The regulation of interleukin (IL)-6 synthesis by cAMP-increasing agents remains an unresolved issue. Since an increase in cAMP levels via activation of histamine H₂ receptors does not induce IL-1β synthesis but enhances self-induction of IL-1 (Vannier, E., and Dinarello, C. A. (1983) J. Clin. Invest. 72, 281–287), we investigated whether histamine regulates IL-6 synthesis. Human peripheral blood mononuclear cells were stimulated with IL-1α in the absence or presence of histamine (1 nM to 100 μM). IL-6 was measured using a specific radioimmunoassay. Histamine alone did not induce protein synthesis or mRNA accumulation for IL-6. Histamine (1–100 μM) enhanced IL-1α-induced synthesis of IL-6 (p < 0.001). Cimetidine and ranitidine, H₂ receptor antagonists structurally unrelated to each other, completely reversed the histamine-mediated increase in IL-1α-induced IL-6 synthesis. However, diphenhydramine, an H₁ receptor antagonist, did not reverse this effect. Prostaglandin E₂, an activator of adenylate cyclase, also enhanced IL-1α-induced synthesis of IL-6. Histamine increased and sustained steady-state levels of IL-6 mRNA in IL-1α-stimulated cells, but reduced IL-6 mRNA half-life (3.5 h versus 1.8 h). Our results indicate that cAMP-increasing agents, such as histamine or prostaglandin E₂, fail to induce IL-6 synthesis but rather enhance IL-1-induced IL-6 synthesis.

Interleukin (IL)-6 is a multifunctional cytokine produced primarily by monocytes and macrophages (1–3), endothelial cells (4, 5), vascular smooth muscle cells (6), and fibroblasts (7, 8). Among its pleiotropic properties, IL-6 activates T cells, promotes B cell growth and immunoglobulin synthesis, stimulates hepatocyte synthesis of acute-phase proteins, is an endogenous pyrogen, induces maturation of megakaryocytes, stimulates proliferation of keratinocytes, and acts as a growth factor for human myeloma and murine plasmacytoma (9). More recently, IL-6 has been described as a growth factor for acquired immunodeficiency syndrome (AIDS)-related Kaposi's sarcoma cells (10) and melanoma cells from advanced stage lesions (11). Elevated IL-6 gene expression and protein synthesis have been measured in patients with various pathological situations, including sepsis (12, 13), rheumatoid arthritis (14), Kaposi's sarcoma (15), and psoriasis (16).

In addition to its well-known physiological properties and role in inflammation, histamine displays immunoregulatory effects. Histamine inhibits the synthesis of IL-2 and interferon-γ in peripheral blood mononuclear cells (PBMC) stimulated with Staphylococcal enterotoxin A (17), suppresses lipopolysaccharide-induced synthesis of tumor necrosis factor (TNF-α) in human monocytes (18), and reduces lipopolysaccharide-induced synthesis of IL-1α in human PBMC, but enhances IL-1α-induced synthesis of IL-1β in the same cells (19). Interestingly, histamine modulates synthesis of each of these cytokines via activation of H₂ receptors.

Recent reports, based on pilot studies, have described the efficacy of H₂ receptor antagonists in the treatment of advanced melanoma patients (20) and in the treatment of Kaposi's sarcoma in patients with AIDS (21). Since IL-6 is a growth factor for both advanced stage melanoma cells and AIDS-related Kaposi's sarcoma cells and because both types of cells proliferate in tissues containing numerous mast cells, we investigated the effect of histamine on IL-6 synthesis. Modulation by histamine of gene expression and total cellular synthesis of IL-6 were studied in human PBMC stimulated with IL-1.

**EXPERIMENTAL PROCEDURES**

**Human PBMC Culture**—Blood was drawn from healthy human volunteers who had not taken any histamine receptor antagonists or cyclooxygenase inhibitors for at least 2 weeks. 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; Hypaque-M 90%, Winthrop Breon Laboratories, New York) gradients. Cells were washed twice in 0.15 M NaCl and resuspended at 5 x 10⁶ cells/ml in ultraltrafiltration RPMI culture medium 1640 (Whittaker M.A. Bioproducts, Walkersville, MD) supplemented with 2 mg/ml glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). PBMC (2.5 x 10⁶ cells/ml in RPMI containing 1% heat-inactivated human AB serum) were stimulated with human recombinant IL-1α (10 ng/ml), kindly provided by Dr. P. Lomedico, Hoffman-LaRoche, Nutley, NJ, in the absence or presence of either histamine (10⁻⁴ to 10⁻⁸ M; Sigma) or prostaglandin E₂ (PGE₂) (0.1 to 1000 ng/ml; Sigma). PBMC cultures were incubated in 12 x 75-mm polypropylene round bottom tubes (Becton Dickinson and Co., Lincoln Park, NJ) for 24 h at 37 °C in a humidified atmosphere containing 5% CO₂.

