Histamine Inhibits the Production of Interleukin-12 through Interaction with H₂ Receptors

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Abstract

IL-12 is essential for T helper 1 (Th1) development and inhibits the induction of Th2 responses. Atopic diseases, which are characterized by Th2 responses, are associated with the overproduction of histamine. Here we present evidence that histamine, at physiological concentrations, strongly inhibits human IL-12 p40 and p70 mRNA and protein production by human monocytes. The use of specific histamine receptor antagonists reveals that this inhibition is mediated via the H₂ receptor and induction of intracellular cAMP. The inhibition of IL-12 production is independent of IL-10 and IFN-γ. The observation that histamine strongly reduces the production of the Th1-inducing cytokine IL-12 implies a positive feedback mechanism for the development of Th2 responses in atopic patients. (*J. Clin. Invest.* 1998. 102:1866–1873.) Key words: cytokines • allergy and immunology • Th1 cells • monocytes • interferon gamma

Introduction

Two distinct subsets of CD4⁺ T helper (Th)[1] cells with different cytokine profiles and functions have been shown to develop from naive precursors. Th1 cells produce IL-2, IFN-γ, and TNF-β, activate macrophages, and cause delayed type hypersensitivity reactions, whereas Th2 cells produce IL-4, IL-5, IL-10, and IL-13, cause eosinophilia, and induce an immunoglobulin class switch to IgG1 (mouse) or IgG4 (human) and IgE (1–3). Aberrant regulation of Th1 or Th2 responses leads to immunopathology; in patients with HIV infection and allergic patients, T helper cells are believed to have shifted toward

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1. *Abbreviations used in this paper:* β2-M, β2-microglobulin; CRE, cAMP responsive element; DTH, delayed-type hypersensitivity; ICER, inducible cAMP early repressor; PGE₂, prostaglandin E₂; RT, reverse transcriptase; SAC, *Staphylococcus aureus* Cowan I strain; Th1, T helper 1 cell.

a Th2 response (4–6), while various organ-specific autoimmune diseases are characterized by Th1-like responses (7).

Recently, it has become clear that the APC-derived factor IL-12 is instrumental for development of a Th1 response (8). Only the IL-12 p70 heterodimer, consisting of a p40 and p35 chain, encoded by two separate genes, is biologically active (9). No biological function is known for the single p40 chain, which is secreted in excess over the p70 protein. IL-12 induces proliferation and IFN-γ production and enhances cytolytic activity in T cells and NK cells (10, 11). In contrast, in the absence of IL-12, a Th2 response is able to develop (8, 12). Therefore, modulation of IL-12 production during an immune response is crucial for the outcome of disease. The Th1 product IFN-γ provides a positive feedback on Th1 development by its capacity to strongly upregulate the production of IL-12 by monocytes (13–15). Endogenous inhibitors of human IL-12 production are IL-10, IL-4, IL-13, and TGF-β (16, 17) and members of the complement system. Ligation of iC3b with complement receptor 3 and cross-linking of CD46 by C3b or measles virus also inhibits the production of IL-12 (18, 19). We previously stated that cAMP-inducing agents such as prostaglandin E₂ (PGE₂) are strong inhibitors of IL-12 p40 production, while upregulating IL-10 production in LPS-stimulated human monocytes (20). Thereafter, it was reported that β₂ agonists also inhibited human IL-12 production and Th1 development by increasing intracellular cAMP (21).

Histamine is an endogenous mediator of many (patho)physiological processes, such as the regulation of gastric acid secretion and cardiac output, while functioning as a neurotransmitter in the brain (for reviews see references 22 and 23). During allergic reactions, histamine is released in large quantities from intracellular stores from mast cells and basophils after cross-linking of cell surface IgE by allergens. Based on the activity of various selective agonists and antagonists, three types of histamine receptors have been described, designated H₁, H₂, and H₃. Signaling through the H₁ receptor involves the activation of PLC, leading to the generation of 1,4,5-inositol phosphate and DAG, whereas H₂ receptor stimulation results in cAMP generation (22, 23). H₁ and H₂ receptors are expressed on many cell types, including lymphoid cells (24–26). The immune modulating effects of histamine include inhibition of IL-2 and IFN-γ production by T cells and inhibition of TNF-α and IL-1 production by monocytes (27–29). Less is known about the H₃ receptor; its expression is observed in the brain (in addition to H₁ and H₂ receptors), but on lymphoid cells its expression is not well studied. Indirect evidence indicates the presence of H₃ receptors on eosinophils (30). The signaling pathway of this receptor is largely unknown. In the present study, we report that H₂ receptor stimulation inhibits IL-12 production by monocytes. This observation leads to new

concepts on the role of histamine during allergic reactions and T helper development.

