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

By Edouard Vannier, Laurie C. Miller, and Charles A. Dinarello

From the Departments of Medicine and Pediatrics, Tufts University School of Medicine and New England Medical Center, Boston, Massachusetts 02111

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 H₁ receptor antagonist, but was reversed by cimetidine or ranitidine, H₂ 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 H₂ receptor-bearing cells.

Histamine is released from basophils and mast cells during immediate-type hypersensitivity reactions. Histamine H₁ receptors mediate many of the inflammatory effects of histamine, whereas H₂ 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% CO₂. 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.

**Cytotoxicity 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 an 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

histamine suppresses synthesis of Tumor Necrosis Factor α

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 H₁ receptors for histamine characterized by a KD in the nanomolar range (8). PBMC have also

![Figure 1](https://example.com/figure1.png)  **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 2](https://example.com/figure2.png)  **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.

![Figure 3](https://example.com/figure3.png)  **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 < 0.005). 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.
been shown to express low affinity H₁ receptors (9) as well as H₂ receptors for histamine (10), both characterized by a kD in the micromolar range. PBMC were incubated in the presence of diphenhydramine, an H₁ receptor antagonist, or cimetidine, an H₂ 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 H₁ receptor mediates the suppressive effect of histamine on TNF-α synthesis. In contrast, cimetidine reversed the histamine (10⁻³ M)-mediated decrease in LPS-induced synthesis of TNF-α (Fig. 3). Ranitidine, an H₂ 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 ± 0.1 × 10⁻⁷ M before vs. 1.8 ± 0.3 × 10⁻⁷ M after incubation, p < 0.01, n = 4). Histamine levels in 24-h supernatants of LPS-stimulated PBMC (1.6 ± 0.4 × 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 ± 3.26 [untreated cells] vs. 5.05 ± 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 E₂ suppresses multiple aspects of macrophage activation, including synthesis of TNF-α (12). Furthermore, activation of H₁ 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 × 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 H₁ receptors does not contribute to the suppressive effects of histamine on TNF-α synthesis.
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 observed by dilutional analysis (Fig. 5B). 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 H₂ receptors results in generation of cAMP (14), likely to be a transcriptional event, since the activation of histamine H₂ receptors results in generation of cAMP (14), 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, PGE₂-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 H₂ 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 H₂ receptor-bearing cells, i.e., mast cells themselves or inflammatory cells in their vicinity, such as synovial cells, chondrocytes, and joint or alveolar macrophages.

References

1. Beer, D.J., and R.E. Rocklin. 1987. Histamine modulation of lymphocyte biology: membrane receptors, signal transduction, and functions. Crit. Rev. Immunol. 7:55.
2. Dohlsten, M., H.O. Sjögren, and R. Carlsson. 1987. Histamine acts directly on human T cells to inhibit interleukin-2 and interferon-gamma production. Cell. Immunol. 109:65.
3. Dohlsten, M., T. Kalland, H.O. Sjögren, and R. Carlsson. 1988. Histamine inhibits interleukin-1 production by lipopolysaccharide-stimulated human peripheral blood monocytes. Stand. J. Immunol. 27:527.
4. Beutler, B., and A. Cerami. 1989. The biology of cachectin/TNF: a primary mediator of the host response. Annu. Rev. Immunol. 7:625.
5. Wershil, B.K., Z.S. Wang, J.R. Gordon, and S.J. Galli. 1991. Recruitment of neutrophils during IgG-dependent cutaneous late phase reactions in the mouse is mast cell-dependent. Partial inhibition of the reaction with antiserum against tumor necrosis factor-alpha. J. Clin. Invest. 87:446.
6. Schindler, R., B.D. Clark, and C.A. Dinarello. 1990. Dissociation between interleukin-1β mRNA and protein synthesis in human peripheral blood mononuclear cells. J. Biol. Chem. 265:10232.
7. van der Meert, J.W.M., S. Endres, G. Lonnenmann, J.G. Cannon, T. Ikejima, S. Okusawa, J.A. Gelfand, and C.A. Dinarello. 1988. Concentrations of immunoreactive human tumor necrosis factor alpha produced by human mononuclear cells in vitro. J. Leukocyte Biol. 43:216.
8. Cameron, W., K. Doyle, and R.E. Rocklin. 1986. Histamine type I (H₁) receptor radioligand binding studies on normal T cell subsets, B cells, and monocytes. J. Immunol. 136:2116.
9. Casale, T.B., S.Wescott, D. Rodbard, and M. Kaliner. 1985. Characterization of histamine H₁ receptors on human mononuclear cells. Int. J. Immunopharmac. 7:639.
10. Gespach, C., A. Courillon-Mallet, J.M. Launay, H. Cost, and J.P. Abita. 1986. Histamine H₂ receptor activity and histamine metabolism in human U-937 monocyte-like cells and human peripheral monocytes. Agents Actions. 18:124.
11. Aoi, R., I. Nakashima, Y. Kitamura, H. Asai, and K. Nakano. 1989. Histamine synthesis by mouse T lymphocytes through induced histidine decarboxylase. Immunology. 66:219.
12. Kunkel, S.L., M. Spengler, M.A. May, R. Spengler, J.W. Larrick, and D. Remick. 1988. Prostaglandin E₂ regulates macrophage-derived tumor necrosis factor gene expression. J. Biol. Chem. 263:5380.
13. Juan, H., and W. Sametz. 1980. Histamine-induced release of arachidonic acid and of prostaglandins in the peripheral vascular bed. Arch. Pharmacol. 314:183.
14. Roszkowski, W., M. Plaut, and L. Lichtenstein. 1977. Selective display of histamine receptors on lymphocytes. Science (Wash. DC). 195:683.
15. Taffet, S.M., K.J. Singhel, J.F. Overholtzer, and S.A. Shurtleff. 1989. Regulation of tumor necrosis factor factor expression in a macrophage-like cell line by lipopolysaccharide and cyclic AMP. Cell. Immunol. 120:291.
16. Gordon, J.R., and S.J. Galli. 1990. Mast cells as a source of both preformed and immunologically inducible TNF-α/cachectin. Nature (Lond.). 346:274.