

## **Histamine enhances interleukin (IL)-1-induced IL-1 gene expression and protein synthesis via H2 receptors in peripheral blood mononuclear cells. Comparison with IL-1 receptor antagonist.**

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### Research Article

Histamine and IL-1 have been implicated in the pathogenesis of chronic inflammatory diseases, such as pulmonary allergic reactions and rheumatoid arthritis. We therefore investigated whether histamine modulated the synthesis of IL-1 beta. Human PBMC were stimulated with IL-1 alpha (10 ng/ml) in the absence or presence of histamine (10(-9)-10(-4) M). Histamine alone did not induce protein synthesis or mRNA accumulation for IL-1 beta. IL-1 alpha-induced IL-1 beta synthesis was enhanced two to threefold by histamine concentrations from 10(-6)-10(-4) M. Cimetidine, an H2 receptor antagonist, reversed the histamine (10(-5) M)-mediated increase in IL-1 alpha-induced IL-1 beta synthesis. Diphenhydramine, an H1 receptor antagonist, had no effect. Indomethacin, a cyclooxygenase inhibitor, significantly reduced IL-1 alpha-induced IL-1 beta synthesis, but had no effect on the histamine-mediated increase in IL-1 alpha-induced IL-1 beta synthesis. Histamine (10(-5) M) enhanced and sustained IL-1 beta mRNA levels in IL-1 alpha-stimulated PBMC. However, histamine reduced IL-1 beta mRNA half-life (2.4 vs 1.2 h), suggesting that histamine enhances IL-1 alpha-induced IL-1 beta synthesis at the level of transcriptional activation. On the other hand, histamine (10(-5) M) did not affect IL-1 alpha-induced synthesis of IL-1 receptor antagonist. These results suggest that mast cells may sustain chronic inflammatory processes by upregulating self-induction of IL-1 through histamine release.

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# Histamine Enhances Interleukin (IL)-1-induced IL-1 Gene Expression and Protein Synthesis via H<sub>2</sub> Receptors in Peripheral Blood Mononuclear Cells

## Comparison with IL-1 Receptor Antagonist

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### Abstract

Histamine and IL-1 have been implicated in the pathogenesis of chronic inflammatory diseases, such as pulmonary allergic reactions and rheumatoid arthritis. We therefore investigated whether histamine modulated the synthesis of IL-1 $\beta$ . Human PBMC were stimulated with IL-1 $\alpha$  (10 ng/ml) in the absence or presence of histamine (10<sup>-9</sup>–10<sup>-4</sup> M). Histamine alone did not induce protein synthesis or mRNA accumulation for IL-1 $\beta$ . IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis was enhanced two to threefold by histamine concentrations from 10<sup>-6</sup>–10<sup>-4</sup> M. Cimetidine, an H<sub>2</sub> receptor antagonist, reversed the histamine (10<sup>-5</sup> M)-mediated increase in IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis. Diphenhydramine, an H<sub>1</sub> receptor antagonist, had no effect. Indomethacin, a cyclooxygenase inhibitor, significantly reduced IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis, but had no effect on the histamine-mediated increase in IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis. Histamine (10<sup>-5</sup> M) enhanced and sustained IL-1 $\beta$  mRNA levels in IL-1 $\alpha$ -stimulated PBMC. However, histamine reduced IL-1 $\beta$  mRNA half-life (2.4 vs 1.2 h), suggesting that histamine enhances IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis at the level of transcriptional activation. On the other hand, histamine (10<sup>-5</sup> M) did not affect IL-1 $\alpha$ -induced synthesis of IL-1 receptor antagonist. These results suggest that mast cells may sustain chronic inflammatory processes by upregulating self-induction of IL-1 through histamine release. (*J. Clin. Invest.* 1993; 92:281–287.) Key words: allergy • mast cells • prostaglandins • cytokines • mRNA stability

### Introduction

Although mast cell activation is the initial event in acute allergic asthma, the event is often followed by a sustained inflammatory reaction that is characterized by a predominantly eosinophilic infiltration. Cross-linkage of the high affinity receptors for IgE (Fc $\epsilon$ RI)<sup>1</sup> on mast cells induces release of preformed and newly synthesized mediators, such as histamine and arachidonic acid metabolites. However, in response to Fc $\epsilon$ RI cross-

linkage, mast cells also synthesize a wide array of cytokines, including IL-1, TNF- $\alpha$ , IL-3, IL-5, and GM-CSF (1–4) which, in turn, activate eosinophil functions. Stimulation of the low affinity receptors for IgE (Fc $\epsilon$ RII) on monocytes also result in the production of IL-1 $\beta$  and TNF- $\alpha$  (5). Therefore, cytokine synthesis by mast cells and monocytes may contribute to the onset of the late phase response.

IL-1 may play an important role in eosinophil accumulation during the late phase response. Recent evidence supports this concept since blockade of IL-1 by the naturally occurring IL-1 receptor antagonist (IL-1Ra) reduces eosinophil infiltration in lungs after inhalation of antigen by sensitized guinea pigs (6). Related to these events, IL-1 upregulates the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 on endothelial cells (7, 8). Eosinophils constitutively express the integrin very late antigen-4 (9) that recognizes vascular cell adhesion molecule-1 (10). Blockade of intracellular adhesion molecule-1 prevents both the eosinophil accumulation in the primate lung and the subsequent bronchial hyperreactivity to acetylcholine (11). Moreover, high affinity receptors for human IL-3, IL-5 or GM-CSF are comprised of an  $\alpha$  chain, specific for each cytokine, and of a common  $\beta$  chain (12, 13). A role for IL-1 also exists in this response since IL-1 upregulates the expression of the  $\beta$  chain (14).

