

# Molecular Regulation of Interleukin-13 and Monocyte Chemoattractant Protein-1 Expression in Human Mast Cells by Interleukin-1 $\beta$

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Mast cells play pivotal roles in immunoglobulin (Ig) E-mediated airway inflammation, expressing interleukin (IL)-13 and monocyte chemoattractant protein-1 (MCP-1), which in turn regulate IgE synthesis and/or inflammatory cell recruitment. The molecular effects of IL-1 $\beta$  on cytokine expression by human mast cells (HMC) have not been studied well. In this report, we provide evidence that human umbilical cord blood-derived mast cells (CBDMC) and HMC-1 cells express the type 1 receptor for IL-1. We also demonstrate that IL-1 $\beta$  and tumor necrosis factor- $\alpha$  are able to induce, individually or additively, dose-dependent expression of IL-13 and MCP-1 in these cells. The induction of IL-13 and MCP-1 gene expression by IL-1 $\beta$  was accompanied by the activation of IL-1 receptor-associated kinase and translocation of the transcription factor, nuclear factor (NF)  $\kappa$ B into the nucleus. Accordingly, Bay-11 7082, an inhibitor of NF- $\kappa$ B activation, inhibited IL-1 $\beta$ -induced IL-13 and MCP-1 expression. IL-1 $\beta$  also induced IL-13 promoter activity while enhancing the stability of IL-13 messenger RNA transcripts. Dexamethasone, a glucocorticoid, inhibited IL-1 $\beta$ -induced nuclear translocation of NF- $\kappa$ B and also the secretion of IL-13 from mast cells. Our data suggest that IL-1 $\beta$  can serve as a pivotal costimulus of inflammatory cytokine synthesis in human mast cells, and this may be partly mediated by IL-1 receptor-binding and subsequent signaling via nuclear translocation of NF- $\kappa$ B. Because IL-1 $\beta$  is a ubiquitously expressed cytokine, these findings have important implications for non-IgE-mediated signaling in airway mast cells as well as for innate immunity and airway inflammatory responses, such as observed in extrinsic and intrinsic asthma.

Mast cells are multifunctional, tissue-residing cells derived from bone marrow. Upon maturation, mast cells express the high-affinity receptor for immunoglobulin E (IgE), Fc epsilon receptor 1 (Fc $\epsilon$ RI), and, after cross-linkage of Fc $\epsilon$ RI by IgE and antigen, mast cell activation occurs. These activated mast cells degranulate, expressing preformed and newly synthesized mediators. These include histamine, lipid mediators, and various cytokines that regulate inflammatory responses, which have been reviewed by us (1). Important to the subject of this article are the cytokines, interleukin (IL)-13 and monocyte chemoattractant protein-1 (MCP-1). Mast cells have been shown to express IL-13 (2). IL-13 has been shown to induce B lymphocyte class-switching

to IgE and also induces vascular cell adhesion molecule expression on endothelium cells (3, 4). IL-13 has also been shown to be pivotal to airway remodeling and mucus hypersecretion in murine transgenic models (5–7). MCP-1 on the other hand is a C-C chemokine that is important in mononuclear recruitment as well as mast cell and basophil activation (8, 9). Together, these cytokines have important effects on regulating allergic airway inflammation as seen in asthma and on innate immune responses (8). In this article, we demonstrate that the monokine, IL-1 $\beta$ , activates the expression of both IL-13 and MCP-1 from the human mast cell line, HMC-1. We also show IL-1 $\beta$  induced MCP-1 expression in cord blood-derived mast cells (CBDMC) and IL-1 $\beta$ -Fc $\epsilon$ RI cross-linkage-induced expression of IL-13 in CBDMC. Because IL-1 $\beta$  is a ubiquitous cytokine expressed in airway macrophages and in many adventitial cells such as fibroblasts, this finding has important implications for innate immunity.

IL-1 $\beta$  mediates its effects by binding to its receptor on inflammatory cells. Two types of receptors, type 1 (IL-1RI) and type II, have been cloned (10). Of these two, IL-1RI is considered to be the biologically active (11–13). Before signal transduction can occur, IL-1 must bind to IL-1RI as well as a co-receptor known as IL-1 receptor accessory protein there by creating a transmembrane heterodimeric protein complex (11, 14). This interplay leads to the intracellular signaling cascade that recruits several early adaptor proteins such as MyD88 (myeloid differentiation factor 88) and IL-1 receptor-associated kinase (IRAK). IRAK becomes hyperphosphorylated moving into the cytoplasmic region of the cell and forms a signalsome with tumor necrosis factor (TNF) receptor-associated factor 6. This signalsome mediates signaling of many downstream events through several regulatory kinases, such as the I $\kappa$ B kinase complex and the mitogen-activated protein kinases (13–15). This can lead to the activation NF- $\kappa$ B, culminating in inflammatory gene expression and subsequent molecular mast cell events (15). However, the presence of IL-1R on human mast cells has not been demonstrated clearly, and the molecular effects of IL-1 on human mast cell signaling and gene expression have not been studied in any great detail.

IL-1 has been shown to have important effects on mast cell biology. For instance, Hultner and colleagues recently demonstrated that IL-1 induces the secretion of T helper type 2 cytokines, IL-3, IL-5, IL-6, and IL-9 from murine mast cells (16). Lu-Kuo and coworkers showed that IL-1 $\beta$  stabilized the message for IL-6 mRNA in murine mast cells (17). Hogaboam and colleagues demonstrated that IL-1 induced activation of rat peritoneal mast cells and expression of nitric oxide and platelet activating factor (18). However, molecular effects of IL-1 on human mast cells and the signaling pathways are unclear. Using human umbilical CBDMC, we recently demonstrated a profound effect of IL-1 on enhancing IgE-mediated expression of IL-5, granulocyte macrophage colony-stimulating factor, and IL-8 (19). In this study, we have extended these observations and demonstrate the molecular effects of IL-1 $\beta$  on mast cells and its role in the

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**Abbreviations:** cord blood-derived mast cells, CBDMC; ethylenediaminetetraacetic acid, EDTA; enzyme-linked immunosorbent assay, ELISA; electrophoretic mobility shift assay, EMSA; Fc epsilon receptor 1, Fc $\epsilon$ RI; immunoglobulin, Ig; interleukin, IL; IL-1 receptor-associated kinase, IRAK; IL-1 receptor type 1, IL-1RI; monocyte chemoattractant protein-1, MCP-1; nuclear factor- $\kappa$ B, NF- $\kappa$ B; phosphate-buffered saline, PBS; phorbol 12-myristate 13-acetate, PMA; PMA/ionomycin, PMA/iono; reverse transcriptase-polymerase chain reaction, RT-PCR.

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regulation of IL-13 and MCP-1 expression from these cells. We show that IRAK and NF- $\kappa$ B may be involved in this induction.

## Materials and Methods

### Cell Culture and Stimulation

HMC-1 cells were grown in RPMI 1,640 (Gibco BRL, Frederick, MD) supplemented with 11.1% fetal bovine serum and 1% 1M Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer solution (Gibco, Rockville, MD). Cells ( $1 \times 10^6$ /ml) were stimulated for 6, 12 and/or 24 h with various concentrations of recombinant IL-1 $\beta$  (1, 10, 100 ng/ml) and/or of TNF- $\alpha$  (1, 10, 100 U/ml; kindly provided by National Cancer Institute's Biological Resources Branch, Rockville, MD). Activation of the cells with phorbol 12-myristate 13-acetate (PMA; 50 ng/ml) (Sigma, St. Louis, MO) and ionomycin (5  $\mu$ M) (Sigma) (PMA/iono) was performed in parallel as a positive control. To investigate inhibition of NF- $\kappa$ B translocation, cells were treated with 10  $\mu$ M final concentration of Bay-11 7082 (Biomol, Plymouth Landing, PA) for 1.5 h before addition of IL-1 $\beta$ . In some experiments, cells were incubated with varying concentrations of dexamethasone (Dex, 10 $^{-6}$  and 10 $^{-7}$  M; Sigma) for 24 h before the addition of IL-1 $\beta$ . Cell viability remained intact after all treatments, as determined by trypan blue exclusion.

