Histamine Suppresses Gene Expression and Synthesis of Tumor Necrosis Factor α via Histamine H2 Receptors

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Summary

Histamine and tumor necrosis factor α (TNF-α) can each contribute to the pathogenesis of allergic reactions and chronic inflammatory diseases. We now report the effect of histamine on gene expression and total cellular synthesis of TNF-α. Lipopolysaccharide (LPS)-induced synthesis of TNF-α in peripheral blood mononuclear cells (PBMC) from 18 healthy donors was suppressed by histamine concentrations from 10^{-6} to 10^{-4} M, levels comparable with those measured in tissues after mast cell degranulation. Histamine (10^{-5} M) markedly suppressed LPS-induced synthesis of TNF-α in both unfractionated PBMC (83% inhibition, $p < 0.001$) and monocytes purified by positive selection of LeuM3$^+$ cells (62% inhibition, $p < 0.05$). The suppressive effect of histamine on TNF-α synthesis did not require the presence of T cells. The histamine-mediated decrease in TNF-α synthesis was not affected by indomethacin, nor by diphenhydramine, an H_1 receptor antagonist, but was reversed by cimetidine or ranitidine, H_2 receptor antagonists, in a dose-dependent manner. Suppression of TNF-α synthesis by histamine is likely to be a transcriptional event, since histamine (10^{-5} M) reduced TNF-α mRNA levels fourfold. These results suggest that histamine release from mast cells may paradoxically limit the extent of inflammatory and immune reactions by suppressing local cytokine synthesis in H_2 receptor-bearing cells.

H istamine is released from basophils and mast cells during immediate-type hypersensitivity reactions. Histamine H_1 receptors mediate many of the inflammatory effects of histamine, whereas H_2 receptors mediate various immunoregulatory effects. In the latter case, histamine inhibits several lymphocyte functions (1) and reduces the production of lymphokines, including macrophage migration–inhibitory factor (MIF) (1), leukocyte migration–inhibitory factor (LIF) (1), IL-2, and IFN-γ (2). Histamine also inhibits the production of IL-1-like activity by LPS-stimulated human monocytes (3).

TNF-α, also named cachectin, displays a multitude of inflammatory and immunological functions (4). Recently, TNF-α has been shown to contribute to the mast cell–dependent recruitment of leukocytes during the IgE-dependent cutaneous late phase reactions (5). We investigated whether histamine could modulate the synthesis of TNF-α. Modulation by histamine of gene expression and total cellular synthesis of TNF-α were studied in human PBMC and purified monocytes stimulated with LPS.

Materials and Methods

Human PBMC Culture. PBMC were separated from heparinized blood as described previously (6). PBMC (2.5 × 10⁶ cells/ml in

RPMI added with 1% heat-inactivated human AB serum) were stimulated with LPS from *Escherichia coli* O55:B5 (10 ng/ml; Sigma Chemical Co., St. Louis, MO) in the presence or absence of histamine (Sigma Chemical Co.) for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. Cell cultures were then assayed for total (cell-associated + secreted) TNF-α synthesis using a specific RIA (7). The sensitivities (defined as 95% binding) of RIAs for TNF-α were 71 ± 6 pg/ml ($n = 12$).

In other experiments, PBMC (5 × 10⁶ cells/ml) were preincubated for 1 h at 37°C in the presence of either indomethacin (1.3 × 10^{-6} M; Sigma Chemical Co.), diphenhydramine (10^{-5} M; Elkins-Sinn, Inc., Cherry Hill, NJ), cimetidine (10^{-6} to 10^{-4} M; Smith Kline & French, Philadelphia, PA), ranitidine (10^{-5} M; Glaxo, Inc., Research Triangle Park, NC), or RPMI.

Histamine RIA. PBMC supernatants were assayed for secreted histamine by specific RIA. The limit of detection was 3 ng/ml (1.6 × 10^{-8} M). Crossreactivity studies showed negligible reactivity (≤0.5%) with L-histidine, spermidine, or serotonin.