Since acute allergic asthma is characterized by both mast cell degranulation and IL-1 production, we investigated the effect of histamine on the synthesis of IL-1 $\beta$  and IL-1Ra. Modulation by histamine of gene expression and total cellular synthesis of IL-1 $\beta$  and IL-1Ra were studied in human PBMC stimulated with either LPS or IL-1 itself.

### Methods

**Human PBMC culture.** Blood was drawn from healthy human volunteers who had not taken any histamine receptor antagonists or cyclooxygenase inhibitors for -2 wk. The study was approved by the Human Investigative Review Committee of The New England Medical Center Hospitals. PBMC were separated from heparinized blood by centrifugation on Ficoll-Hypaque (Ficoll Type 400; Sigma Chemical Co., St. Louis, MO, and Hypaque-M 90%; Winthrop Breon Laboratories, New York) gradients. Cells were washed twice in 0.15 M NaCl and resuspended at 5  $\times$  10<sup>6</sup> cells/ml in ultrafiltered RPMI culture medium 1640 (Whittaker Bioproducts, Walkersville, MD) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin (Gibco Laboratories, Grand Island, NY). PBMC (2.5  $\times$  10<sup>6</sup> cells/ml in RPMI containing 1% heat-inactivated human AB serum) were stimulated with LPS from *Escherichia coli* O55:B5 (10 ng/ml; Sigma Chemical Co.) or human recombinant IL-1 $\alpha$  (10 ng/ml; kindly provided by Dr. P. Lomedico, Hoffmann-LaRoche Inc., Nutley, NJ) in the absence or presence of either histamine (10<sup>-9</sup>–10<sup>-4</sup> M, Sigma Chemical Co.) or PGE<sub>2</sub> (0.1–1,000 ng/ml, Sigma Chemical Co.). PBMC cultures were incubated in 12  $\times$  75-mm polypropylene round bottom tubes (Becton Dickinson Co., Lincoln Park, NJ) for 24 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

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1. Abbreviations used in this paper: Fc $\epsilon$ RI, high affinity receptors for IgE; Fc $\epsilon$ RII, low affinity receptors for IgE; IL-1Ra, IL-1 receptor antagonist.

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In other experiments, PBMC ( $5 \times 10^6$  cells/ml) were pre-incubated for 1 h at 37°C in the presence of either  $1.3 \times 10^{-6}$  M indomethacin (Sigma Chemical Co.),  $10^{-5}$  M diphenhydramine (Elkins-Sinn, Inc., Cherry Hill, NJ), from  $10^{-6}$  to  $10^{-4}$  M cimetidine (Smith Kline & French, Philadelphia, PA),  $10^{-5}$  M ranitidine (Glaxo Inc., Research Triangle Park, NC), 1 µg/ml IL-1Ra (kindly provided by Dr. R. C. Thompson, Synergen, Inc., Boulder, CO), or RPMI as a control.

**Cytokine RIAs.** After incubation, cultures were subjected to three freeze-thaw cycles. This procedure is optimal for recovery and measurement of total (cell-associated and secreted) cytokines by specific RIAs as described for IL-1β (15) and IL-1Ra (16). The sensitivities (defined as 95% binding) of RIAs for IL-1β and IL-1Ra were  $63 \pm 12$  ( $n = 13$ ) and  $78 \pm 11$  ( $n = 4$ ) pg/ml, respectively.

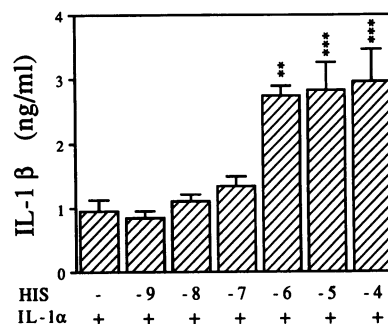
**RNA isolation and Northern analysis.** PBMC were stimulated for 4, 8, 16, or 32 h at 37°C in 50 ml polypropylene tubes with IL-1α (10 ng/ml) in the absence or presence of histamine ( $10^{-7}$ – $10^{-4}$  M). Total cellular RNA was then extracted from PBMC by lysis with 4 M guanidium isothiocyanate followed by ultracentrifugation on a 5.7-M cesium chloride gradient (17). Total RNA (10 µg) was subjected to electrophoresis in 6.6% formaldehyde (Sigma Chemical Co.), 1.2% agarose (International Biotechnology Inc., New Haven, CT) gel and transferred to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, IL) by capillary blotting. Serial dilutions of total RNA (2.50, 1.25, and 0.62 µg) were also applied directly on nylon membranes using a microsample filtration manifold (Minifold; Schleicher & Schuell, Inc., Keene, NH). The membranes were exposed to short-wave ultraviolet light for 5 min to fix the RNA to the nylon matrix, and treated for 2 h at 42°C with prehybridization solution containing 10 mg/ml salmon sperm DNA. Membranes were then treated at 42°C overnight with prehybridization solution containing 10 mg/ml salmon sperm DNA and  $^{32}$ P-labeled nucleic acid probe. The probes used were a 1,075-bp fragment of human IL-1β precursor cDNA subcloned into pGEM2, and the full length of chicken β-actin cDNA subcloned in pGEM3 (gift of Dr. B. Huber, Tufts University, Boston, MA). The DNA was labeled using 3,000 Ci/mmol  $^{32}$ P-dCTP (New England Nuclear, Boston, MA) and a random primed DNA labeling kit (Boehringer Mannheim GmbH, Mannheim, Germany). After incubation, membranes were washed in 0.1% SDS,  $1 \times$  SSC at 42°C. Washed membranes were exposed to x-ray film (XAR5; Kodak, Rochester, NY) at -70°C with an intensifying screen.