Methods

Antibodies and reagents

The anti–IL-12 mAbs, reacting with both IL-12 p40 and p70, C11.79 and C8.6 (31), were kindly provided by Dr. G. Trinchieri (The Wistar Institute, Philadelphia, PA). mAb 20C2, specific for IL-12 p70, was a kind gift from Dr. M.K. Gately (Hoffmann-LaRoche, Nutley, NJ). Anti–IL-10 mAb B-T10 and B-N10 were a kind gift from Dr. J. Wijdenes (Diaclone, Besancon, France). PGE$_2$ was obtained from Sigma Chemical Co. (St. Louis, MO). rhIL-10 was a kind gift from Dr. R. de Waal Malefijt (DNAX, Palo Alto, CA). *Staphylococcus aureus* Cowan I strain (SAC, Pansorbin) was obtained from Calbiochem (La Jolla, CA), histamine dihydrochloride and triprolidine dihydrochloride from Sigma Chemical Co., and ranitidine dihydrochloride from Glaxo (Middlesex, UK). Clobenpropit dihydrobromide and amthamine dihydrobromide were synthesized at the Free University (Department of Pharmacochemistry, Amsterdam, The Netherlands).

Whole blood cultures

Whole blood was obtained by venepuncture from normal healthy donors in sodium-heparin–containing sterile blood collecting tubes (VT-100SH tubes, Venoject; Terumo Europe N.V., Leuven, Belgium). To prevent spontaneous production of cytokines by endotoxin or endotoxin-like substances present in culture media, IMDM was ultrafiltrated by means of a hollow-fiber dialyzer (32) (Hemoflow F5; Fresenius A.G., Bad Homburg, Germany). Whole blood was diluted 1:10 in ultrafiltrated IMDM, supplemented with 0.1% FCS, penicillin (100 IU/ml), streptomycin (100 μg/ml), and 15 IU/ml sodium heparin (Leo Pharmaceutical Products B.V., Weesp, The Netherlands). Diluted whole blood was cultured in triplicate in 200-μl flat-bottomed culture plates (Nunc, Roskilde, Denmark) and stimulated with SAC (0.01% wt/vol). Histamine, agonist, antagonists, or PGE$_2$ were added simultaneously with SAC. Supernatants were harvested after 18–24 h of culture, and cytokine levels were determined.

Assays for cytokines

IL-12 p70 ELISA. Flat-bottomed microtiter plates (Maxisorb; Nunc) were coated overnight with mAb 20C2 (0.5 μg/ml in 0.1 M carbonate buffer, pH 9.6, 100 μl/well). All subsequent incubations were in 100-μl volumes at room temperature. The plates were washed twice with PBS, 0.02% (vol/vol) Tween 20, and incubated for 30 min with PBS containing 2% (vol/vol) cow's milk as a blocking step. After washing, biotinylated purified mAb C8.6 was added (final concentration 0.25 μg/ml) together with IL-12 containing samples diluted in high-performance ELISA buffer (CLB, Amsterdam, The Netherlands) for 1.5 h. Thereafter, the plates were washed five times and incubated with poly-streptavidin-horseradish peroxidase (CLB), 1:10,000 diluted (according to the manufacturer's instructions) in PBS containing 2% (vol/vol) cow's milk for 0.5 h, washed, and developed with a solution of 100 μg/ml of 3,5,3′,5′-tetramethylbenzidine (Merck, Darmstadt, Germany) with 0.003% (vol/vol) H$_2$O$_2$ in 0.11 M sodium acetate, pH 5.5 (100 μl/well). The reaction was stopped by adding an equal volume of 2 M H$_2$SO$_4$ to the wells. Plates were read at 450 nm in a Titer-tek Multiskan reader. Background absorbance at 540 nm was subtracted. rhIL-12 p70, provided by Dr. S.F. Wolf (Genetics Institute Inc., Cambridge, MA), was used as a standard and the detection limit was 0.5 pg/ml (twice the background). This assay does not detect free p40 (provided by Dr. S.F. Wolf, Genetics Institute, Inc.) up to 20 ng/ml.

IL-12 ELISA. IL-12 ELISA detects both p40 and p70 (20) and was performed identical to the p70-specific ELISA with the exception that the coating antibody was replaced by mAb C11.79, which was used at 2 μg/ml. IL-12 containing culture supernatant, standardized to rhIL-12 p70 (provided by Dr. S.F. Wolf, Genetics Institute,

Inc.), was used as a standard. The detection limit was 4 pg/ml (twice the background).

IL-10 ELISA. IL-10 ELISA assay was performed identical to the IL-12 ELISA, except that the blocking step was omitted (20). For coating, mAb B-N10 was used at 0.5 μg/ml in PBS, and for detection, biotinylated mAb B-T10 was used at 0.125 μg/ml. rhIL-10 was used as a standard.