CBDMC were harvested from fresh cord blood obtained by patient consent and institutional review board approval. Blood was diluted 1:1 phosphate-buffered saline (PBS), layered over Lymphoprep, centrifuged, and washed with more PBS. The cells were then grown in DMEMF12 media supplemented with 20% fetal bovine serum (Atlanta Biologicals, Atlanta, GA);  $5 \times 10^{-5}$  M 2-mercaptoethanol (Fisher, Pittsburgh, PA); 0.5 ml insulin-transferin-sodium selenite solution (Sigma-Aldrich, St. Louis, MO); 25 mM HEPES (Gibco, Carlsbad, CA); 300 nM PGE $_2$  (Cayman, Ann Arbor, MI); 100 ng/ml recombinant human IL-6 (kindly provide by Amgen, Thousand Oaks, CA); and 80 ng/ml stem cell factor (kindly provided by Amgen) for  $\sim$ 16 wk or until mature (19). Maturity of CBDMC was observed by antichymase (kindly provided by Dr. Andrew Walls, University of Southampton, Southampton, UK) and antitrypsin (kindly provided by Promega, Madison, WI) antibody staining. Cross-linking of Fc $\epsilon$ RI on CBDMC surface was done using myeloma IgE at 1  $\mu$ g/ml and anti-IgE at 1.5  $\mu$ g/ml. IgE was added overnight at 37°C before the addition of anti-IgE.

### Immunocytochemistry Staining

For immunocytochemistry staining, cytospin preparations of resting or stimulated cells (50 ng/ml PMA and 5  $\mu$ M Ionomycin) were performed, and incubated with a primary rat anti-human IL-1RI antibody (Antigenix, Huntington Station, NY), followed by a 30-min incubation with a mouse anti-rat IgG fluorescein isothiocyanate-conjugated secondary antibody (Antigenix). Slides were kept in the dark until observed under a fluorescence microscope (Olympus BX60, Melville, NY).

### Western Blotting Analysis

For analysis of IRAK expression, mast cells were lysed by 10% Nonidet P-40 in hypotonic buffer, and the cytoplasmic fractions were isolated and stored at  $-80^\circ\text{C}$ . A 10 mg quantity of total protein was added to equal amounts of Laemmli's buffer (Bio-Rad Laboratories, Hercules, CA), heated for 5 min at  $100^\circ\text{C}$ , resolved on 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to nitrocellulose membrane (Bio-Rad). The membrane was blocked with 5% bovine serum antigen in PBS, pH 7.4, for 1 h and incubated in 1:500 dilution of a primary mouse antihuman IRAK antibody (BD Biosciences, San Diego, CA) overnight at  $4^\circ\text{C}$ . After washing, membranes were incubated with peroxidase-conjugated secondary antibody (Amgen) for 1 h and Super Signal substrate (Pierce, Rockford, IL). Immunoreactive proteins were detected by enhanced chemiluminescence.  $\beta$ -Actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used as a loading control.

### IL-13 and MCP-1 Gene and Protein Expression

Gene expression for IL-13 or MCP-1 was assessed using reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was extracted by the RNeasy technique from cultured cells according to manufacturer's instructions (Tel-Test, Inc., Friendswood, TX). First strand cDNA was synthesized following a reverse-transcription step at  $42^\circ\text{C}$  for 20 min

in the presence of murine leukemia virus RT (2.5 U/ $\mu$ l), 1 mM each of the nucleotides dATP, dCTP, dGTP, and dTTP, RNase inhibitor (1 U/ $\mu$ l),  $10\times$  PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3), and MgCl $_2$  (5 mM), using oligo(dT) $_{16}$  (2.5  $\mu$ M) as a primer. PCR amplification was done on aliquots of the cDNA in the presence of MgCl $_2$  (1.8 mM), each of the dNTPs (0.2 mM), AmpliTaq polymerase (1 U/50  $\mu$ l), and paired cytokine-specific primers (0.2 nM of each primer) in a total volume of 50  $\mu$ l. PCR consisted of the following: 1 cycle at  $95^\circ\text{C}$  for 2 min, 45 cycles at  $95^\circ\text{C}$  for 45 s,  $60^\circ\text{C}$  for 45 s, and  $72^\circ\text{C}$  for 1 min 30 s, and, lastly, 1 cycle at  $72^\circ\text{C}$  for 10 min for G3PDH; and 1 cycle of  $95^\circ\text{C}$  for 2 min, 36 cycles at  $95^\circ\text{C}$  for 45 s,  $60^\circ\text{C}$  for 45 s, and  $72^\circ\text{C}$  for 1 min 30 s, and 1 cycle at  $72^\circ\text{C}$  for 10 min for IL-13 (5' GGAA GCTT CTCC TCAA TCCT CTCC TGTT-3'), IL-1RI (5' GAAG CTGG ACCC CTTG GTAA-3'), and MCP-1 (5' AGAA CTGT GGTT CAAG AGG-3'). A total of 12  $\mu$ l of the amplified products were subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide. IL-1RI, IL-13, and MCP-1 bands were compared with expected base pair migration distances from Phi 174 Hae III DNA maker (Promega, Madison, WI). IL-13 and MCP-1 levels in cell-free culture supernatants were assayed by enzyme-linked immunosorbent assay (ELISA) as previously described using commercially available kits (R&D Systems, Minneapolis, MN) (20–22). Densitometry was done by normalizing gel band intensities to housekeeping genes on UN-SCAN-IT software (Silk Scientific, Orem, UT).

### mRNA Stability Assay for IL-1 $\beta$ -Induced IL-13 Gene Expression

To examine whether IL-1 $\beta$  regulates IL-13 gene expression post-transcriptionally, analysis of mRNA stability was performed using a transcription inhibitor, actinomycin D, a potent inhibitor of RNA polymerase II-dependent transcription, as were semi-quantitative RT-PCR analyses at various time points with the inhibitor. The cells ( $1 \times 10^6$  per condition) were treated with IL-1 $\beta$  (10 ng/ml) for 2 hrs to induce IL-13 expression, followed by extensive washes. The cells were then cultured with or without IL-1 $\beta$  in the presence of actinomycin D (2  $\mu$ g/ml; Sigma). Total RNAs were isolated using the RNeasy kit (Qiagen, Santa Clarita, CA) at various time points after the addition of actinomycin D. RT-PCR analysis was performed using a standard protocol and pairs of human IL-13 and G3PDH primers. DNA strands were denatured at  $95^\circ\text{C}$  for 45 s, followed by PCR at  $60^\circ\text{C}$  for 45 s,  $72^\circ\text{C}$  for 45 s, for 32 cycles for IL-13 and 26 cycles for G3PDH. The intensities of PCR products on 2% ethidium bromide-containing agarose gel with optimized exposure were evaluated by OpiQuant Acquisition and Analysis (Packard Bioscience Co., Meriden, CT). The relative level of gene expression was quantified by calculating the ratio of densitometric readings (optical densities) of the band intensity for IL-13 and G3PDH from the same sample at each time point, and was then normalized to the ratio at time 0.

### Induction of IL-13 Promoter Activity in IL-1-Stimulated Mast Cells

To investigate the minimal promoter activity of IL-13 gene in IL-1 $\beta$ -stimulated cells, transient transfection assays were performed using a reporter gene construct containing the minimal promoter sequence of IL-13, in which the promoter sequence ( $-233$  to  $+50$ , relative to the transcription initiation site) of the IL-13 gene was fused to the luciferase coding sequence. A reporter gene construct containing a minimal promoter ( $-217$  to  $+51$ , relative to the transcription initiation site) of the IL-4 gene was included in the assays for comparison. Plasmid DNA was obtained with purification by double cesium chloride gradient (BioServe Biotechnologies, Laurel, MD). The SuperFect reagent (Qiagen) was used for transient transfections of HMC-1 cells according to the manufacturer's directions. A total of 2  $\mu$ g of plasmid DNA and 8  $\mu$ l SuperFect reagent were used for transfection of  $1 \times 10^6$  HMC-1 cells. The transfected cells were stimulated with 10-ng/ml IL-1 $\beta$ . Luciferase expression was monitored by chemiluminescence of cell lysates 24 h after transfections using the Enhanced Luciferase Assay Kit (Analytical Luminescence Laboratory, Ann Arbor, MI), as recommended by the manufacturer. Total protein content of cell lysates was determined with Bio-Rad protein assays (Bio-Rad).