**Statistical analysis.** Data were expressed as mean ± SEM of the indicated number of experiments. Differences between histamine- or drug-treated groups and RPMI-treated groups were analyzed for significance by Student's *t* test for paired samples, Wilcoxon signed rank test, or two-way ANOVA.

## Results

**Histamine enhances IL-1β synthesis in IL-1α-stimulated PBMC.** PBMC stimulated with IL-1α synthesized  $0.95 \pm 0.16$  ng/ml IL-1β (Fig. 1). Histamine alone did not induce IL-1β synthesis (data not shown). However, as little as  $10^{-6}$  M histamine significantly enhanced IL-1α-induced IL-1β synthesis (188% increase,  $P < 0.01$ ) (Fig. 1). No further significant increase in IL-1α-induced IL-1β synthesis was observed at higher concentrations of histamine ( $10^{-5}$  and  $10^{-4}$  M). Unstimulated PBMC cultures contained  $0.11 \pm 0.01$  ng/ml IL-1β ( $n = 4$ ).

**Histamine enhances IL-1β synthesis via activation of H<sub>2</sub> receptors.** To determine whether histamine regulates IL-1β synthesis via H<sub>1</sub> or H<sub>2</sub> receptors, PBMC were incubated in the presence of diphenhydramine, an H<sub>1</sub> receptor antagonist, or cimetidine, an H<sub>2</sub> receptor antagonist. Diphenhydramine ( $10^{-5}$  M) or cimetidine ( $10^{-4}$  M) alone did not induce IL-1β synthesis (data not shown). Furthermore, diphenhydramine modified neither IL-1α-induced IL-1β synthesis nor the his-

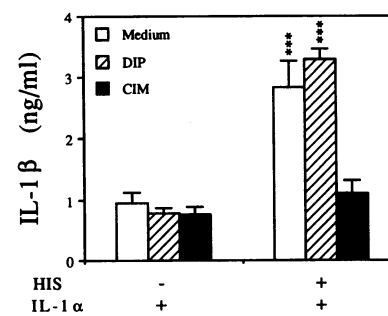


**Figure 1.** Effect of histamine on IL-1α-induced synthesis of IL-1β. PBMC were stimulated for 24 h with IL-1α (10 ng/ml) in the absence (-) or presence of histamine (HIS:  $10^{-9}$ – $10^{-4}$  M). Differences in IL-1β synthesis were analyzed for significance by ANOVA (\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Data are expressed as mean ± SEM for four donors.

mine ( $10^{-5}$  M)-mediated increase in IL-1β synthesis (Fig. 2). In contrast, cimetidine blocked the histamine-mediated increase in IL-1α-induced synthesis of IL-1β (Fig. 2). Similar results were observed for histamine  $10^{-6}$  M (data not shown). Ranitidine ( $10^{-5}$  M), an H<sub>2</sub> receptor antagonist structurally unrelated to cimetidine, also prevented the histamine ( $10^{-6}$  M)-mediated increase in IL-1α-induced IL-1β synthesis (87% increase,  $P < 0.001$  [untreated cells] vs 29% increase,  $P > 0.05$  [ranitidine-treated cells],  $n = 4$ ). Finally, self-induction of IL-1 was not modified by either cimetidine (Fig. 2) or ranitidine (data not shown) in the absence of exogenous histamine.

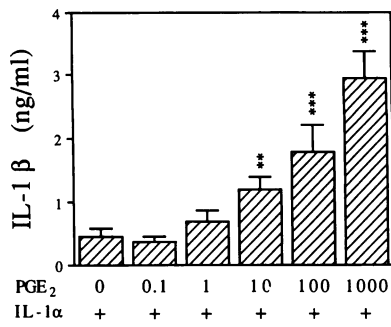
The histamine-mediated increase in IL-1β synthesis was reversed by cimetidine in a dose-dependent manner. The histamine ( $10^{-6}$  M)-mediated increase in IL-1β synthesis was reversed by cimetidine at  $10^{-5}$  M (49% increase,  $n = 4$ ,  $P > 0.05$ ) and at  $10^{-4}$  M (17% increase,  $n = 4$ ,  $P > 0.05$ ). However, cimetidine at  $10^{-6}$  M failed to reverse the histamine-mediated increase in IL-1β synthesis (104% increase,  $n = 4$ ,  $P < 0.01$ ).