IL-6 ELISA. Procedures were identical to the IL-12 ELISA, except that the blocking step was eliminated and for coating anti–IL-6, mAb CLB.IL6/16 was used at 1 μg/ml in PBS. Affinity-purified biotinylated polyclonal sheep anti–IL-6 was used for detection at 0.25 μg/ml (33). rhIL-6 (34) was used as a standard. The detection limit was 1 pg/ml.

Competitive reverse transcriptase (RT)-PCR

Whole blood (see above) was stimulated with SAC in the absence or presence of 10 ng/ml IFN-γ (Boehringer Mannheim, Almere, The Netherlands). Total cellular RNA was extracted using TRIzol Reagent (GIBCO BRL/Life Technologies, Gaithersburg, MD) according to manufacturer's instructions. After determination of A$_{260}$ and A$_{280}$, 1 μg of RNA was transcribed to cDNA for 1 h at 37°C in 20-μl reaction volumes containing 1× RT buffer, 200 U of Superscript RT (GIBCO BRL), oligo(dT) primer (GIBCO BRL), 0.01 M dithiothreitol, and 0.5 mM dNTP's (Promega, Madison, WI). The reaction was terminated by a 10-min incubation at 95°C.

Cellular cDNA was amplified together with twofold diluted titrations of an internal competitive standard of plasmid IL-12 and β2-microglobulin (β2-M) cDNA, pQB3, a kind gift from D. Shire (Sanofi ELF Bio Recherches, Labège, France) (35). The internal standard binds to the same primer set but yields a PCR product of a different length. PCR reactions were carried out in 1× PCR buffer supplemented with 1.5 mM MgCl$_2$, 0.2 mM dNTP's, 750 nM of each primer; for IL-12 p40: sense primer; ATTGAGGTCATGGTGGATGC, antisense primer; AATGCTGGCATTTTTGCGGC, and β2-M: sense primer: CCAGCAGAGAATGGAAAGTC, antisense primer: GATGCTGCTTACATGTCTCG (GIBCO BRL), and 1.25 U of Taq polymerase (GIBCO BRL) in a final volume of 25 μl. cDNA was denatured for 5 min at 95°C, followed by 35 cycles of amplification: 95°C for 45 s, 59°C for 45 s, and 72°C for 2 min. After the last cycle, the samples were incubated at 72°C for 10 min. The amplified products were separated by agarose gel electrophoresis in the presence of ethidium bromide and visualized by ultraviolet light to identify bands of equal intensity.