### NF- $\kappa$ B Assays

Nuclear proteins were extracted from HMC-1 cells by a previously described method with modification (23). HMC-1 cells were centrifuged, then washed three times in cold PBS and collected in a 1.5 ml microcentri-

fuge tube. Added to this was 100  $\mu$ l of ice-cold hypotonic buffer: 10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM ethyleneglycoltetraacetic acid, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1  $\mu$ M aprotinin, 1  $\mu$ M pepstatin, 14  $\mu$ M leupeptin, 50 mM NaF, 30 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , and 20 mM *p*-nitrophenyl phosphate. Cells were incubated on ice for 30 min and vortexed after addition of 6.25 ml of 10% nonidet P-40. After 2 min of centrifugation at  $30,000 \times g$ , the supernatants were decanted off the top and kept at  $-80^\circ\text{C}$ . Whereas the pellets were resuspended and vortexed every 20 min for 3 h in 60  $\mu$ L of a hypertonic salt solution: 20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM ethyleneglycoltetraacetic acid, 12 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1  $\mu$ M aprotinin, 1  $\mu$ M pepstatin, 14 mM leupeptin, 50 mM NaF, 30 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , and 20 mM *p*-nitrophenyl phosphate. Nuclear fraction protein samples were then spun down, and supernatants, which contain nuclear proteins, were harvested and kept in  $-80^\circ\text{C}$  until used. Total protein concentration for both samples was determined by BCA protein assay reagent. Nuclear translocation of NF- $\kappa$ B was analyzed by the electrophoretic mobility shift assay (EMSA). Briefly, 7 mg of nuclear protein were added to 2  $\mu$ l of  $1 \times$  binding buffer (50 mg/ml of double-stranded poly dI-dC, 10 mM Tris HCl pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM dithiothreitol, 1 mM  $\text{MgCl}_2$ , and 10% glycerol), and 35 fmol of double-stranded NF- $\kappa$ B consensus oligonucleotide (5' AGT TGA GGG GAC TTT CCC AGG C 3') end-labeled with  $\gamma\text{-P}^{32}$  ATP. The reaction mixture was incubated at room temperature for 20 min and analyzed by electrophoresis on a 5% nondenaturing polyacrylamide gel. The gel was then dried on a Gel-Drier (Bio-Rad Laboratories) and exposed to Kodak X-ray film (Eastman Kodak, Rochester, NY) at  $-80^\circ\text{C}$ .

### Statistical Analysis

Data presented are representative of three independent experiments. All individual experiments were done in triplicate samples. RT-PCR experiments were done three times to ensure reproducibility. All values are given as the mean  $\pm$  SD. Statistical analysis was done using the Students *t* test and Statistica version 5 computer software (StatSoft, Inc Tulsa, OK). A *P* value of  $< 0.05$  was considered significant.

## Results

### Mast Cells Express IL-1 Type 1 Receptor

Several studies have shown that IL-1RI is able to transduce a signal and induce cellular activation (11–13). Figure 1 shows immunocytochemistry staining on resting HMC-1 cells (Figures 1A and 1B) and human umbilical CBDMC (Figures 1C and 1D) with a rabbit antibody to IL-1RI. Figure 1A shows minimal background fluorescence from unlabeled HMC-1 cells. Figure 1B shows resting HMC-1 cells express IL-1 receptor type one (IL-1RI) constitutively. Human umbilical CBDMCs constitutively express the IL-1RI as well (Figure 1C unlabeled and Figure 1D labeled with antibody). To confirm constitutive expression of IL-1RI in HMC-1 we performed RT-PCR on resting and PMA- and ionomycin-stimulated HMC-1 (Figure 1E). Whereas constitutive gene expression of IL-1RI is seen in resting cells, activation is accompanied by induction of IL-1RI transcripts at 12 h. This is in keeping with the immunocytochemistry data and suggests that human mast cells may constitutively express IL-1RI.

### IL-1 $\beta$ Induces IL-13 and MCP-1 Gene Expression in Mast Cells

To investigate the ability of IL-1 $\beta$  (10 ng/ml) and TNF- $\alpha$  (100 U/ml) to induce IL-13 and MCP-1 gene expression in human mast cells, HMC-1 cells were activated for 6 or 12 h with IL-1 $\beta$  and/or TNF- $\alpha$ , or with PMA/iono as positive control. RNA was extracted and reverse transcribed to cDNA. cDNA was amplified by PCR for 36 cycles. GAPDH was used as a housekeeping gene. To ensure equal RNA loading, RNA was quantitated by optical density readings at 260 nm, and the integrity of the 28S and 18S RNA bands determined by electrophoresis in ethidium

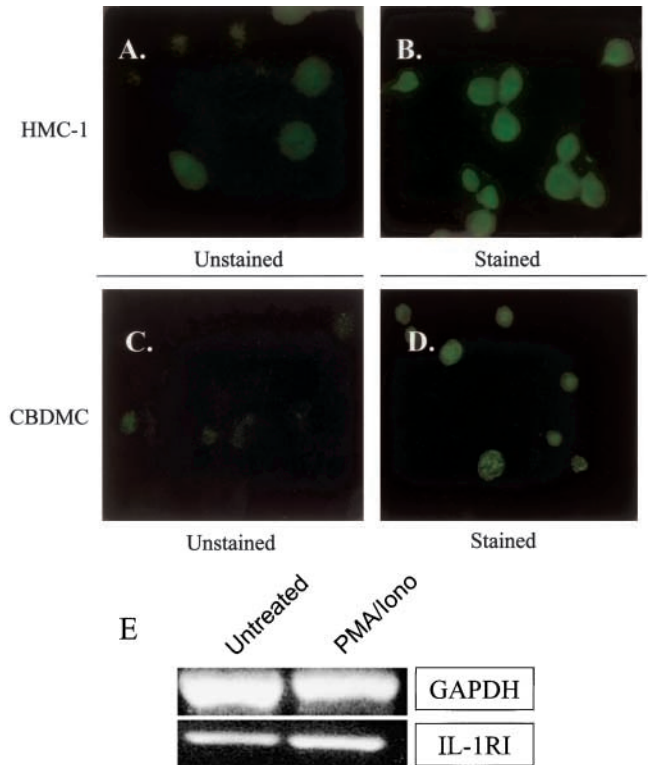


**Figure 1.** Expression of IL-1 receptor on resting and stimulated HMC-1 cells. Cells were harvested onto a slide by cytospin. Cells were then fixed and stained with a rat anti-human IL-1RI antibody and a secondary antibody of mouse anti-rat IgG with fluorescein isothiocyanate-conjugate or an isotype control. Pictures were obtained under fluorescence microscope at  $40\times$  magnification at 0.25 sec exposure. (A) Unstained HMC-1 cells. (B) HMC-1 cells stained with primary and secondary antibodies. (C) CBDMCs unstained. (D) CBDMCs stained primary and secondary antibodies. (E) For confirmation of immunocytochemistry results, IL-1RI gene expression was evaluated in HMC-1 cells either resting or activated with PMA/iono for 12 h before RNA harvest. RT-PCR analysis was performed using primers specific for IL-1RI and GAPDH (housekeeping gene), and specificity of amplification was determined by electrophoresis on 3% agarose gel.

bromide-stained 2% agarose gel. Figure 2A shows IL-13 gene expression induced by IL-1 $\beta$  and TNF- $\alpha$  at 6 and 12 h. Resting cells demonstrated no expression of IL-13. PMA/iono strongly and consistently induced IL-13 transcripts, as would be expected. However, when HMC-1 cells were activated with PMA/iono, IL-13 gene expression message peaked at 6 h and then decreased by 12 h. On the other hand, IL-1 $\beta$ -activated cells intensified their expression of IL-13 mRNA from 6–12 h, suggesting that IL-1 $\beta$  may prolong IL-13 mRNA stability (2.4-fold to roughly a 6.0-fold increase, respectively).

In studying the effects of IL-1 $\beta$  and TNF- $\alpha$  on MCP-1 expression in HMC-1, the same treatments and procedures were used for MCP-1 as with IL-13. When HMC-1 cells were incubated with PMA/iono or IL-1 $\beta$ , MCP-1 gene expression peaked at 6 h and diminished by 12 h (Figure 2B). There was a 3.2-fold increase in MCP-1 gene expression in mast cells treated with IL-1 $\beta$  for 6 h, which decreased by 12 h. This expression contrasted with the later expression of IL-13 transcripts, suggesting differential regulation and/or altered RNA decay with the two cytokines. Nevertheless, these data confirm that IL-1 $\beta$  induces IL-13 and MCP-1 transcription in human mast cells.