**PGE<sub>2</sub> enhances IL-1β synthesis in IL-1α-stimulated PBMC.** Since activation of H<sub>2</sub> receptors results in an increased activity of adenylate cyclase (18), we next investigated whether PGE<sub>2</sub>, another activator of adenylate cyclase, enhanced IL-1β synthesis in IL-1α-stimulated PBMC. PBMC stimulated with IL-1α synthesized  $0.46 \pm 0.12$  ng/ml IL-1β (Fig. 3). PGE<sub>2</sub> alone (1,000 ng/ml) did not induce IL-1β synthesis (data not shown). However, IL-1α-induced synthesis of IL-1β was increased by PGE<sub>2</sub> in a dose-dependent manner (Fig. 3). PGE<sub>2</sub> at 10 ng/ml significantly enhanced IL-1β synthesis by 2.6-fold,



**Figure 2.** Effects of histamine receptor antagonists on the histamine-mediated increase in IL-1β synthesis. PBMC were preincubated for 1 h with diphenhydramine (DIP;  $10^{-5}$  M) or cimetidine (CIM;  $10^{-4}$  M), or without drugs. PBMC were then stimulated for 24 h with

IL-1α (10 ng/ml) in the absence (-) or presence (+) of histamine (HIS;  $10^{-5}$  M). Differences in IL-1β synthesis within drug-treated groups were analyzed for significance by Student's *t* test for paired samples (\*\*\*)  $P < 0.001$ ). Data are expressed as mean ± SEM for four donors.



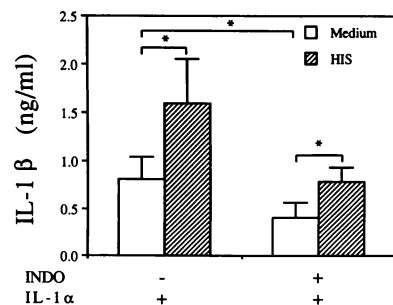
**Figure 3.** Effect of PGE<sub>2</sub> on IL-1 $\alpha$ -induced synthesis of IL-1 $\beta$ . PBMC were stimulated for 24 h with IL-1 $\alpha$  (10 ng/ml) in the absence (-) or presence of PGE<sub>2</sub> (0.1-1,000 ng/ml). Differences in IL-1 $\beta$  synthesis were analyzed for significance by ANOVA (\*\* $P < 0.001$ ; \*\*\* $P < 0.001$ ). Data are expressed as mean $\pm$ SEM for six donors.

whereas higher concentrations of PGE<sub>2</sub> further increased IL-1 $\beta$  synthesis. These observations further strengthen the concept that an increase in cAMP levels enhances self-induction of IL-1.

Since IL-1 induces PGE<sub>2</sub> synthesis in PBMC, we next investigated whether the endogenous production of PGE<sub>2</sub> by IL-1 $\alpha$ -stimulated PBMC contributed to the self-induction of IL-1 and to the histamine-mediated increase in IL-1 synthesis. At a concentration of indomethacin, which completely inhibits prostaglandin synthesis (19), we observed a significant reduction in IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis (51% inhibition,  $P < 0.05$ ) (Fig. 4). However, indomethacin did not affect the histamine-mediated increase in IL-1 $\beta$  synthesis by IL-1 $\alpha$ -stimulated PBMC (97% increase,  $P < 0.05$  [untreated cells] vs 99% increase,  $P < 0.05$  [indomethacin-treated cells],  $n = 4$ ). Indomethacin alone did not induce IL-1 $\beta$  synthesis (data not shown).

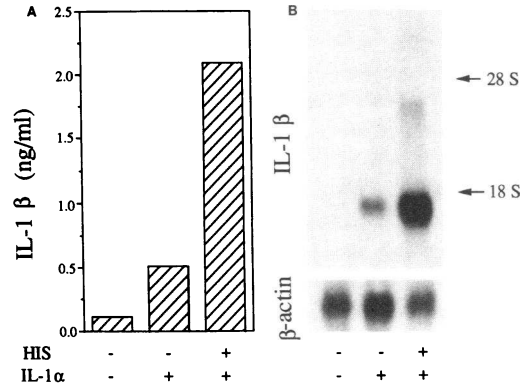
*Histamine enhances and sustains IL-1 $\beta$  mRNA levels in IL-1 $\alpha$ -stimulated PBMC.* Unstimulated, cultured PBMC did not express detectable levels of IL-1 $\beta$  mRNA (Fig. 5 and 6). Histamine (10<sup>-5</sup> M) strongly enhanced both IL-1 $\beta$  protein and IL-1 $\beta$  mRNA levels in IL-1 $\alpha$ -stimulated PBMC (Fig. 5). Histamine alone did not induce mRNA accumulation for IL-1 $\beta$  (data not shown).