Results

Human IL-12 production is strongly reduced by histamine. Various bacterial stimuli induce cytokine production in whole blood cultures with different efficacies; both LPS, heat-killed *Escherichia coli* and formalin-fixed SAC lead to IL-12 p40 production, but only SAC leads to significant IL-12 p70 production (14, 36, 37). Histamine, a mediator tightly connected to the effector phase of atopy, caused a strong reduction of IL-12 p40 and p70 production induced by SAC (Fig. 1). IL-12 p40 production induced by *E. coli* was inhibited to the same extend by histamine (not shown). In 15 donors, concentrations as low as 10^{-8} M histamine led to a mean inhibition of IL-12 p70 production of more than 50% (Fig. 1). In the same cultures, production of IL-10 was approximately twofold enhanced in the presence of 10^{-7} M histamine, whereas IL-6 production was not affected. To rule out the possibility that the effect of histamine on IL-12 is indirect via IL-10, we repeated the experiment in the presence of excess neutralizing antibodies to IL-10. Fig. 2 shows that IL-10 is not involved in the inhibition of IL-12 production by histamine.
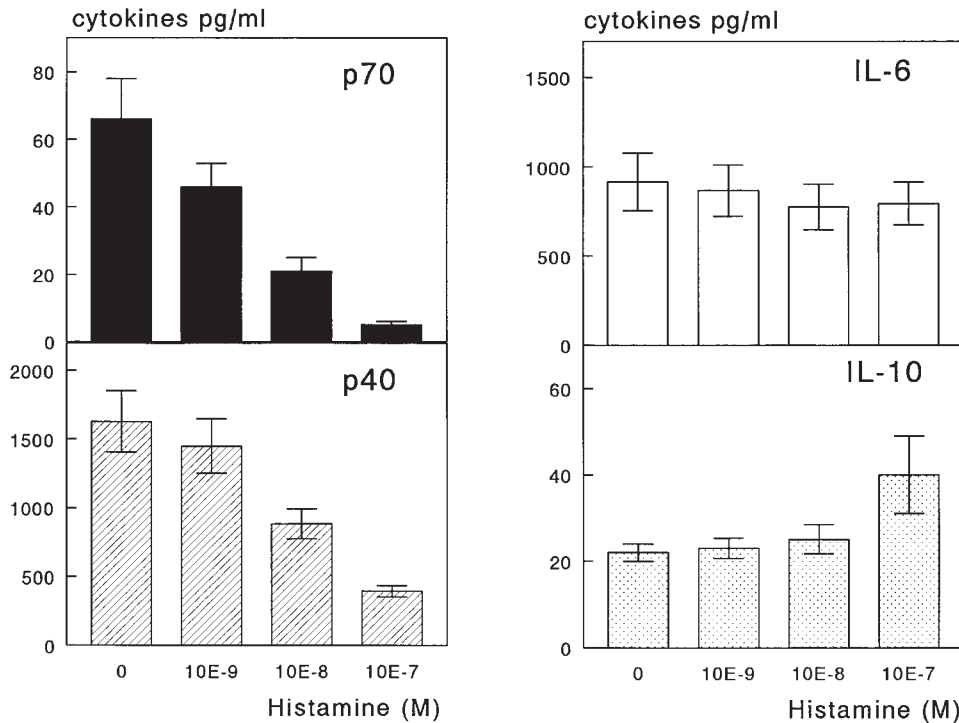
Figure 1. Effects of histamine on IL-12 p40 and p70, IL-6, and IL-10 production. Whole blood (1:10 diluted) was stimulated with SAC in the absence or presence of indicated concentrations of histamine. Results are the mean cytokine production± SE of 15 donors (p40, p70, and IL-6), for IL-10 the mean of six donors± SE is shown.
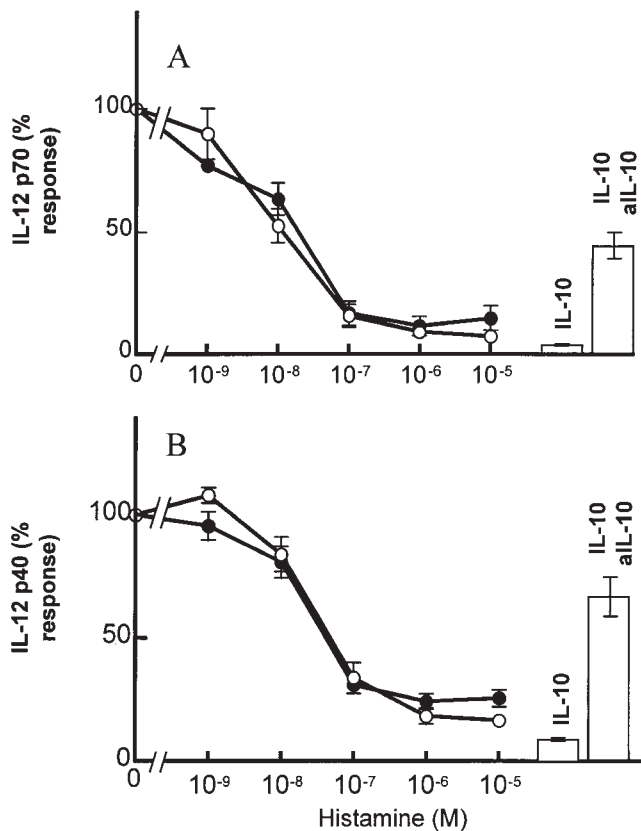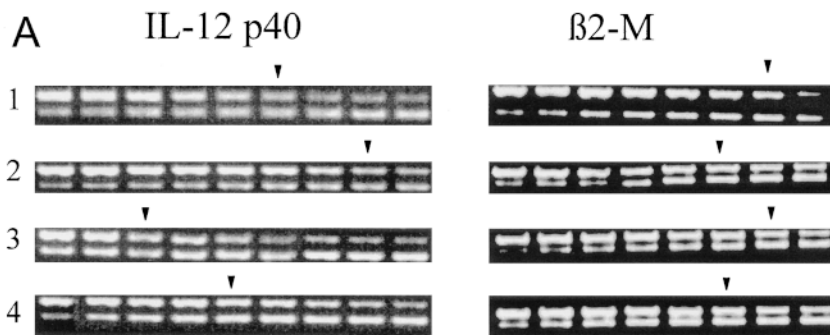

Figure 2. Inhibition of IL-12 p70 (*A*) and p40 (*B*) is independent of IL-10. Whole blood was stimulated with SAC in the absence (○) or presence (●) of histamine and anti–IL-10. Anti–IL-10 mAb BT-10 was used at 5 μg/ml, IL-10 at 600 pg/ml. The results are the mean production (±SE) of four donors. The mean IL-12 p70 and p40 production without addition of histamine or anti–IL-10 were 45 pg/ml and 1045 pg/ml, respectively.