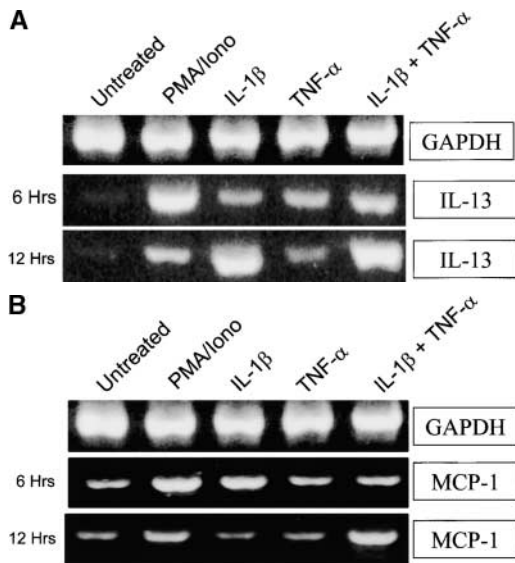


**Figure 2.** IL-1 $\beta$  and TNF- $\alpha$  induce IL-13 and MCP-1 gene expression. Mast cells were incubated with PMA/iono, IL-1 $\beta$  (10 ng/ml), TNF- $\alpha$  (100 U/ml), or IL-1 $\beta$  (10 ng/ml) and TNF- $\alpha$  (100 U/ml) for 6 and 12 h before RNA harvest. Total mRNA was extracted after 6 and 12 h. RT-PCR analysis was performed using either IL-13 primer or MCP-1 primer, and product was determined by expected base pair migration on an agarose gel. GAPDH was used as a housekeeping gene. (A) IL-13 transcripts expressed at 6 and 12 h after activation. (B) MCP-1 transcripts at 6 and 12 h after activation. IL-1-induced IL-13 mRNA expression peaked at 12 h whereas IL-1-induced MCP-1 expression peaked at 6 h but persisted at hour 12.

#### IL-1 Enhances IL-13 mRNA Stability and IL-13 Promoter Activity

To investigate the ability of IL-1 $\beta$  to stabilize IL-13 mRNA transcripts, HMC-1 cells were stimulated with IL-1 $\beta$  (10 ng/ml) for 2 hours, at which point actinomycin D was added to stop *de novo* RNA synthesis, and total RNAs were harvested at 0, 1, 4, 8, and 12 h in the presence or absence of IL-1 $\beta$ . Figure 3A shows the decay kinetics of IL-13 mRNA. In the absence of IL-1 $\beta$  in the culture, the relative level of IL-13 mRNA decreased to around 50% at the 4-h time point after the treatment with actinomycin D, and at the 12-h time point only ~10% of IL-13 mRNA remained. In contrast, the relative level of IL-13 mRNA remained stable in the entire 12-h time period when IL-1 $\beta$  is present in the culture. To evaluate further the ability of IL-1 $\beta$  to induce IL-13 transcription at a molecular level, we transiently transfected HMC-1 cells with minimal promoter sequences of both IL-4 and IL-13 as described in MATERIALS AND METHODS. As shown in Figure 3B, IL-1 $\beta$  was able to induce about a 2.7-fold increase in IL-13 promoter activity as compared with that seen for media control, whereas a slight decrease in the promoter activity of IL-4 was found in IL-1 $\beta$ -treated cells, but the difference did not reach statistical significance (Figure 3B). These results suggest, therefore, that the functional effect of IL-1 $\beta$  on the expression of IL-13 is operative at both transcriptional and post-transcriptional levels.

#### CBDMC Express IL-13 and MCP-1 in Response to IL-1 $\beta$ and Cross-Linking

CBDMCs were stimulated with PMA/iono, IgE/anti-IgE, IL-1 $\beta$ , and IL-1 $\beta$  plus IgE/anti-IgE for 24 h and assayed for IL-13 and MCP-1 production by ELISA (Figures 4A and 4B). PMA/iono is a potent stimulator of IL-13, whereas Fc $\epsilon$ RI cross-linking and IL-1 $\beta$  alone had no effect. Untreated CBDMC produced

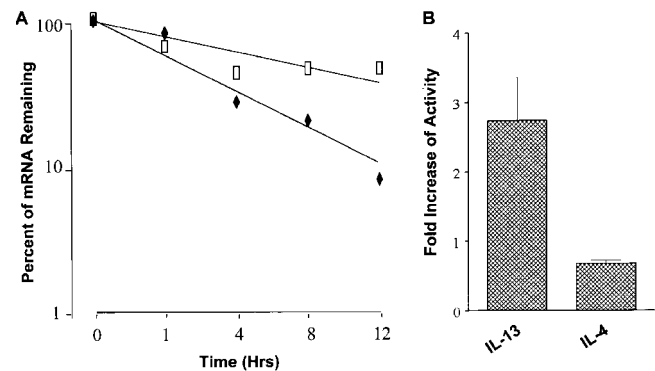


**Figure 3.** IL-1 $\beta$  enhances mRNA stability and induces IL-13 promoter activity. (A) Analysis of IL-13 mRNA stability. Cells were stimulated with IL-1 $\beta$  (10 ng/ml) for 2 h and then actinomycin D was added to stop *de novo* RNA synthesis, and RNA from cells was harvested at 0, 1, 4, 8, and 12 h (actinomycin D, diamonds; actinomycin D + IL-1 $\beta$ , rectangles). RT-PCR analysis was performed, and the ratio of IL-13 mRNA steady-state level to that of GAPDH was calculated by densitometric analysis for each time point. Data were expressed as the optical density ratio of the PCR products for IL-13 and GAPDH. The Y axis demonstrates % of mRNA remaining and the X axis indicates the time points of RNA harvest after activation. (Please see text for interpretation.) (B) IL-1 $\beta$ -induced promoter activity of IL-13. HMC-1 cells were transiently transfected with each of the luciferase reporter constructs, pGL3.IL4p, bearing bp -217 to +51 (relative to the transcription start site) of human IL-4 gene, and pGL3.IL13p, bearing bp -233 to +50 (relative to the transcription start site) of human IL-13 gene. Results are indicated as mean fold-increase  $\pm$  SD in duplicate. (Please see text for interpretation.)

0.886  $\pm$  1.53 pg/ml of IL-13, whereas PMA/iono-treated cells produced 580.7  $\pm$  19.3 pg/ml ( $P < 0.000005$  compared with untreated). IL-1 $\beta$  and IgE/anti-IgE cross-linkage alone both produced 0.00 pg/ml of IL-13. Cross-linking of Fc $\epsilon$ RI along with IL-1 $\beta$  stimulation together, however, greatly increased IL-13 production over untreated samples (339.7  $\pm$  16.5 pg/ml,  $P < 0.00005$  compared with untreated). This shows a need for both stimuli to trigger IL-13 production in CBDMCs (Figure 4A).

CBDMCs are also capable of producing MCP-1, but due to the growth factors needed for mast cell maturation and the highly prolific nature of these cells, MCP-1 baseline production is higher than normal. PMA/iono treatment is a potent activator of MCP-1 (2,859.6  $\pm$  85.4 pg/ml compared with 1,138.2  $\pm$  71.2 pg/ml for untreated cells,  $P < 0.00002$  compared with untreated). IgE/anti-IgE and IL-1 $\beta$  alone both significantly increased MCP-1 production over untreated levels (1,715.2  $\pm$  86.5 pg/ml and 1,983.3  $\pm$  125.1 pg/ml, respectively, both  $P$  values  $< 0.001$  compared with untreated). IL-1 $\beta$  plus IgE/anti-IgE together (2,109.8  $\pm$  117.0 pg/ml) did not have a significant enhancing effect over IL-1 $\beta$  alone, but did have a significant enhancing effect over IgE/anti-IgE alone ( $P < 0.01$ ).