IL-1 $\beta$  mRNA reached peak levels within the first 8 h after exposure to IL-1 $\alpha$  and gradually returned to basal levels (Fig. 6). IL-1 $\beta$  protein levels increased during the first 16 h of incubation; no further increase was observed at 32 h. Histamine



**Figure 4.** Effect of indomethacin on the histamine-mediated increase in IL-1 $\beta$  synthesis. PBMC were preincubated for 1 h with (+) or without (-) indomethacin (INDO; 1.3  $\times 10^{-6}$  M). PBMC were then stimulated for 24 h with IL-1 $\alpha$  (10 ng/ml) in the absence

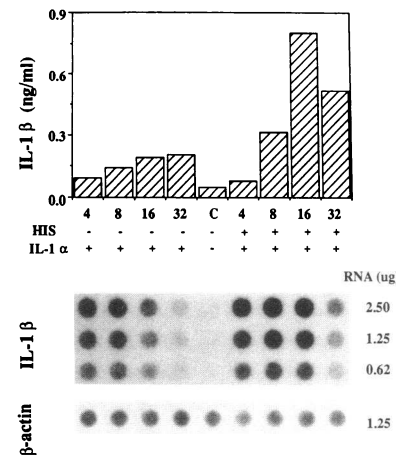
(open bars) or presence (hatched bars) of histamine (HIS; 10<sup>-5</sup> M). Differences in IL-1 $\beta$  synthesis within or between indomethacin-treated group and control group were analyzed for significance by Wilcoxon signed-rank test (\* $P < 0.05$ ). Data are expressed as mean $\pm$ SEM for four donors.



**Figure 5.** Effect of histamine on IL-1 $\alpha$ -induced mRNA accumulation for IL-1 $\beta$ . PBMC were stimulated for 16 h with IL-1 $\alpha$  (10 ng/ml) in the absence (-) or presence (+) of histamine (HIS; 10<sup>-5</sup> M). mRNA levels for IL-1 $\beta$  (B, upper panel) and  $\beta$ -actin (B, lower panel) were analyzed by Northern blotting. Levels of IL-1 $\beta$  protein in the corresponding 16 h PBMC cultures were assayed by RIA (A). Panels depict Northern blot analysis and protein levels of one experiment representative of the results from three donors.

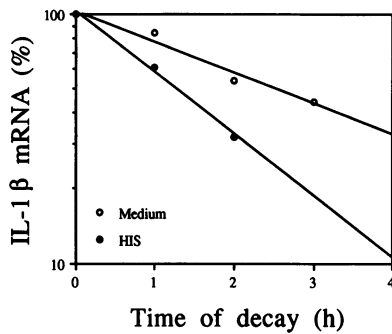
(10<sup>-5</sup> M) did not modify IL-1 $\beta$  mRNA and protein levels 4 h after exposure to IL-1 $\alpha$  (Fig. 6), but increased by twofold those observed 8 h after IL-1 $\alpha$  stimulation. Moreover, histamine sustained mRNA accumulation for IL-1 $\beta$ . IL-1 $\beta$  mRNA levels from histamine-treated PBMC were increased more than fourfold at 16 h after exposure to IL-1 $\alpha$ . IL-1 $\beta$  protein levels in the corresponding 16 h PBMC cultures were increased to the same extent. Finally, IL-1 $\beta$  mRNA and protein levels from histamine-treated PBMC were higher at 32 h after exposure to IL-1 $\alpha$ , more than twofold greater than those of the corresponding untreated cells.

*Effect of histamine on IL-1 $\beta$  mRNA stability in IL-1 $\alpha$ -stimulated PBMC.* To investigate whether histamine enhances steady-state mRNA levels for IL-1 $\beta$  by increasing the half-life



**Figure 6.** Kinetics of the histamine-mediated regulation of IL-1 $\beta$  gene expression in IL-1 $\alpha$ -stimulated PBMC. PBMC were stimulated by IL-1 $\alpha$  (10 ng/ml) in the absence (-) or presence (+) of histamine (HIS; 10<sup>-5</sup> M). mRNA levels for IL-1 $\beta$  and  $\beta$ -actin were analyzed 4, 8, 16, and 32 h after IL-1 $\alpha$  stimulation by dilutional analysis. Serial dilutions of total RNA (2.50, 1.25, and 0.62  $\mu$ g) for

each experimental point are shown. Levels of IL-1 $\beta$  from PBMC cultures at the corresponding time points were assayed by RIA. Unstimulated PBMC (C) were cultured for 32 h and then analyzed for IL-1 $\beta$  mRNA and protein levels. Panels depict the dilutional analysis and protein levels of one experiment representative of the results from three donors.



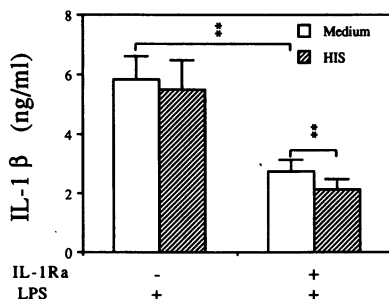
**Figure 7.** Half-life of IL-1 $\beta$  mRNA in IL-1 $\alpha$ -stimulated PBMC. PBMC were stimulated with IL-1 $\alpha$  (10 ng/ml) in the absence (open symbols) or presence (closed symbols) of histamine (HIS; 10<sup>-5</sup> M). New transcription was stopped by addition of actinomycin D (10  $\mu$ g/ml)

4 h after IL-1 $\alpha$  stimulation. Relative steady-state mRNA levels for IL-1 $\beta$  and  $\beta$ -actin were measured at 1, 2, and 3 h thereafter. IL-1 $\beta$  mRNA levels are normalized to the corresponding  $\beta$ -actin mRNA levels. Data are plotted as log of percent remaining IL-1 $\beta$  mRNA vs time of decay in hours. IL-1 $\beta$  mRNA half-lives were determined by regression analysis.

of IL-1 $\beta$  mRNA in IL-1 $\alpha$ -stimulated PBMC, new transcription was arrested by addition of actinomycin D to PBMC cultures 4 h after exposure to IL-1 $\alpha$ . Exponential regression analyses (Fig. 7) indicated that histamine (10<sup>-5</sup> M) actually reduced the half-life of IL-1 $\beta$  mRNA from 2.4 h to 1.2 h in IL-1 $\alpha$ -stimulated PBMC. IL-1 $\beta$  mRNA levels in the 16 h PBMC cultures were too low to perform mRNA decay studies.