In other experiments, PBMC (5 x 10⁶ cells/ml) were preincubated for 1 h at 37 °C in the presence of either diphenhydramine (10⁻⁴ M; Elkins-Sinn, Inc., Cherry Hill, NJ), cimetidine (10⁻² to 10⁻¹ M; Smith Kline & French Laboratories), ranitidine (10⁻⁶ M; Glaxo, Inc., Research Triangle Park, NC), 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 + secreted) cytokines by specific RIAs as described for IL-6 (22) and TNF-α (23). The sensitivities (defined as 95% binding) of the RIAs for IL-6 and TNF-α were 40 ± 1 pg/ml (n = 8) and 20 ± 1 pg/ml (n = 8), respectively.
PBMC-After 24 h of culture, unstimulated PBMC synthesized IL-6 (19), we investigated whether the histamine-mediated PBMC cultures contained TNFα stimulation with IL-1α. PBMC synthesized IL-2 in the absence of stimulation with IL-1α. PBMC synthesized IL-6 (113% increase, p < 0.001). However, a concentration of histamine as low as 10-8 M did not modify the histamine-mediated increase in IL-6 synthesis (Fig. 2). However, cimetidine (10-4 M) completely prevented the histamine (10-8 M)-mediated increase in IL-6 synthesis. Similar results were observed for histamine 10-4 M (data not shown). Ranitidine (10-6 M), an H2 receptor antagonist structurally unrelated to cimetidine, also suppressed the histamine-mediated increase in IL-1α-induced IL-6 synthesis (83% increase, p < 0.001 (untreated cells)) versus 4% reduction, p = 0.05 (ranitidine-treated cells). Neither cimetidine (Fig. 2) nor ranitidine (Fig. 3) modified IL-1α-induced synthesis of IL-6 in the absence of exogenous histamine. Cimetidine antagonized the histamine-mediated increase in IL-1α-induced IL-6 synthesis in a dose-dependent manner (Fig. 4). The histamine-mediated increase in IL-6 synthesis was completely suppressed by cimetidine from 10-5 M (11% increase, n = 3, p > 0.05) to 10-4 M (8% reduction, p < 0.05). Cimetidine at 3 x 10-4 M partially prevented the histamine-mediated increase in IL-6 synthesis (36% increase, p < 0.01), whereas cimetidine at 10-4 M failed to reverse the histamine-mediated increase in IL-6 synthesis (75% increase, p < 0.001).

PGE2 Enhances IL-1α-induced IL-6 Synthesis—Activation of H2 receptors results in increased adenylate cyclase activity (25). To strengthen further the hypothesis that increased cAMP levels do not directly induce IL-6 synthesis but rather enhance IL-1α-induced IL-6 synthesis, we attempted to mimic the regulatory effect of histamine on IL-6 synthesis using PGE2, another activator of adenylate cyclase. PGE2 alone did not induce IL-6 synthesis at concentrations as high as 1000 ng/ml (n = 9). However, as in the case of histamine, PGE2 enhanced IL-1α-induced IL-6 synthesis (Fig. 5). The PGE2-mediated increase in IL-6 synthesis was dose-dependent. IL-6 synthesis was enhanced by 10 ng/ml of PGE2 (91% increase, p < 0.001), whereas higher concentrations of PGE2 further increased IL-6 synthesis (151% increase at 100 ng/ml PGE2 and 212% increase at 1000 ng/ml PGE2, p < 0.001).
Histamine Enhances IL-1-induced IL-6 Synthesis

Histamine Enhances and Sustains IL-6 mRNA Accumulation in IL-1α-stimulated PBMC—Unstimulated cultured PBMC did not express detectable IL-6 mRNA levels (see Figs. 6 and 7). Histamine (10^{-5} M) alone failed to induce detectable IL-6 mRNA levels (data not shown). However, histamine (10^{-5} M) enhanced both IL-6 steady-state mRNA levels and IL-6 protein levels assessed 16 h after exposure to IL-1α (Fig. 6). It is noteworthy that IL-6 mRNA levels in IL-1α-stimulated PBMC at this particular time point were dramatically increased in the presence of histamine, whereas corresponding IL-6 protein levels were increased only 3-fold.