Histamine inhibits IL-12 production at the level of mRNA production. We studied the effect of histamine on IL-12 p40 mRNA production using a competitive RT-PCR. First, we examined the kinetics of IL-12 mRNA production. A 6-h stimulation (in the absence or presence of IFN-γ) turned out to be optimal for IL-12 mRNA production (data not shown). We subsequently analyzed the effect of histamine on the production of IL-12 p40 mRNA after a 6-h stimulation with SAC with or without IFN-γ (Fig. 3). The amount of IL-12 p40 cDNA is expressed relative to the amount of β2-microglobulin cDNA as a measure for the amount of mRNA isolated (Fig. 3 *B*). The production of IL-12 p40 mRNA was reduced to < 10% by histamine, irrespective of the presence of IFN-γ. The reduction of IL-12 p40 mRNA was accompanied by an equal reduction in IL-12 p40 protein production (measured after 24 h of stimulation; Fig. 3 *B*). The fact that this inhibition also occurred in the presence of IFN-γ indicates that the inhibition of histamine is not indirect through an inhibitive effect on IFN-γ production.

Histamine directly affects monocyte IL-12 production. We have shown before that in whole blood, IL-12 production is derived from monocytes (20, 37). It could be argued that the observed inhibition of IL-12 production by histamine in whole blood cultures is indirect through another cell type. Therefore, we examined the effect of histamine on purified monocytes. Because the production of IL-12 is very low in isolated monocytes (36), IFN-γ was added as a costimulator of SAC or LPS. Under these conditions, histamine inhibited the production of IL-12 to a similar extent as was observed in whole blood cultures (data not shown). Therefore, we conclude that histamine targets the monocyte directly.

Histamine-induced inhibition of IL-12 production can be reversed by an H₂ antagonist. Three different receptors, H₁, H₂, and H₃, mediate the biological effects of histamine. We analyzed which receptor was involved in the inhibition of IL-12 production using specific receptor antagonists. Triprolidine,

A IL-12 p40   ß2-M

Figure 3. Inhibition of IL-12 p40 mRNA and protein by histamine. (*A*) Whole blood was stimulated with: lane *1*, SAC; lane *2*, SAC and histamine 10^{-6} M; lane *3*, SAC and IFN-γ; lane *4*, SAC, IFN-γ and histamine 10^{-6} M. RT products of cellular IL-12 p40 and β2-M mRNA were quantified by coamplification with graded amounts of standard cDNA. The upper band represents plasmid cDNA, and the lower band represents cellular cDNA. For β2-M, 0.1 μl of the RT product was used in each PCR reaction. For IL-12, the quantities of RT products were: lane *1*, 0.1 μl; lane *2*, 0.2 μl; lane *3*, 0.05 μl; and lane *4*, 0.1 μl for the same standard cDNA titrations. (*B*) The amount of IL-12 p40 mRNA is expressed relative to the amount of β2-M. The amount of IL-12 p40 protein is measured after 24 h of culture. Representative results of one out of three experiments are shown.

B

	mRNA:		protein:	
	IL-12p40/ B2-M	% prod.	IL-12 p40 pg/ml	% prod.
SAC	0.02	100	1622	100
SAC Hist	0.0013	6	171	10.5
SAC IFNg	0.32	100	6181	100
SAC IFNg Hist	0.02	6	743	12

ranitidine, and clobenpropit selectively compete with the activity of histamine on H_1, H_2, and H_3 receptors, respectively (22). Their antagonistic capacities are expressed as pA_2 values, i.e., the negative logarithm of the concentration required to get 50% reduction of the efficacy of histamine. For triprolidine, a pA_2 value of 9.7 has been found, for ranitidine 7.2, and for clobenpropit 9.9 (22). For all three antagonists, we tested the effect of 100-fold the pA_2 value. Only ranitidine, the H_2 antagonist, reversed the inhibition by histamine of IL-12 p40 as well as p70 production. Neither the H_1 nor the H_3 antagonist had any effect (Fig. 4, *A* and *B*). More detailed analysis of the ranitidine effect (Fig. 4, *C* and *D*) allowed calculation of the pA_2 value for ranitidine in this system. The obtained pA_2 values, 7.2 and 7.3 for p70 and p40, respectively, correspond to the reported histamine-antagonizing potency of ranitidine. In this respect, the H_2 receptor on monocytes functions identically to H_2 receptors on nonlymphoid cells and is responsible for the inhibition of IL-12 production by histamine.

Specific stimulation of histamine H_2 receptors by an H_2 agonist inhibits the production of IL-12. To confirm that the inhibition of IL-12 production was due to specific interaction of histamine with H_2 receptors, we also examined the effect of amthamine, a specific H_2 agonist. Amthamine has been described to be 1.6 times more potent than histamine on H_2 receptors but to have hardly any activity on H_1 and H_3 receptors (22). This was confirmed in our experiments because the IC50 values for amthamine and histamine were 1.8×10^{-8} and 5×10^{-8} for p40 production and 1.4×10^{-8} and 3.6×10^{-8} for p70 production, respectively, in the same donors (Fig. 5). As expected, the inhibition of IL-12 production by amthamine (and histamine) could again be reversed by an excess of the H_2 antagonist ranitidine.

