-labeled cDNA probes for the mouse RARs and RXRs, as described in MATERIALS AND METHODS. B: GAPDH mRNA level was also measured by Northern blot analysis using a cDNA for human GAPDH and was used to quantify the mRNA level of α-, β-, and γ-subtypes of RAR and RXR by densitometric analysis. Values are means ± SD (n = 3). *Significantly increased vs. before treatment (time 0). P < 0.01. ○, 1 ng/ml IL-1β; ●, 1 μM RA; ■, 1 ng/ml IL-1β + 1 μM RA.
It is possible to speculate that proliferation signals induced by these factors may interfere with RA-mediated expression of bone-type ALP in IEC-6 cells.

**IL-1β itself could not stimulate bone-type ALP expression, and it could augment this expression only when RA-mediated pathways were active, suggesting that IL-1β may act as a cofactor that specifically enhances the distinct RA signaling pathway in IEC-6 cells. Lipopolysaccharide and vitamin D₃ have been**

**Regulation of bone-type ALP expression in IEC-6 cells**

**Fig. 4. Detection of type 1 IL-1 receptor mRNA in IEC-6 cells by RT-PCR.** A: total RNA (1 µg) was prepared from IEC-6 cells treated with vehicle (lanes 1 and 5), 1 µM RA (lanes 2 and 6), 1 ng/ml IL-1β (lanes 3 and 7), or 1 µM RA + 1 ng/ml IL-1β (lanes 4 and 8) for 3 days. RT-PCR was performed using the oligonucleotide couples specific for type 1 IL-1 receptor or β-actin. The amplification was terminated after 28 cycles of PCR. The amplified products were run on a 1.5% agarose gel, blotted on nylon membranes, and hybridized to the 32P-labeled synthetic oligonucleotides specific for type 1 IL-1 receptor or β-actin, as described in MATERIALS AND METHODS. B: the β-actin mRNA level was used to quantify the mRNA level of type 1 IL-1 receptor by densitometric analysis. Values are means ± SD (n = 3). IL-1R, IL-1 receptor.

**Fig. 5. Detection of RXR response element (RXRE)-binding activity after treatment with RA and IL-1β.** IEC-6 cells were cultured with vehicle (lanes 1 and 5), 1 µM RA (lanes 2 and 6), 1 ng/ml IL-1β (lane 4), or 1 µM RA + 1 ng/ml IL-1β (lanes 3, 7–13) for the indicated days. Whole cell proteins were extracted on the indicated days, and gel mobility shift assay was performed as described in MATERIALS AND METHODS. Lanes 9–11 contained a 10-, 50-, or 100-fold molar excess of the unlabeled RXRE oligonucleotide, respectively. Lanes 12 and 13 contained a 50- or 100-fold molar excess of the mutant RXRE oligonucleotide, respectively. An RXRE-protein complex is indicated by “RXRE.” Similar results were obtained in 3 separate experiments.

**Fig. 6. Effects of protein kinase inhibitors on ALP activity in IEC-6 cells treated with RA and IL-1β.** IEC-6 cells were treated with vehicle, 1 µM RA, 1 ng/ml IL-1β, and 1 ng/ml IL-1β plus 1 µM RA for 5 days. They were also treated with IL-1β and RA in the presence of H-8, H-7, or GF-109203X at the indicated concentrations. ALP activity in these cells was measured as described in MATERIALS AND METHODS. Values are means ± SD; n = 5. Means with different superscripts are significantly different; P < 0.01.
shown to exhibit similar stimulatory effects on RA-mediated ALP activity in IEC-6 cells (5, 11). However, those studies evaluated the effects by measuring ALP activity; therefore, the molecular mechanism(s) underlying the interaction is not fully understood. At first, we examined whether the stimulatory effect of IL-1β on the RA action occurred at the level of the membrane receptor or nuclear receptors. Treatment with IL-1β and/or RA did not change the expression of type 1 IL-1 receptor transcript, although it was reported that stimulation of the IEC-6 cells by IL-1β downregulated the expression of type 1 IL-1 receptor at 24 h (18). We also examined the changes in RAR and RXR transcript expressions. IL-1β alone significantly increased the level of RAR-β and RXR-β mRNAs, and it also enhanced the RA-mediated expression of these receptors. Among the changes in expressions of all retinoid receptors tested, RAR-β and RXR-β mRNAs responded most rapidly to the simultaneous treatment within 4 h, and their enhanced expressions were well correlated with the expression of bone-type ALP. The present study also demonstrated that IL-1β further enhanced the RA-mediated increase of the binding activity to an RXRE, at which a homodimer of RXR or heterodimer of RAR and RXR can interact (4). These results indicate that IL-1β may stimulate the RA-mediated signaling pathway by upregulating the RAR-β- and/or RXR-β-dependent transcription cascade.

An apparent retinoic acid receptor response element (RARE) has not been documented in the 5′-flanking regions of exon 1 (bone type) and exon 2 (liver type) of rat LBK ALP gene (28). An RXRE is located in the upstream of the liver-type exon, but not the bone-type exon, of rat LBK ALP gene (28). Although an RARE or RXRE has not been reported to be present in the 5′-flanking region of exon 1A (corresponding to rat exon 1) of mouse LBK ALP gene, RA has also been shown to induce LBK ALP protein by increasing the amount of the mRNA-containing exon 1A in mouse F9 teratocarcinoma cells (26). Thus the upregulation of RAR-β and RXR-β mRNA expressions and the activation of RXRE binding may evoke the secondary responses required for bone-type ALP expression rather than directly stimulating the transcriptional activation of the ALP gene. At present, the underlying mechanism for the retinoid receptor-mediated transcription of the rat bone-type ALP gene remains to be elucidated.

We next examined whether protein kinases were involved in the stimulatory effect of IL-1β on RA-mediated ALP expression because protein kinase inhibitors suppressed the synergistic effects between peptide growth factors and nuclear hormones and catalytic molecules downstream of both receptors are usually phosphorylated by protein kinases for their activation (1, 9, 31). Several recent investigations reported that a cAMP response element as well as RARE was involved in RA-dependent RAR-β promoter activation in embryonal carcinoma cells (13) and that cAMP response element binding protein is known to be activated by PKA (15). However, a high concentration (20 μM) of a PKA inhibitor, H-8, was required to significantly inhibit the increased ALP activity by 13%. Furthermore, dbcAMP did not affect ALP activity in IEC-6 cells even in the presence of IL-1β or RA (data not shown), suggesting that PKA may not be involved in the interaction between IL-1β and RA in IEC-6 cells. In contrast, relatively specific PKC inhibitors, H-7 and GF-109203X, significantly suppressed the synergism between IL-1β and RA on ALP activity at the lower concentrations. IL-1 is known to increase PKC activity in various types of cells, such as human astroglialoma cells (2), mouse osteoblast-like cells (10), and mouse fibroblast cells (29). Furthermore, Kurie et al. (14) reported that the stimulation of PKC by phorbol ester could upregulate RAR-β expression and potentiate the effects of RA on RAR-β-mediated transcription (14). On the basis of this information, PKC may be one of the putative mediators for the interaction between IL-1β and RA signaling pathways in IEC-6 cells.

At present, we do not know whether RA similarly regulates transcription of distinct genes by interacting with growth factors and/or cytokines in vivo. However, our results suggest that overproduction of inflammatory cytokines under specific situations, such as infection and inflammation, may greatly modify RA-dependent gene expression; therefore, it may disturb the normal development of tissues including the small intestine.

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