#### IL-1 Induction of IL-13 and MCP-1 Secretion from HMC-1 Cells

HMC-1 cells were incubated with various concentration of IL-1 $\beta$  (1, 10, 100 ng/ml) for 24 h and cell-free supernatants were assayed for IL-13 and MCP-1 proteins by ELISA. Constitutively, HMC-1's produced a mean value of 30.3  $\pm$  35.9 pg/ml of IL-13 protein (Figures 5A and 5B). Cells stimulated with IL-1 $\beta$  at various concentrations showed an induction of IL-13 protein production between the 1 and 10 ng/ml concentrations (94.9  $\pm$  17.1 pg/ml and 122.0  $\pm$  41.0 pg/ml, respectively), but the effect reached a plateau at a concentration of 100 ng/ml, 123.0  $\pm$  46.5 pg/ml (Figure 5A). All concentrations of IL-1 $\beta$  induced a significant increase in IL-13

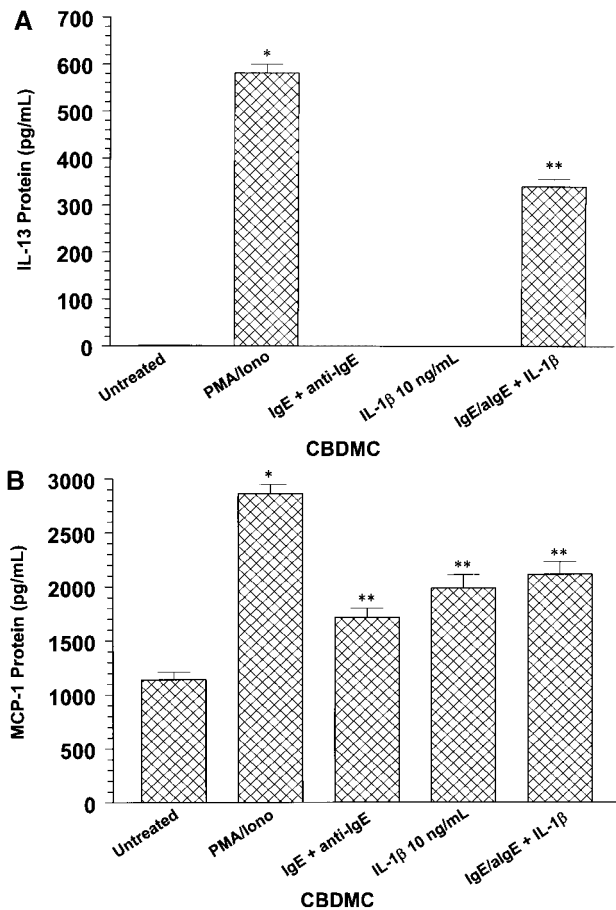


**Figure 4.** Demonstration of IL-13 and MCP-1 production in CBDMC. CBDMCs were treated for 24 h with PMA/iono, IgE/anti-IgE, IL-1 $\beta$ , and IL-1 $\beta$  plus IgE/anti-IgE. (A) PMA/iono was a good activator of IL-13 in CBDMCs, whereas IgE/anti-IgE and IL-1 $\beta$  had no effect. However, IL-1 $\beta$  and IgE/anti-IgE together produced a significant increase in IL-13 (\* $P < 0.000005$  compared with CBDMC-untreated and \*\* $P < 0.00005$  compared with CBDMC-untreated). (B) MCP-1 is produced in CBDMC treated with PMA/iono (\* $P < 0.00002$  compared with untreated). Fc $\epsilon$ RI cross-linking with IgE/anti-IgE and IL-1 $\beta$  also produced a significant increase in MCP-1 over control levels (\*\* $P < 0.001$  compared with untreated).

protein production as compared with media control ( $P < 0.005$ ; Figure 5A).

HMC-1 cells activated with PMA (50 ng/ml) and ionomycin (5  $\mu$ M) produced  $400.3 \pm 11.1$  pg/ml of IL-13 ( $P < 0.000001$  as compared with media control; Figure 5B). Cells stimulated with TNF- $\alpha$  did not show a significant increase in IL-13 protein production at 100 U/ml concentration ( $34.3 \pm 35.8$  pg/ml,  $P < 0.057$  when compared with the media control; Figure 5B). The induction of IL-13 transcript in response to TNF- $\alpha$  in the absence of concomitant IL-13 secretion is interesting, and could be partly explained as follows: expression of cytokine transcript in the absence of secreted protein might represent complicated intracellular post-transcription/translational/secretory processes that are poorly understood. Cells stimulated with IL-1 $\beta$  and TNF- $\alpha$  together, however, showed an increase in IL-13 protein production ( $268.5 \pm 112.6$  pg/ml,  $P < 0.0008$ ; Figure 5B).

Similar experiments were conducted to measure MCP-1 secretion by HMC-1 cells. HMC-1 cells secreted  $645.1 \pm 21.8$  pg/ml of MCP-1 constitutively. HMC-1 showed a dose-dependent response to IL-1 $\beta$ : they produced  $1,321.0 \pm 93.3$ ,  $1,916 \pm 691.9$ ,

and  $2,118 \pm 353.6$  pg/ml of MCP-1 when treated with 1, 10, and 100 ng/ml ( $P < 0.000001$ ,  $P < 0.0002$ , and  $P < 0.000003$ , respectively; Figure 5C). IL-1 $\beta$  at concentrations of 100 ng/ml also increased MCP-1 significantly as compared with that induced by IL-1 $\beta$  at concentrations of 1 ng/ml ( $P < 0.05$ ). Cells incubated with PMA/iono secreted a mean value of  $2,005.3 \pm 586.6$  pg/ml of MCP-1 ( $P < 0.0003$  as compared with media control; Figure 5D).

IL-1 $\beta$  alone induced a significant increase in MCP-1 secretion ( $1,916.3 \pm 691.9$  pg/ml,  $P < 0.0002$  as compared with media control; Figure 5D). Cells treated with TNF- $\alpha$  (100 U/ml) alone showed an increase in MCP-1 protein secretion ( $1,119 \pm 165.6$  pg/ml,  $P < 0.005$  as compared with media control; Figure 5D). HMC-1 treated with both IL-1 $\beta$  and TNF- $\alpha$  had no further effect on MCP-1 protein production ( $2,251 \pm 489.8$  pg/ml,  $P < 0.00002$  compared with the control; Figure 5D).

#### IL-1 Regulates NF- $\kappa$ B Nuclear Translocation and IRAK Induction in Mast Cells

To further understand the molecular consequences between IL-1 $\beta$  and IL-1RI in mast cells, we evaluated two signaling mechanisms, IRAK and NF- $\kappa$ B. Immunoblot analysis demonstrated that there was only a slight expression of IRAK in untreated cells, whereas there was an enhanced expression in cells stimulated with PMA/iono or IL-1 $\beta$  (10 ng/ml) at 30 min (Figure 6A). We next evaluated the nuclear translocation of NF- $\kappa$ B in these cells after activation. HMC-1 cells were treated the same as previously described and nuclear proteins were separated and analyzed by EMSA. As shown in Figure 6B, nuclear translocation of NF- $\kappa$ B is seen after activation of mast cells by IL-1 $\beta$ . When cells were pretreated with Bay-11 7082, inhibition of nuclear translocation of NF- $\kappa$ B was seen after IL-1-induced activation (32% decrease as seen by densitometry) (Figure 6B). These data suggest that some of the effects after the binding of IL-1 $\beta$  to its receptor, IL-1RI, on mast cells, may be mediated by a pivotal transcription factor, NF- $\kappa$ B. To look at the functional aspects of NF- $\kappa$ B translocation on IL-13 expression in mast cells, we pre-incubated cells with Bay-11 7082 (10  $\mu$ M) for 1.5 h before treatment with IL-1 $\beta$  (10 ng/ml) for 24 h. Cell-free supernatants were harvested and protein production was determined by ELISA. Pretreatment of HMC-1 with Bay-11 7082 (10  $\mu$ M) significantly decreased IL-1 $\beta$  (10 ng/ml)-induced IL-13 protein production ( $32.9 \pm 4.3$  pg/ml vs.  $132.5 \pm 35.5$  pg/ml of IL-1 $\beta$  alone,  $P < 0.005$ ; Figure 6C). Bay-11 7082 also decreased IL-1 $\beta$ -induced MCP-1 protein production ( $948 \pm 90.2$  pg/ml vs.  $1,440 \pm 120.9$  pg/ml of IL-1 $\beta$  alone,  $P = 0.017$ ; Figure 6D) but not as profoundly as that for IL-13. The differences in sensitivities of IL-13 and MCP-1 to Bay-11 7082 could be explained by complicated underlying regulatory mechanisms that govern specific signaling for cytokines.