Isolation of LeuM3$^+$ Cells or CD3$^-$ Cells. PBMC were washed twice in ice-cold PBS buffer (1% heat-inactivated human AB serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in PBS) and incubated for 30 min on ice with 2 μl (per 10⁶ PBMC) murine anti–human LeuM3 (monocyte/macrophage antigen) or CD3 (T cell antigen) mAb (Becton Dickinson & Co., Mountain View, CA). PBMC were then washed and mixed with sheep anti–mouse IgG–coated magnetic particles (Dynal, Oslo, Norway), at a ratio of three beads per LeuM3$^+$ cell or six beads per CD3$^+$ cell. After

rotation at 4°C for 2 h, rosetted cells were separated from the whole cell suspension using a magnetic particle concentrator (Dynal). Fractionated cells (5 × 10⁶ cells/ml) were resuspended in RPMI and incubated as described above.

Cytofluorometry Analysis. Unfractionated PBMC, LeuM3⁻, and CD3⁻ cells (0.5 × 10⁶) were incubated first with murine anti-human LeuM3 and CD3 mAbs, and then with FITC-conjugated goat anti-human IgG (Tago Inc., Burlingame, CA). Cells were then washed, fixed with 1% formaldehyde, and analyzed on a Epics 541 flow cytometer (Coulter Electronics, Hialeah, FL).

RNA Isolation and Northern Analysis. Preparation of total cellular RNA and Northern blot analysis with a ³²P-labeled nucleic acid probe were performed as described previously (6). The probes used were a 575-bp fragment of human TNF-α cDNA (American Type Culture Collection, Rockville, MD) and a full-length (2,000-bp) chicken β-actin cDNA subcloned in pGEM3.

Results and Discussion

Unstimulated PBMC did not synthesize detectable amounts of TNF-α. LPS-stimulated PBMC synthesized 8.41 ± 2.05 ng/ml of TNF-α. Histamine itself did not induce TNF-α synthesis (data not shown), but decreased the LPS-induced synthesis of TNF-α (Fig. 1). Suppression of cytokine synthesis was observed at histamine concentrations from 10^{-6} to 10^{-4} M.

To determine whether the histamine-mediated decrease in TNF-α synthesis resulted from a direct effect of histamine on LPS-stimulated monocytes, LeuM3⁺ cells were isolated from PBMC. Unfractionated PBMC from five donors contained 16.5 ± 1.2% LeuM3⁺ cells and 59.9 ± 3.2% CD3⁺ cells. After LeuM3⁺ selection, the LeuM3⁻ population contained 2.7 ± 1.2% LeuM3⁺ cells and 73.5 ± 3.9% CD3⁺ cells. Unstimulated LeuM3⁺ cells did not synthesize detectable amounts of TNF-α. Histamine (10^{-5} M) reduced, to a comparable extent, the LPS-induced synthesis of TNF-α by both LeuM3⁺ cells (62% inhibition, $p < 0.05$) and unfractionated PBMC (79% inhibition, $p < 0.05$) (Fig. 2). Since
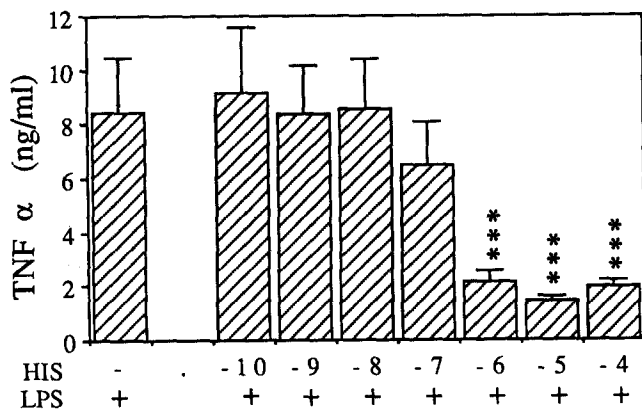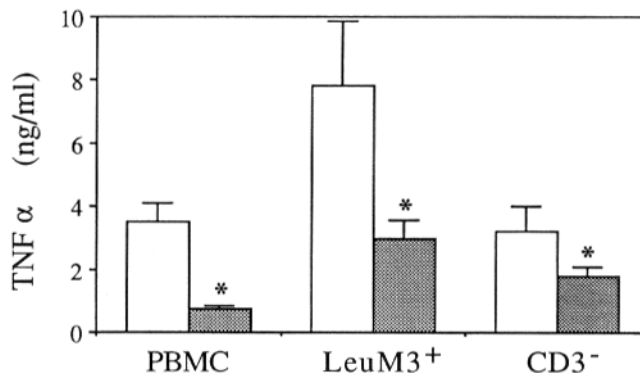
Figure 2. Effect of histamine on the LPS-induced synthesis of TNF-α in fractionated PBMC. Unfractionated PBMC, LeuM3⁺ cells, or CD3⁻ cells were stimulated with LPS (10 ng/ml) in the absence (*open bars*) or presence (*stippled bars*) of histamine (10^{-5} M). Differences in TNF-α synthesis within each cell population were analyzed for significance by Wilcoxon signed-rank test (* = $p < 0.05$). Data are expressed as mean ± SEM for five donors.