Histamine and PGE_2 have additive effects on the inhibition of IL-12 production. The inhibitive activities of histamine and PGE_2 on IL-12 production were compared and examined for possible synergistic effects (Fig. 6). PGE_2 appeared to be more potent than histamine at 10^{-9} and 10^{-8} M in inhibiting IL-12 production, but at 10^{-7} M there was no difference in activity. The combination of histamine and PGE_2 resulted in a cumulative effect on the inhibition of IL-12 production, without any synergism.

Discussion

The present data indicate that histamine is an important inhibitor of the production of IL-12 by monocytes. Although SAC is an activator of both B cells and monocytes, B cells hardly contribute to the production of IL-12 (38). In whole blood cultures, monocytes are considered the main IL-12– and IL-6–producing cells, because CD14-depleted MNC or granulocytes did not produce IL-12 or IL-6 after stimulation with SAC. In agreement with a direct inhibitive effect of histamine on the production of TNF-α and IL-1 in isolated monocytes (28, 29), we also observed an inhibition of IL-12 production by histamine in isolated monocytes, which could be reversed by an H_2 antagonist (data not shown).

PGE_2 (after interaction with the PGE_2 receptor) and histamine (through interaction with the H_2 receptor) activate adenylate cyclase, which leads to the generation of increased levels of cAMP in various cell types (22, 23). In previous reports, we showed that the effects of PGE_2 on IL-12 p40, TNF-α, and IL-10 production by LPS-stimulated human monocytes in whole blood cultures could all be mimicked by cAMP (20, 36). In agreement with others (39), we observed increased levels of
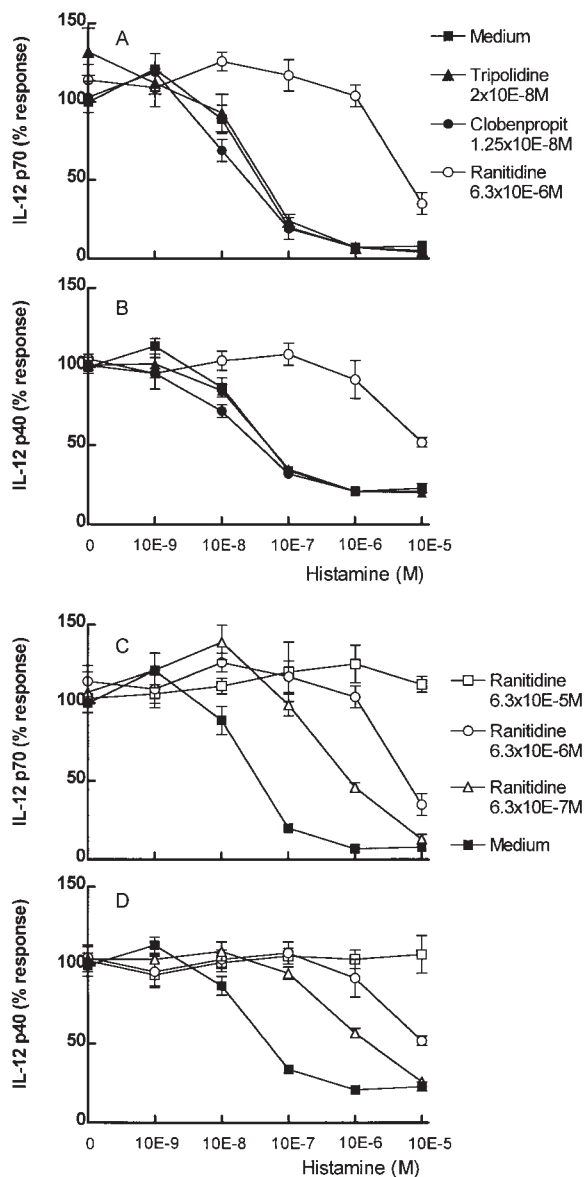
Figure 4. Reversal of histamine-induced inhibition of IL-12 production by a H_2 antagonist. Whole blood (1:10 diluted) was stimulated with SAC and graded concentrations of histamine, in the absence or presence of different histamine antagonists as indicated. (*A* and *B*) Ranitidine (H_2 antagonist, pA_2 7.2), triprolidine (H_1 antagonist, pA_2 = 9.7), and clobenpropit (H_3 antagonist, pA_2 = 9.9) were all tested at 100-fold the pA_2 value. (*C* and *D*) Ranitidine was used at 10-, 100-, and 1,000-fold its pA_2 value. Results are the mean production of four donors±SE. In the absence of histamine or antagonists, mean p70 production was 83 pg/ml, and mean p40 production was 1,696 pg/ml.