**Effect of histamine on LPS-induced IL-1 $\beta$  synthesis.** Self-induction of IL-1 significantly contributes to IL-1 $\beta$  synthesis in LPS-stimulated PBMC (20). Because histamine enhances self-induction of IL-1, we investigated the effect of histamine on LPS-induced IL-1 $\beta$  synthesis in the presence of saturating concentrations of the naturally occurring IL-1 receptor antagonist, IL-1Ra (Fig. 8). IL-1Ra itself significantly reduced LPS-induced IL-1 $\beta$  synthesis (54% inhibition,  $P < 0.01$ ). In the absence of IL-1Ra, histamine (10<sup>-5</sup> M) did not affect LPS-induced IL-1 $\beta$  synthesis (6% inhibition,  $P > 0.05$ ). However, histamine significantly reduced LPS-induced IL-1 $\beta$  synthesis in the presence of IL-1Ra (22% inhibition,  $P < 0.01$ ).

**Effect of histamine on IL-1Ra synthesis in LPS- or IL-1 $\alpha$ -stimulated PBMC.** Since exogenous IL-1Ra inhibits self-induction of IL-1 in human PBMC by 50% at equimolar concentrations (21), endogenous IL-1Ra synthesis may reduce self-induction of IL-1 in LPS-stimulated PBMC. We, therefore,



**Figure 8.** Effect of histamine on LPS-induced synthesis of IL-1 $\beta$ . PBMC were preincubated for 1 h with (+) or without (-) IL-1Ra (1  $\mu$ g/ml). PBMC were then stimulated for 24 h with LPS (10 ng/ml) in the absence (open bars) or presence (hatched bars) of histamine (HIS; 10<sup>-5</sup> M). Differences in IL-1 $\beta$  synthesis within drug-treated groups were analyzed for significance by Student's  $t$  test for paired samples (\*\* $P < 0.01$ ). Data are expressed as mean $\pm$ SEM for eight donors.

Differences in IL-1 $\beta$  synthesis within drug-treated groups were analyzed for significance by Student's  $t$  test for paired samples (\*\* $P < 0.01$ ). Data are expressed as mean $\pm$ SEM for eight donors.

**Table I.** Effect of Histamine on IL-1Ra Synthesis in LPS- or IL-1 $\alpha$ -stimulated PBMC

Stimulus	Medium	Histamine
LPS	11.48 $\pm$ 1.99	10.59 $\pm$ 1.85
IL-1 $\alpha$	1.72 $\pm$ 0.36	2.07 $\pm$ 0.35

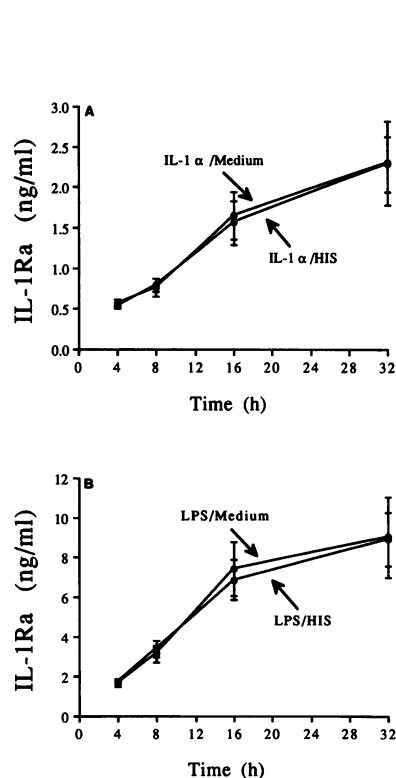
PBMC were stimulated for 24 h with LPS (10 ng/ml) or IL-1 $\alpha$  (10 ng/ml) in the absence (medium) or presence of histamine (10<sup>-5</sup> M). Concentrations of IL-1Ra are expressed in ng/ml. Differences in IL-1Ra synthesis within LPS- or IL-1 $\alpha$ -stimulated groups were analyzed for significance by Student's  $t$  test for paired samples (all  $P > 0.05$ ). Data are expressed as mean $\pm$ SEM for four donors.

assessed the effect of histamine on IL-1Ra synthesis induced by either LPS or IL-1 $\alpha$ . Histamine (10<sup>-5</sup> M) did not affect IL-1Ra synthesis induced by either LPS or IL-1 $\alpha$  (Table I). As described above, histamine did not affect LPS-induced synthesis of IL-1 $\beta$  (data not shown), but significantly enhanced (88% increase,  $P < 0.05$ ) IL-1 $\alpha$ -induced synthesis of IL-1 $\beta$ .