histamine activates T suppressor cells to release the histamine-induced suppressor factor, which, in turn, could inhibit lymphokine production (1), CD3⁺ cells were removed from PBMC, and CD3⁻ cells were then stimulated for TNF-α synthesis. After CD3⁺ selection, the CD3⁻ population contained 13.6 ± 3.2% LeuM3⁺ cells and 9.5 ± 1.4% CD3⁺ cells. Histamine (10^{-5} M) again reduced LPS-induced synthesis of TNF-α by CD3⁻ cells (44% inhibition, $p < 0.05$) (Fig. 2), indicating that the histamine-mediated decrease in TNF-α synthesis by LPS-stimulated PBMC does not require T cells.

Human monocytes, B cells, T helper cells, and T suppressor cells express high affinity H_1 receptors for histamine characterized by a kD in the nanomolar range (8). PBMC have also

Figure 1. Effect of histamine on the LPS-induced synthesis of TNF-α. PBMC were stimulated with LPS (10 ng/ml) in the absence (−) or presence of histamine (HIS; 10^{-10} to 10^{-4} M). Differences in TNF-α synthesis were analyzed for significance by ANOVA (*** = $p < 0.001$). Data are expressed as mean ± SEM for five donors.
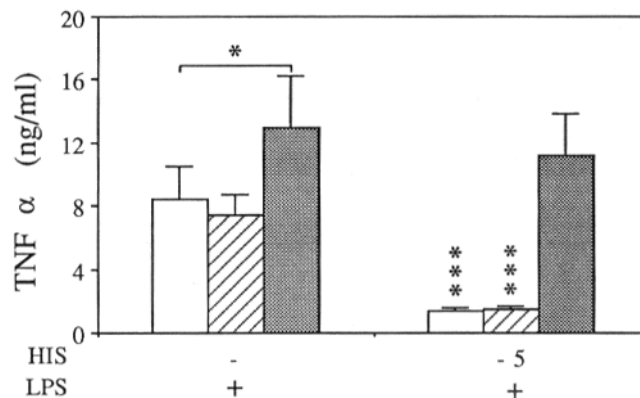
Figure 3. Effects of histamine receptor antagonists on the histamine-mediated decrease in TNF-α synthesis. PBMC were preincubated with diphenhydramine (10^{-5} M; *hatched bars*) or cimetidine (10^{-4} M; *dotted bars*), or without drugs (*open bars*). PBMC were then stimulated with LPS (10 ng/ml) in the absence (−) or presence of histamine (HIS; 10^{-5} M). Differences in TNF-α synthesis between drug-treated groups were analyzed for significance by ANOVA (* = $p < 0.05$). Differences in TNF-α 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 five donors.
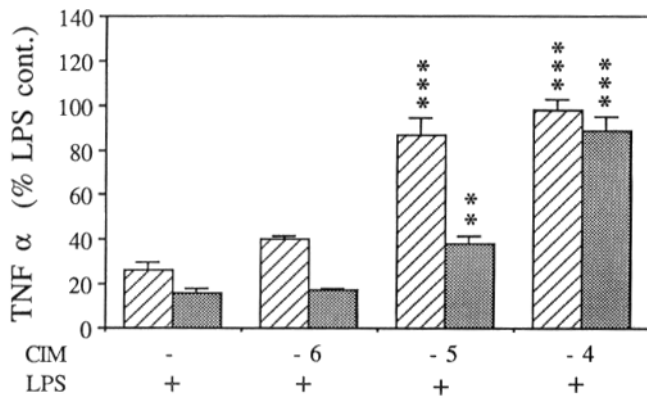
Figure 4. Dose-dependent effect of cimetidine on the histamine-mediated decrease in TNF-α synthesis. PBMC were preincubated with cimetidine (CIM; 10^{-6} to 10^{-4} M), and then stimulated with LPS (10 ng/ml) in the absence or presence of histamine (10^{-6} M; hatched bars; 10^{-5} M; stippled bars). TNF-α levels from histamine-treated groups are expressed as percentage of those from the corresponding control groups. Differences in TNF-α synthesis between cimetidine-treated groups were analyzed for significance by ANOVA (** = $p < 0.01$; *** = $p < 0.001$). Data are expressed as mean $\pm$ SEM for three donors.