#### Dexamethasone Inhibits IL-13 Expression in Mast Cells

Dexamethasone is a glucocorticoid commonly used in allergic and inflammatory disease. It stabilizes the NF- $\kappa$ B-I $\kappa$ B complex by increasing I $\kappa$ B production. We therefore sought to determine the effects of dexamethasone on IL-1 $\beta$  induced cytokine synthesis and NF- $\kappa$ B nuclear translocation. HMC-1 cells were incubated overnight with dexamethasone at either  $10^{-6}$  M or  $10^{-7}$  M concentrations. The concentrations were chosen because they are in the physiologic ranges when dexamethasone is administered therapeutically. At these concentrations, all cells retained their viability as determined by trypan exclusion. Cells pretreated with dexamethasone were stimulated with IL-1 $\beta$  (10 ng/ml) and incubated for either 1 h for nuclear fractions or 12 and 24 h for ELISA. Cell-free supernatants were harvested and subjected to IL-13 and MCP-1 measurements by ELISA. To investigate the mechanism

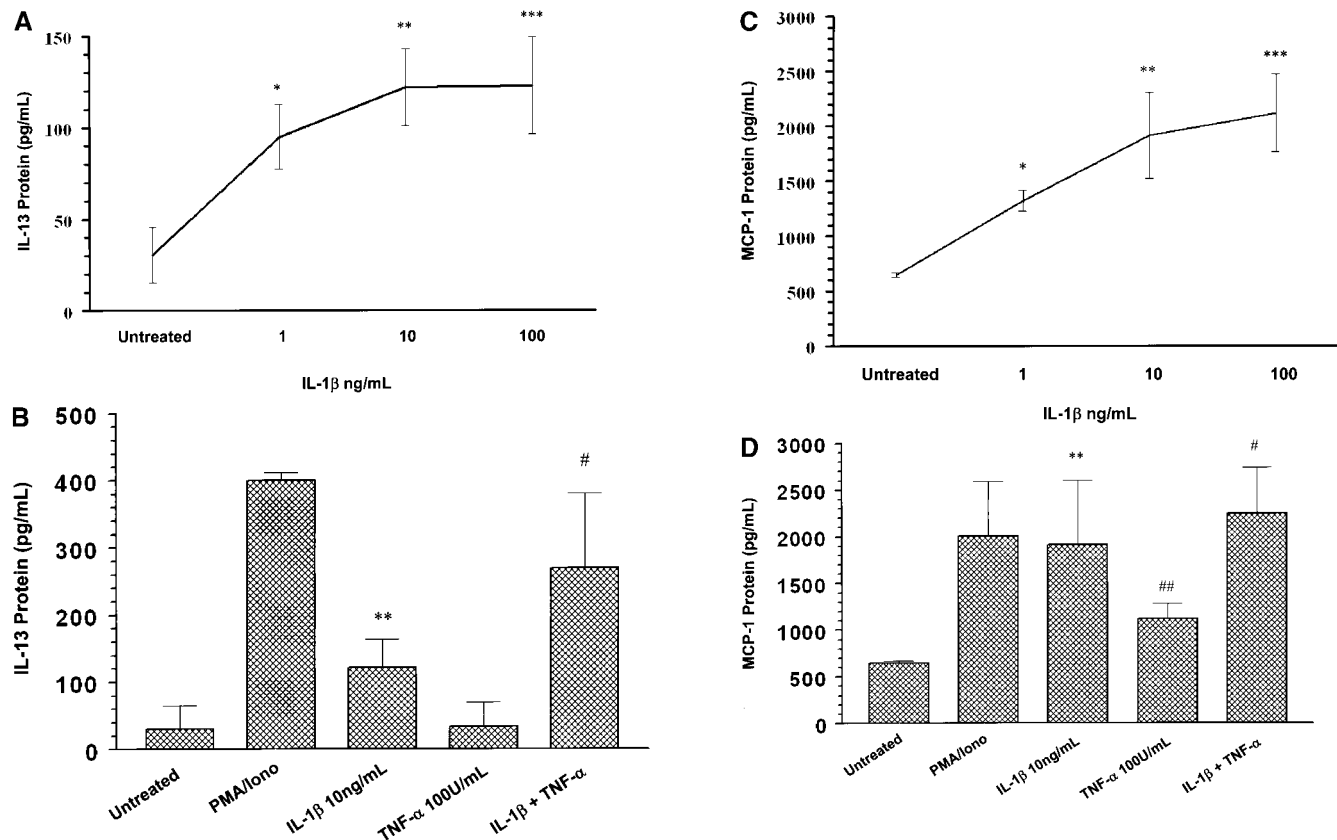


**Figure 5.** IL-1 $\beta$  and TNF- $\alpha$  induce IL-13 and MCP-1 protein production. Mast cells were incubated with PMA/iono, IL-1 $\beta$  (1, 10, 100 ng/ml), TNF- $\alpha$  (1, 10, 100 U/ml), or IL-1 $\beta$  (10 ng/ml) and TNF- $\alpha$  (100 U/ml) for 24 h, cell-free supernatants were harvested, and protein production was determined by ELISA. (A and B) IL-1 $\beta$  induction of IL-13 protein production (\* $P$  < 0.002, \*\* $P$  < 0.002, \*\*\* $P$  < 0.003, and # $P$  < 0.001, compared with control); (A) dose-response curve using varying concentrations of IL-1 $\beta$ ; (B) Fixed concentrations of IL-1 $\beta$  (10 ng/ml) and TNF- $\alpha$  (100 U/ml); (C and D) IL-1 $\beta$  induction of MCP-1 protein production (\* $P$  < 0.001, \*\* $P$  < 0.002, \*\*\* $P$  < 0.00003, # $P$  < 0.005, and ## $P$  < 0.001 compared with control); (C) dose-response curve using varying concentrations of IL-1 $\beta$ ; (D) fixed concentrations of IL-1 $\beta$  (10 ng/ml) and TNF- $\alpha$  (100 U/ml).

of protein reduction by dexamethasone, we isolated the nuclear fractions from the treated HMC-1 at 1 h incubation time for NF- $\kappa$ B analysis by EMSA. Figure 6A shows that dexamethasone inhibited the nuclear translocation of NF- $\kappa$ B, which may indicate one mechanism of action of these drugs in inflammatory disease and is consistent with our earlier data demonstrating that inhibition of NF- $\kappa$ B results in decreased IL-13 and MCP-1 expression.

Dexamethasone significantly inhibited the IL-1 $\beta$ -induced IL-13 production in HMC-1 in a dose-dependent fashion at both the 12 and 24 h incubations (Figure 7B). After 12 h of incubation, cells treated with IL-1 $\beta$  in the presence of dexamethasone at  $10^{-7}$  M or  $10^{-6}$  M concentration showed a  $25 \pm 6\%$  and a  $72 \pm 6\%$  reduction of IL-13 protein, respectively ( $P$  < 0.002 and 0.00003, respectively, as compared with IL-1-treated cells). A comparable result was also shown at the 24 h time-point. IL-1 $\beta$ -treated cells are represented as 100%, whereas cells treated with IL-1 $\beta$  in the presence of dexamethasone at  $10^{-7}$  M or  $10^{-6}$  M showed a  $29 \pm 1\%$  and  $58 \pm 4\%$  reduction of IL-13 protein, respectively ( $P$  < 0.002 and 0.00002, respectively, as compared with IL-1 treated cells). In similar experiments dexamethasone also showed a significant inhibition of MCP-1 protein production in a dose-dependent manner at both the 12 h and 24 h time periods (Figure 7C). Cells treated and incubated for 12 h with IL-1 $\beta$  in the presence of dexamethasone at  $10^{-7}$  M or  $10^{-6}$  M concentration showed a significant reduction in MCP-1 protein production ( $30 \pm 10\%$ ,  $P$  < 0.02, and  $42 \pm 11\%$ ,  $P$  < 0.003, respectively), while cells treated and incubated for 24 h with

IL-1 $\beta$  in the presence of dexamethasone at  $10^{-7}$  M or  $10^{-6}$  M concentration showed a significant reduction in MCP-1 protein production ( $41.3 \pm 10\%$ ,  $P$  < 0.007, and  $74 \pm 3\%$ ,  $P$  < 0.0008, respectively).