We then investigated whether histamine modified the kinetics of IL-1Ra synthesis in PBMC stimulated with either LPS or IL-1 $\alpha$ , thus reducing self-induction of IL-1. As shown in Fig. 9, histamine (10<sup>-5</sup> M) did not modify the kinetics of IL-1Ra synthesis in PBMC stimulated with either IL-1 $\alpha$  (A) or LPS (B).

## Discussion

In these studies, we demonstrate that histamine enhances IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis via activation of histamine H<sub>2</sub> receptors. IL-1 $\beta$  synthesis was increased two to threefold at



**Figure 9.** Effect of histamine on the kinetics of IL-1Ra synthesis. PBMC were stimulated with either IL-1 $\alpha$  (10 ng/ml, A) or LPS (10 ng/ml, B) in the absence (medium; open symbols) or presence of histamine (HIS, 10<sup>-5</sup> M; closed symbols). IL-1Ra levels were analyzed 4, 8, 16, and 32 h after addition of either IL-1 $\alpha$  or LPS. Unstimulated PBMC cultures contained 0.49 $\pm$ 0.11 ng/ml IL-1Ra after 32 h of incubation. Differences in IL-1Ra synthesis between the histamine-treated group and the control group were analyzed for significance by Student's  $t$  test for paired samples (all  $P > 0.05$ ). Data are expressed as mean $\pm$ SEM for six donors.

histamine concentrations from  $10^{-6}$  to  $10^{-4}$  M. Histamine increased and sustained the steady-state mRNA levels for IL-1 $\beta$  in IL-1 $\alpha$ -stimulated PBMC, but reduced the half-life of IL-1 $\beta$  mRNA in the same cells, suggesting that the histamine-mediated increase in IL-1 $\beta$  synthesis is a transcriptional event.

Histamine enhanced self-induction of IL-1 at concentrations as low as  $10^{-6}$  M. Histamine concentrations in the micromolar range have been reported to suppress the LPS-induced synthesis of TNF- $\alpha$  in PBMC (22), to reduce the LPS-induced synthesis of IL-1 in human monocytes purified by adherence (23) and to inhibit the synthesis of IL-2 and IFN- $\gamma$  in PBMC stimulated with Staphylococcal Enterotoxin A (24). Thus, the increase in self-induction of IL-1 occurs at histamine concentrations required to observe other immunomodulatory effects of histamine. However, the biological relevance of these concentrations remains uncertain, since histamine levels in tissue have yet to be determined. It is indeed difficult to evaluate histamine levels in tissue after mast cell degranulation because of the rapid degradation of histamine. Nevertheless, based on the histamine concentrations reached *in vitro* after basophil degranulation (25), histamine levels are thought to be as high as  $10^{-6}$  M in the vicinity of degranulated mast cells.

Human monocytes, B cells, T helper, and T suppressor cells express high affinity H<sub>1</sub> receptors for histamine characterized by a  $K_d$  in the nanomolar range (26). PBMC have also been shown to express low affinity H<sub>1</sub> receptors (27), as well as H<sub>2</sub> receptors for histamine (28), both characterized by a  $K_d$  in the micromolar range. Since the H<sub>1</sub> receptor antagonist diphenhydramine did not modify the histamine-mediated increase in IL-1 $\beta$  synthesis, neither the high affinity nor the low affinity H<sub>1</sub> receptor mediates the histamine-mediated increase in IL-1 $\beta$  synthesis. In contrast, both cimetidine and ranitidine, H<sub>2</sub> receptor antagonists structurally unrelated to each other, prevented the histamine-mediated increase in self-induction of IL-1. Thus, histamine enhances IL-1 $\beta$  synthesis via activation of H<sub>2</sub> receptors. However, neither cimetidine nor ranitidine modified the IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis in the absence of exogenous histamine, ruling out a contribution of endogenous histamine released by contaminating basophils in our experimental conditions.

To strengthen further the concept that an increase in cAMP levels enhances self-induction of IL-1, we investigated whether PGE<sub>2</sub>, another activator of adenylate cyclase, increased self-induction of IL-1. PGE<sub>2</sub> itself did not induce IL-1 $\beta$  synthesis but enhanced IL-1 $\beta$  synthesis in IL-1 $\alpha$ -stimulated PBMC. Similarly, PGE<sub>2</sub> has been reported to increase the IL-1 $\beta$ -induced synthesis of IL-1 $\alpha$  in PBMC (29). In support of this observation, indomethacin, a cyclooxygenase inhibitor, reduced the IL-1 $\alpha$ -induced synthesis of IL-1 $\beta$ . Of critical importance to this finding is the fact that IL-1 increases both enzymatic activity and gene expression of cyclooxygenase (30–32). Thus, endogenous production of PGE<sub>2</sub> by IL-1-stimulated PBMC likely explains the suppressive effect of indomethacin on self-induction of IL-1. Recently, IL-1Ra has been shown to exert beneficial effects in experimental arthritis (33). Deleterious effects of prostaglandins, PGE<sub>2</sub> in particular, may contribute to the worsening of inflammatory diseases since PGE<sub>2</sub> increases self-induction of IL-1. Thus, the beneficial effects of nonsteroidal antiinflammatory drugs in arthritis may result, in part, from a suppression of a positive regulatory effect of PGE<sub>2</sub> on IL-1 synthesis.