been shown to express low affinity H_1 receptors (9) as well as H_2 receptors for histamine (10), both characterized by a kD in the micromolar range. PBMC were incubated in the presence of diphenhydramine, an H_1 receptor antagonist, or cimetidine, an H_2 receptor antagonist. Diphenhydramine (10^{-5} M) or cimetidine (10^{-4} M) alone did not induce synthesis of TNF-α ($n = 5$, data not shown). Diphenhydramine modified neither LPS-induced TNF-α synthesis nor the histamine (10^{-5} M)-mediated decrease in LPS-induced TNF-α synthesis (Fig. 3), indicating that neither the high affinity nor the low affinity H_1 receptor mediates the suppressive effect of histamine on TNF-α synthesis. In contrast, cimetidine reversed the histamine (10^{-5} M)-mediated decrease in LPS-induced synthesis of TNF-α (Fig. 3). Ranitidine, an H_2 receptor antagonist structurally unrelated to cimetidine, also reversed the histamine-mediated decrease in TNF-α synthesis (data not shown).

Cimetidine significantly enhanced LPS-induced synthesis

of TNF-α (154% of LPS values, $p < 0.05$) (Fig. 3) in the absence of exogenous histamine. However, levels of histamine in supernatants of unstimulated PBMC rose during 24 h of incubation (0.4 $\pm$ 0.1 $\times$ 10^{-7} M before vs. 1.8 $\pm$ 0.3 $\times$ 10^{-7} M after incubation, $p < 0.01$, $n = 4$). Histamine levels in 24-h supernatants of LPS-stimulated PBMC (1.6 $\pm$ 0.4 $\times$ 10^{-7} M) were not statistically different from those of unstimulated PBMC. These results suggest that endogenous histamine production takes place in PBMC cultures and may account for the increased synthesis of TNF-α by cimetidine-treated PBMC. In agreement with this hypothesis, macrophages and T lymphocytes synthesize histamine de novo through induced histidine decarboxylase (11).

Histamine reduces TNF-α synthesis in a competitive manner, since cimetidine partially reversed the decrease in LPS-induced synthesis of TNF-α (12.93 $\pm$ 3.26 [untreated cells] vs. 5.05 $\pm$ 0.39 ng/ml [cimetidine-treated cells]; $p < 0.001$, $n = 5$) when histamine reached 10^{-4} M. The histamine-mediated decrease in TNF-α synthesis was reversed by cimetidine in a dose-dependent manner (Fig. 4). Cimetidine (10^{-6} M) failed to reverse the histamine-mediated decrease in cytokine synthesis, whereas higher concentrations of cimetidine (10^{-5} and 10^{-4} M) partially or completely reversed the effect of histamine 10^{-6} or 10^{-5} M.