As shown in Fig. 7, IL-6 mRNA levels reached peak levels within the first 8 h of stimulation with IL-1α and rapidly returned to basal levels within the following 8 h. IL-6 protein levels steadily increased during the first 16 h of stimulation by IL-1α but increased only slightly thereafter. Histamine (10^{-5} M) strongly enhanced both IL-6 mRNA and protein levels as early as 4 h after exposure to IL-1α. However, IL-6 mRNA levels observed 8 h after IL-1α stimulation were enhanced by more than 4-fold in the presence of histamine, although the corresponding IL-6 protein levels were increased by only 2-fold. Moreover, IL-6 mRNA levels from histamine-treated cells increased by more than 4-fold after 16 h of exposure to IL-1α, when compared with untreated cells. IL-6 levels in histamine-treated cells reached peak levels at 16 h and were increased by only 2-fold. IL-6 mRNA and protein levels in histamine-treated PBMC remained higher at 32 h, when compared with untreated cells.

Effect of Histamine on IL-6 mRNA Stability in IL-1α-stimulated PBMC—To elucidate whether histamine enhances IL-6 mRNA steady-state levels in IL-1α-stimulated cells by increasing IL-6 mRNA stability, actinomycin D was added to PBMC cultures 4 h after exposure to IL-1α. Exponential regression analyses indicate that histamine (10^{-6} M) reduced the half-life of IL-6 mRNA from 3.5 to 1.8 h in cells stimulated with IL-1α.

DISCUSSION

In these studies, we demonstrate that histamine enhances IL-1-induced IL-6 synthesis via activation of H₂ receptors. IL-6 synthesis was increased 2-fold at histamine concentrations from 10^{-4} to 10^{-3} M. Histamine increased and sustained the steady-state mRNA levels for IL-6 in IL-1α-stimulated PBMC, but reduced the half-life for IL-6 in the same cells, suggesting that the histamine-mediated increase in IL-6 synthesis is a transcriptional event.

Human monocytes, B cells, T helper, and T suppressor cells express high affinity H₁ receptors for histamine characterized by a Kᵢ in the nanomolar range (26). PBMC have also been shown to express low affinity H₁ receptors (27), as well as H₂ receptors for histamine (28), both characterized by a Kᵢ in the micromolar range. Since the H₁ receptor antagonist diphenhydramine did not modify the histamine-mediated increase in IL-6 synthesis, neither the high affinity nor the low affinity H₁ receptor mediates the histamine-mediated increase in IL-6 synthesis. In contrast, both cimetidine and ranitidine, H₂ receptor antagonists structurally unrelated to each other, prevented the histamine-mediated increase in IL-6 synthesis in IL-1α-stimulated cells. Thus, histamine enhances IL-1-induced IL-6 synthesis via activation of H₂ receptors. Neither cimetidine nor ranitidine modified IL-1α-induced IL-6 synthesis in the absence of exogenous histamine, ruling out a contribution of endogenous histamine release from contaminating basophils in our experimental conditions.

Activation of histamine H₂ receptors increases adenylyl cyclase activity (25). Although cAMP-increasing agents alone have been reported to induce IL-6 synthesis in the FS-4 cell line.
Histamine Enhances IL-1-induced IL-6 Synthesis

FIG. 5. Effect of histamine on IL-1α-induced mRNA accumulation for IL-6. PBMC were stimulated for 16 h with IL-1α (10 ng/ml) in the absence (−) or presence (+) of histamine (HIS; 10−5 M). mRNA levels for IL-6 (upper right panel) and β-actin (lower right panel) were analyzed by Northern blotting. Levels of IL-1β protein in the corresponding 16-h PBMC cultures were assayed by RIA (left panel). Panels depict Northern blot analysis and protein levels of one experiment representative of the results from three donors.

Histamine Enhances IL-1-induced IL-6 Synthesis

Fig. 6. Effect of histamine on IL-1α-induced mRNA accumulation for IL-6. PBMC were stimulated for 16 h with IL-1α (10 ng/ml) in the absence (−) or presence (+) of histamine (HIS; 10−5 M). mRNA levels for IL-6 (upper right panel) and β-actin (lower right panel) were analyzed by Northern blotting. Levels of IL-1β protein in the corresponding 16-h PBMC cultures were assayed by RIA (left panel). Panels depict Northern blot analysis and protein levels of one experiment representative of the results from three donors.

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