Figure 5. Inhibition of IL-12 production by the H_2 agonist amthamine in comparison with histamine. Whole blood (1:10 diluted) was stimulated with SAC and the indicated concentrations of amthamine and histamine. The H_2 antagonist ranitidine (*R*) was used at 10^{-4} M. Results are the mean production of four donors±SE. In the absence of histamine or agonists/antagonists, mean IL-12 p70 and p40 production were 108 pg/ml and 2,023 pg/ml, respectively.

cAMP in purified monocytes after incubation with histamine, which could be prevented by an H_2 antagonist (data not shown). Because both the inhibition of IL-12 production and enhancement of cAMP levels by histamine were mediated through the H_2 receptor and the combined effects of histamine and PGE_2 on IL-12 production were additive, histamine and PGE_2 most likely inhibit IL-12 production by a similar cAMP-dependent mechanism. The exact pathway of cAMP-induced inhibition of IL-12 production is presently unknown. In T cells, a cAMP-inducible transcriptional repressor protein (ICER, in-
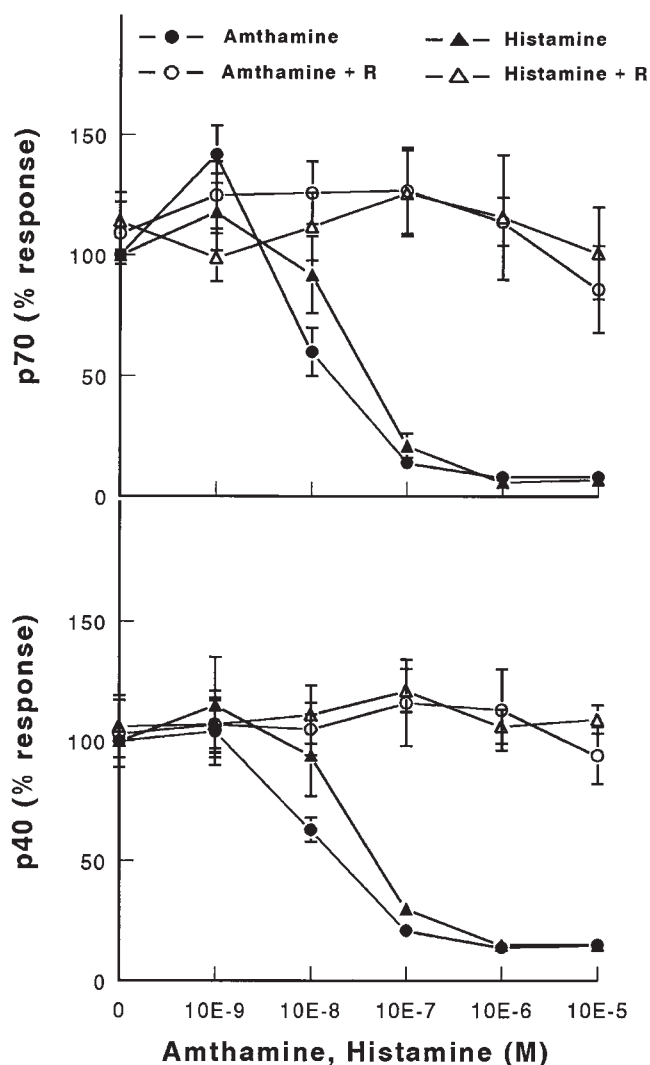
ducible cAMP early repressor) has been described that inhibits IL-2 promoter activity (40). This mechanism probably involves binding of ICER to cAMP responsive elements (CRE), thereby inhibiting transactivating CRE-binding proteins. A similar mechanism may be responsible for the cAMP-induced inhibition of IL-12 production, since the human IL-12 p40 promoter contains a CRE-like motif (15). Another explanation may be reduced activation of NF-κB through cAMP-induced retardation of degradation of the inhibitor of NF-κB, IκBα (41). Murine p40 transcription has been shown to be positively regulated by NF-κB (42). Because the human IL-12 p40 promoter contains a NF-κB site (15), this may also be true for human p40 regulation.