## Discussion

Our data suggest that IL-1 $\beta$  is not only an activator of cytokine gene transcription, but also serves to stabilize cytokine mRNAs, thereby prolonging their half-life. This phenomenon has previously been described in mouse bone marrow mast cells as well as in fibroblast and B-cells, but not in human mast cells (16, 17). Along with enhancement of IL-13 mRNA stability, we illustrate that IL-1 $\beta$  is capable of increasing the activity of the IL-13 promoter, suggesting some specificity in the response. When taken together, the results from both experiments strongly suggest that IL-1 $\beta$  is a potent activator of IL-13 in mast cells, which can magnify the immune response through very late activating antigen-4-vascular cell adhesion molecule-1 monocyte recruitment. These data suggest that IL-1 $\beta$  activates IL-13 gene expression through direct activation of the IL-13 promoter, whereas the IL-4 promoter activity decreased, supporting previous studies that suggest that the IL-4 promoter effects may be more transient (24).

IL-1 $\beta$  is a ubiquitous, potent proinflammatory cytokine that is capable of modulating angiogenesis, lymphokine production, monocyte recruitment, cartilage remodeling, and proliferation

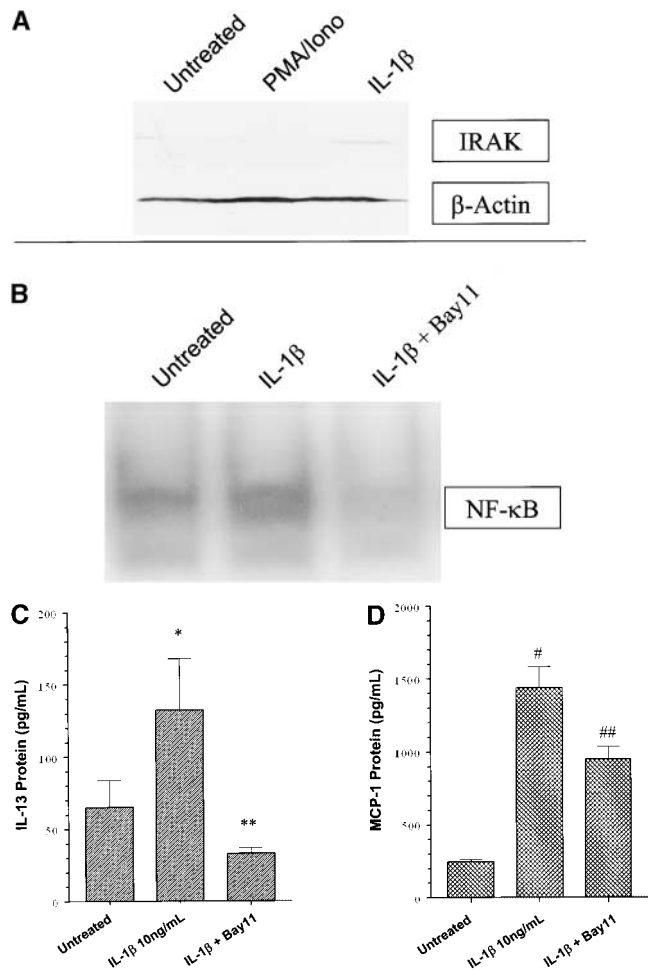


**Figure 6.** IL-1 $\beta$  signaling via IRAK and NF- $\kappa$ B pathways. IL-1RI activation by IL-1 $\beta$  leads to activation of IRAK. (A) Induction of IRAK is seen with IL-1 $\beta$  within 20 min of mast cell activation. (B) Mast cells were stimulated with either IL-1 $\beta$  (10 ng/ml) or preincubated with Bay-11 7082 (10  $\mu$ M) for 1.5 h before IL-1 $\beta$  stimulation. NF- $\kappa$ B nuclear translocation was assessed using EMSA. Bay-11 7082 inhibited nuclear translocation of NF- $\kappa$ B translocation as would be expected. (C) HMC-1 mast cells were stimulated for 24 h with IL-1 $\beta$  (10 ng/ml) or preincubated with Bay-11 (10  $\mu$ M) for 1.5 h before IL-1 $\beta$  stimulation. Cell-free supernatants analyzed for IL-13 protein production by ELISA (\* $P$  < 0.004, \*\* $P$  < 0.005). (D) HMC-1 cells were stimulated for 24 h with IL-1 $\beta$  (10 ng/ml) or preincubated with Bay-11 (10  $\mu$ M) for 1.5 h before IL-1 $\beta$  stimulation. Cell-free supernatants were harvested and assayed for MCP-1 protein production by ELISA (\* $P$  < 0.005, \*\* $P$  < 0.017).

of mesangial cells, fibroblast, and smooth muscle cells (15). IL-1 $\beta$ -responsive activities depend on the interactions of IL-1 $\beta$  with its receptor, IL-1RI, and the formation of a transmembrane heterodimer complex between IL-1RI and IL-1 receptor accessory protein (14). IL-1RI is the signal-transducing receptor, and transduces with a high efficacy, requiring less than 10 bound ligands to produce a signal (13). The ability of IL-1 $\beta$  to directly activate mast cells provides a novel and pivotal pathway of the innate immune response. In infectious or inflammatory states, IL-1 $\beta$  can be expressed by adventitial cells, such as fibroblasts, endothelium, or mononuclear cells. Our data suggest that this binding of IL-1 $\beta$  to IL-1RI on mast cells could lead to a cascade of events culminating in inflammatory-immune responses that may be pivotal to mucosal immunity. Mast cell activation, leading to activation of IRAK and NF- $\kappa$ B followed by inflammatory