Since IL-1 stimulates PGE<sub>2</sub> production in human monocytes, we also investigated whether endogenous PGE<sub>2</sub> contributed to the histamine-mediated increase in IL-1 $\beta$  synthesis. Indomethacin did not modify the effect of histamine on IL-1 $\alpha$ -induced IL-1 $\beta$  synthesis, indicating that histamine does not regulate IL-1 synthesis via an endogenous generation of prostaglandins. Since activation of H<sub>1</sub> receptors leads the generation of PGE<sub>2</sub> (34, 35), these results also support our observation that triggering of H<sub>1</sub> receptors does not participate in the regulatory effects of histamine on IL-1 synthesis.

Activation of H<sub>2</sub> receptors results in an increased activity of adenylate cyclase (18). Whether cAMP-increasing agents suppress or upregulate IL-1 gene expression or protein synthesis remains a controversial issue (23, 36–39). Recently, IL-1Ra has been shown to reduce LPS-induced IL-1 $\beta$  synthesis (20), indicating that self-induction of IL-1 contributes to IL-1 $\beta$  synthesis in LPS-stimulated PBMC. Histamine enhances self-induction of IL-1 via activation of H<sub>2</sub> receptors. However, histamine reduced LPS-induced IL-1 $\beta$  synthesis in the presence of saturating concentrations of IL-1Ra. We, therefore, believe this is the accurate assessment of the histamine effects on the LPS-induced synthesis of IL-1 $\beta$  because of the reciprocal effects of histamine on IL-1 $\beta$  synthesis. Hence, the net effect of histamine (or any cAMP-increasing agent) on IL-1 $\beta$  synthesis in LPS-stimulated PBMC reflects both an increase in self-induction of IL-1 and a decrease in LPS-induced IL-1 $\beta$  synthesis.

Histamine increased steady-state levels for IL-1 $\beta$  mRNA in IL-1 $\alpha$ -stimulated PBMC. Since histamine reduced IL-1 $\beta$  mRNA half-life, histamine likely increases the transcriptional rate of the IL-1 $\beta$  gene. Interestingly, PGE<sub>2</sub> increased IL-1 $\beta$  mRNA levels in LPS-stimulated murine peritoneal macrophages (38) or human monocytes (39). In both reports, elevated cAMP levels did not alter the stability of IL-1 $\beta$  mRNA, but increased the transcriptional rate of the IL-1 $\beta$  gene. The striking similarity in the mechanisms of upregulation of IL-1 $\beta$  gene expression and protein synthesis lead us to postulate that an increase in IL-1 synthesis in LPS-stimulated monocytes is caused in part by an increase in self-induction of IL-1. Both the PGE<sub>2</sub>-mediated increase in self-induction of IL-1 and the reduction of self-induction of IL-1 by indomethacin support our hypothesis. Interestingly, PGE<sub>2</sub> or dibutyl cAMP also enhances mRNA accumulation and protein synthesis for IL-1 $\beta$  in PMA-stimulated human monocytes (37) and myeloid leukemia cells (40). In agreement with our hypothesis, self-induction of IL-1 largely (70%) contributes to PMA-induced IL-1 $\beta$  synthesis (20).

Previous reports indicate that IL-1 may synergize with cAMP-increasing agents (41, 42). In a pre-B cell line 70Z/3, dibutyl cAMP or PGE<sub>2</sub> failed to increase surface IgM or to increase  $\kappa$  chain mRNA level. However, both cAMP-increasing agents increased the IL-1 $\alpha$ -induced  $\kappa$  chain expression. Although signaling pathways for IL-1 remain an open issue (43, 44), it is unlikely that the signal transduction pathway for IL-1 involves cAMP. Accordingly, we report that histamine alone did not induce mRNA accumulation for IL-1 $\beta$ . However, cAMP may act through one or more intermediate steps leading ultimately to a change in transcriptional regulators, since histamine likely increases the transcriptional rate of the IL-1 $\beta$  gene. Such an indirect effect of histamine on IL-1-induced IL-1 gene expression is further supported by the absence of a cAMP re-

sponsive element motif in the 5' regulatory sequences of the IL-1 $\beta$  gene (39).

Our results expand on those of others in that we measured total IL-1 $\beta$  synthesis using a specific immunoreactive assay. Unlike bioassays for IL-1 $\beta$ , the immunoassay is not affected by the presence of IL-1Ra. Reduction in IL-1Ra synthesis may contribute to an increase in self-induction of IL-1 in LPS-stimulated PBMC. However, a specific radioimmunoassay for IL-1Ra revealed that histamine failed to affect the synthesis of IL-1Ra induced by either LPS or IL-1 $\alpha$ . Furthermore, histamine did not accelerate the synthesis of IL-1Ra over a time period of 32 h. Thus, histamine is unlikely to increase self-induction of IL-1 in LPS-stimulated PBMC by affecting the endogenous synthesis of IL-1Ra. Finally, the lack of effect of histamine on IL-1 $\alpha$ -induced IL-1Ra synthesis provides additional evidence that IL-1 $\beta$  and IL-1Ra, two members of the same cytokine gene family (45), are differentially regulated (16, 46).

Our studies indicate that histamine enhances self-induction of IL-1 $\beta$  via H<sub>2</sub> receptors. Because IL-1 contributes to the eosinophil infiltration characteristic of the late phase response to antigen, histamine release by activated mast cells may sustain chronic inflammatory processes, in addition to its immediate inflammatory properties.

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