Prostaglandin E_2 suppresses multiple aspects of macrophage activation, including synthesis of TNF-α (12). Furthermore, activation of H_1 receptors leads to the generation of arachidonic acid metabolites (13). To determine whether prostaglandin synthesis could account for the histamine-mediated decrease in cytokine synthesis, PBMC were incubated in presence of indomethacin, a cyclooxygenase inhibitor. Indomethacin (1.3 $\times$ 10^{-6} M) itself did not induce synthesis of TNF-α ($n = 5$). Indomethacin significantly enhanced LPS-induced synthesis of TNF-α (126% of LPS values, $p < 0.05$) but did not modify the histamine (10^{-5} M)-mediated reduction in LPS-induced synthesis of TNF-α (data not shown). Therefore, the histamine-mediated decrease in TNF-α production by LPS-stimulated PBMC does not require generation of prostaglandins. These results also support our observation that triggering of H_1 receptors does not contribute to the suppressive effects of histamine on TNF-α synthesis.
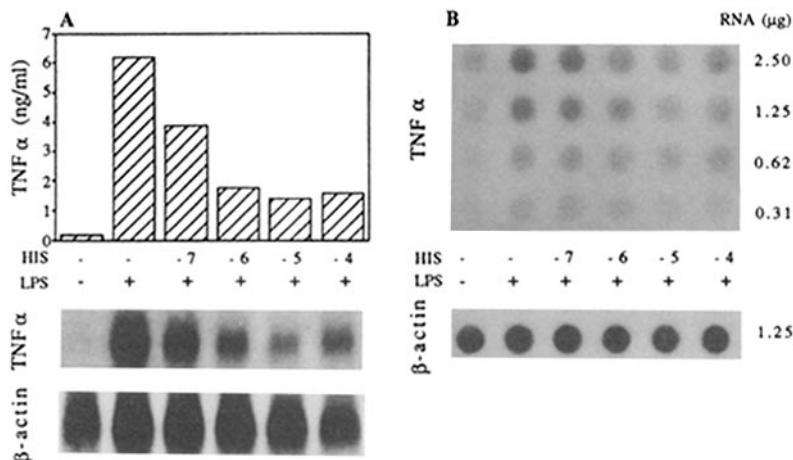

Figure 5. Effect of histamine on the LPS-induced mRNA accumulation for TNF-α. PBMC were stimulated by LPS (10 ng/ml) in the absence (−) or presence of histamine (HIS; 10^{-7} to 10^{-4} M). mRNA levels for TNF-α and β-actin were analyzed 4 h after LPS stimulation by Northern blotting (A) and dilutional analysis (B). (A) Levels of TNF-α protein in the corresponding 4-h PBMC cultures were assayed by RIA (upper panel). Total RNA (10 μg) was subjected to electrophoresis in 6.6% formaldehyde, 1.2% agarose gel, and transferred to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, IL) by capillary blotting (lower panel). Lower and upper panels depict Northern blots and protein levels of one experiment representative of three donors. (B) Serial dilutions of total RNA (2.50, 1.25, 0.62, and 0.31 μg) for an experiment representative of two donors are shown.

Modulation by histamine of mRNA levels and protein concentrations for TNF-α was studied 4 h after LPS stimulation. Histamine itself did not induce mRNA accumulation for TNF-α (data not shown). Histamine (10^{-7} M) slightly reduced LPS-induced synthesis of TNF-α protein, but did not modify LPS-induced mRNA accumulation (Fig. 5). Higher concentrations of histamine (10^{-6} to 10^{-4} M) markedly reduced TNF-α protein synthesis as well as TNF-α mRNA accumulation (Fig. 5 A). Similar results were also observed by dilutional analysis (Fig. 5 B). Histamine (10^{-6} to 10^{-4} M) reduced TNF-α mRNA levels fourfold. The histamine-mediated decrease in TNF-α synthesis is therefore likely to be a transcriptional event, since the activation of histamine H_2 receptors results in generation of cAMP (14), and increased levels of cAMP decrease the LPS-induced accumulation of TNF-α mRNA in the macrophage cell line RAW264, but have no effect on TNF-α mRNA stability

(15). Moreover, PGE_2-induced increase in cAMP levels reduces nuclear transcription of TNF-α in LPS-stimulated macrophages (12).

In these studies, we have demonstrated that histamine modulates gene expression and synthesis of TNF-α by LPS-stimulated PBMC via activation of histamine H_2 receptors. However, inhibitory effects of histamine on TNF-α production are achieved at concentrations comparable to those reached in tissue after mast cell degranulation. Mast cells also synthesize TNF-α in response to stimulation of the FcεRI receptor (16). Therefore, histamine release from mast cells may paradoxically limit the extent of inflammatory and immune reactions by suppressing local cytokine synthesis in H_2 receptor-bearing cells, i.e., mast cells themselves or inflammatory cells in their vicinity, such as synovial cells, chondrocytes, and joint or alveolar macrophages.

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