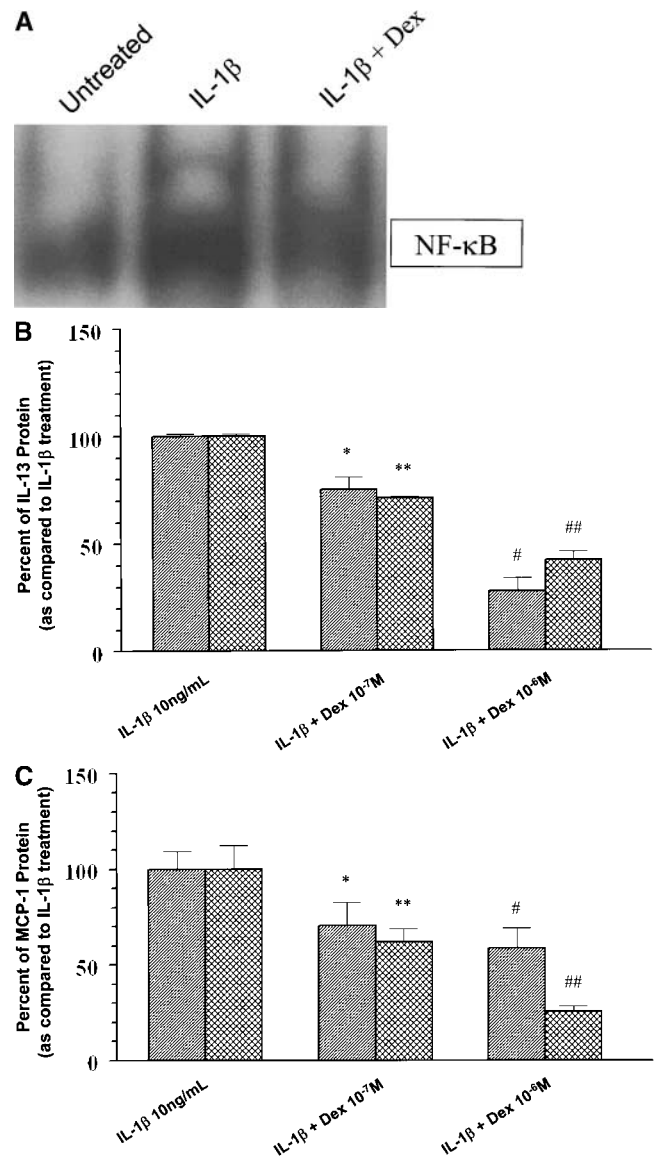


**Figure 7.** Dexamethasone attenuates IL-1 $\beta$  stimulation of IL-13 and MCP-1 production via the NF- $\kappa$ B pathway. (A) Dexamethasone inhibits nuclear translocation of NF- $\kappa$ B. Mast cells were stimulated with either IL-1 $\beta$  (10 ng/ml) or preincubated with dexamethasone (10 $^{-6}$  M) for 24 h before IL-1 $\beta$  stimulation. NF- $\kappa$ B nuclear translocation was assessed using EMSA. Dexamethasone inhibits IL-1 $\beta$ -induced NF- $\kappa$ B nuclear translocation. (B) IL-13 production is attenuated by dexamethasone. Mast cells were treated with dexamethasone at either 10 $^{-6}$  or 10 $^{-7}$  M concentrations overnight, then incubated with IL-1 $\beta$  (10 ng/ml) for 12 or 24 h. Cell-free supernatants were harvested and protein was evaluated by ELISA. Significant inhibition of IL-13 production from mast cells in response to IL-1 $\beta$  was seen with dexamethasone (\* $P$  < 0.002, \*\* $P$  < 0.002, \* $P$  < 0.00003, and \*\* $P$  < 0.00002 compared with cells treated with IL-1 $\beta$  alone). Actual values of IL-13 and IL-1 $\beta$  at 12 (solid bars) and 24 h (hatched bars) are 132.5  $\pm$  35.5 and 105.9  $\pm$  36.4 pg/ml, respectively. Actual values of IL-13 for dexamethasone 10 $^{-7}$  and 10 $^{-6}$  M are 99.38  $\pm$  7.0 and 77.8  $\pm$  7.8 pg/ml, respectively for 12 h, and 65.3  $\pm$  16.6 and 27.7  $\pm$  10 pg/ml, respectively for 24 h. (C) MCP-1 production is attenuated by dexamethasone. Mast cells were treated the same as for IL-13. Significant inhibition of MCP-1 production from mast cells in response to IL-1 $\beta$  was seen with dexamethasone (\* $P$  < 0.02, \*\* $P$  < 0.007, \* $P$  < 0.003, and \*\* $P$  < 0.0008 compared with cells treated with IL-1 $\beta$  alone). Actual values of MCP-1 in for IL-1 $\beta$  at 12 and 24 h are 1,110.4  $\pm$  22.4 and 1,716.3  $\pm$  22.4 pg/ml, respectively. Actual values of MCP-1 for dexamethasone 10 $^{-7}$  and 10 $^{-6}$  M are 792.6  $\pm$  23.5 and 68.65  $\pm$  3.3 pg/ml, respectively for 12 h, and 966.27  $\pm$  70.2 and 441.01  $\pm$  14.2 pg/ml, respectively for 24 h.

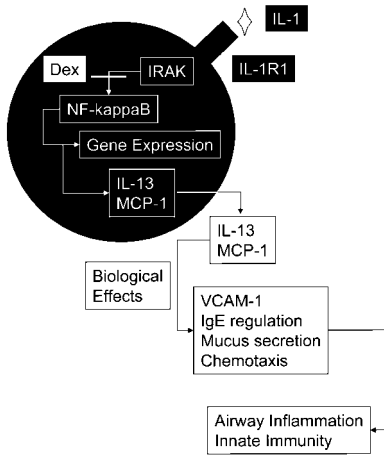


**Figure 8.** Diagram demonstrating the effects of IL-1RI ligation on human mast cells. Activation of IRAK is accompanied by NF- $\kappa$ B nuclear translocation and IL-13 and MCP-1 gene expression. Translation and secretion of these proteins from mast cells can lead to vascular cell adhesion molecule-1 expression, IgE class switching, mucus hypersecretion, and leukocyte/mononuclear cell chemotaxis, which can contribute to airway inflammation and innate immune responses. Dexamethasone as an antiinflammatory drug can inhibit activation of this pathway.

cytokine gene expression and cellular recruitment could provide an important adjunct pathway to defend against pathogens. In the case of the airway, inflammatory cell adhesion and chemotaxis, mucus production, and IgE regulation may all culminate in chronic inflammatory responses. This is summarized in diagrammed form in Figure 8.

In this article, we demonstrate that the human mast cell line HMC-1 expresses IL-1RI, thereby allowing IL-1 $\beta$  to directly activate human mast cells. Previous reports have shown that bone marrow-derived murine mast cells could be coactivated by IL-1 and ionomycin (16). It has been previously hypothesized that induction of IL-13, which induces airway hyperresponsiveness, may be accomplished via surrounding cells (6). In this report, IL-1 $\beta$  significantly upregulated IL-13 and MCP-1 gene expression and protein production independent of IgE, therefore implying that mast cells play a greater role in the orchestration of inflammatory diseases such as rheumatoid arthritis and atherosclerosis as well as asthma in an IgE-independent manner. We also found that IL-1 $\beta$  and TNF- $\alpha$  together increased the stimulation of IL-13 protein and MCP-1 production. This may be important as both cytokines are produced at local sites of inflammation, and could compound the severity of disease (25).

We have also shown that IL-1 $\beta$  alone as well as IL-1 $\beta$  and TNF- $\alpha$  together signal through an NF- $\kappa$ B-dependent pathway as do bacterial pathogens, such as *Moraxella catarrhalis* in mast cells (26). The actions of IL-1 $\beta$  could be attenuated by addition of Bay-11 7082 to cell culture before activation by inhibiting cytokine-induced translocation of NF- $\kappa$ B through the blocking of I $\kappa$ B- $\alpha$  phosphorylation (27). Dexamethasone also inhibited NF- $\kappa$ B nuclear translocation, as would be expected, in response to IL-1 $\beta$  stimulation. Dexamethasone has been shown to inhibit MCP-1 expression in human airway smooth muscle cells (28). Dexamethasone has also been shown to inhibit IL-13 production in HMC-1 and human lung mast cells (29). In our study, dexamethasone strongly inhibited both IL-13 and MCP-1 expression from mast cells. Yet, interestingly, the maximal inhibition for IL-13 was at the 24-h time point, whereas MCP-1 was at the 12-h time point. This suggests differential sensitivities of signaling pathways regulating IL-13 and MCP-1 to the effects of glucocorticoids. Thus, the IRAK-NF- $\kappa$ B pathway in mast cells could represent a dominant mechanism that regulates inflammatory gene expression.

Whereas most of these experiments were performed in HMC-1 cells, we have shown similar enhancing effects of IL-1 $\beta$  on cytokine expression from human CBDMC developed from umbilical cord blood-derived mononuclear cells (19). Because they use some of the same signaling molecules, like MyD88, IRAK, and TNF receptor associated factor 6, the Toll-like receptor (TLR)-IL-1 signaling pathways may in some way converge to induce pivotal effects in human mast cells. Investigators have demonstrated that lipopolysaccharides (LPS) acting upon TLR-4 induced significant release of IL-13 in CBDMC (14, 30). Another group of investigators have also shown that LPS acting through TLR-4 can induce human mast cells to secrete TNF- $\alpha$  and several chemokines specific for T helper type 2 cells and eosinophils (31). Activation of TLR-4 via LPS has also been shown to inhibit apoptosis of CBDMCs by inducing Bcl-xL (32). Studies on the regulation of the TLR pathways in human mast cells are currently underway in our laboratories.

In summary, the ability of IL-1 $\beta$  to directly activate mast cells provides a novel and pivotal pathway of the innate immune response involving human mast cells. Binding of IL-1 $\beta$ , expressed by adventitial cells in response to infection, to IL-1RI on mast cells, leading to activation of IRAK and NF- $\kappa$ B preceding inflammatory cytokine gene expression and cellular recruitment could provide an important adjunct pathway to defend against pathogens. In the case of the airway, inflammatory cell adhesion and chemotaxis, mucus production, and IgE regulation may all culminate in chronic inflammatory responses, which are summarized in Figure 8.

**Conflict of Interest Statement:** S.A.L. has no declared conflicts of interest; S.M.F. has no declared conflicts of interest; S.K.H. has no declared conflicts of interest; C.L. has no declared conflicts of interest; D.S.C. has no declared conflicts of interest; and G.K. has no declared conflicts of interest. This study was supported by National Institutes of Health grants AI-43310 and HL-63070, Research Development Committee grant (ETSU), the Cardiovascular Research Institute (ETSU), and Amgen, Inc., Thousand Oaks, California.

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