Interestingly, both PGE_2 and histamine are associated with Th2-mediated (allergic) diseases. Histamine is released during allergic reactions after interaction of allergens with cell-bound IgE on basophils and mast cells. PGE_2 is overproduced by
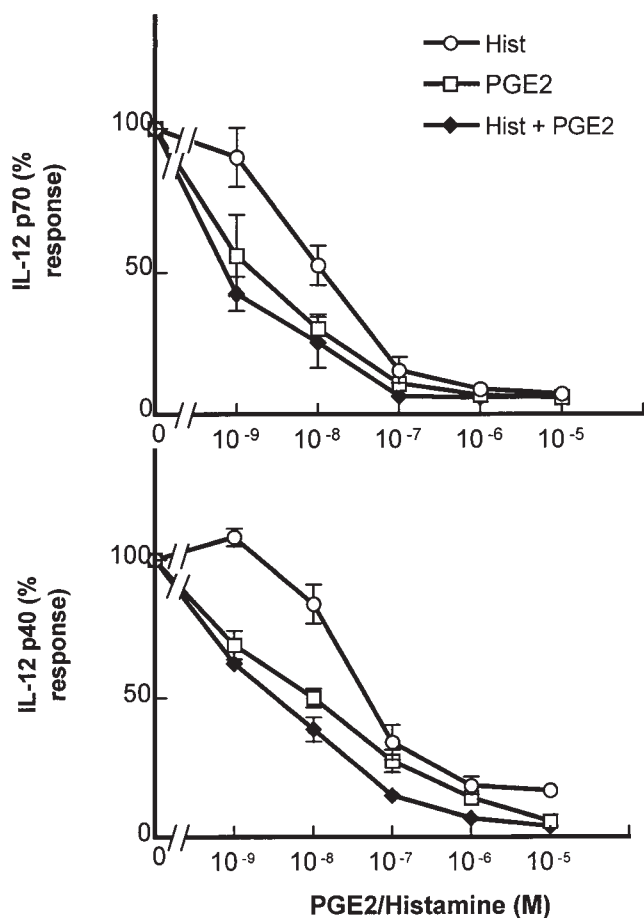
Figure 6. Histamine- and PGE$_2$-induced inhibition of IL-12 production are additive. SAC-stimulated whole blood cultures were incubated with indicated concentrations of histamine and PGE$_2$. Results are the mean production ($\pm$SE) of four donors. Mean IL-12 p70 and p40 production without histamine or PGE$_2$ were 45 pg/ml and 1,045 pg/ml, respectively.

PBMCs from atopic dermatitis and hyper-IgE patients (43, 44). In addition, PBMCs from atopic dermatitis patients show enhanced basal activation of PKA and increased levels of cAMP (45). In allergic asthma patients with enhanced IgE levels, we found a reduced capacity to produce IL-12 p70 in response to SAC (37). The in vivo exposure of monocytes to histamine and PGE$_2$ could have contributed to this. Similar observations were reported on HIV-infected patients. A reduced production of IL-12 and an enhanced production of PGE$_2$ (46–48) by PBMCs from these patients was observed. However, even after stimulation conditions with normal production of PGE$_2$ or after blockage of the production of PGE$_2$, the production of IL-12 is reduced in these patients (49, 50). Because HIV infection is accompanied by increased serum levels of PGE$_2$ (51) and enhanced levels of intracellular cAMP and basal PKA activation (52), the in vivo production of PGE$_2$ by other cell types or other cAMP-inducing agents such as histamine or β-adrenergic agonists may be responsible for the reduced production of IL-12. Although the role of histamine in HIV infection seems far fetched, treatment of AIDS related complex patients with an H$_2$ antagonist has been claimed to yield beneficial effects, including improved T cell proliferation and delayed-type of hypersensitivity (DTH) reactions (53).

Also in mice the suppression of DTH responses caused by histamine can be reversed by H$_2$ antagonists (54, 55). The in vivo role of histamine has also been investigated in allergic rhinitis patients. The systemic or local use of a histamine H$_2$ antagonist decreases the levels of IgE in serum or nasal fluid, respectively (56, 57). Because IgE production can be considered a readout for Th2 responses, while Th1 responses are characterized by DTH responses, histamine is clearly positively influencing Th2 responses in vivo.

Overproduction of histamine and PGE$_2$ can lead to Th2 responses by several mechanisms. Histamine (H$_2$ receptor-mediated) inhibits TNF-α production by monocytes (28), while PGE$_2$ and other cAMP inducers have also been reported to inhibit TNF-α and to enhance IL-10 production by monocytes (20, 58, 59). TNF-α may contribute to Th1 development by potentiating IL-12–induced production of IFN-γ in PBMCs (16). In addition, cAMP, histamine, and PGE$_2$ inhibit secretion of the Th1 cytokines IL-2 and IFN-γ but not the Th2 cytokines IL-4 and IL-5 (27, 60, 61). However, the most important mechanism of induction of Th2 development is probably the inhibition of IL-12 production, because the concentrations of PGE$_2$ and histamine that inhibit the production of IL-12 are approximately 100- and 1000-fold lower, respectively, than those required for inhibition of T cell cytokine production. The levels of histamine that completely inhibit the production of IL-12 are well within the physiological range. Histamine reaches levels as high as 10^{-7} M in serum or nasal washing fluids (62, 63), suggesting that concentrations of histamine in tissue may even be higher. Initial histamine release is dependent on the presence of IgE, induced by Th2 cytokine production. Histamine may provide a positive feedback on continued Th2 differentiation by the inhibition of IL-12. In addition, histamine enhances anti-CD58 plus IL-4– or IL-13–induced IgE production in purified B cells (64). Interestingly, a positive feedback loop of histamine on Th2 differentiation appears to be more evident in allergic patients. Basophils from allergic asthma and atopic dermatitis patients show an increased histamine releasability compared with basophils from normal controls (65–67). This may lead to reduced IL-12 production and Th2 development, and as a result continued overproduction of IgE, a characteristic of these patients.

The observation that the cAMP/PKA pathway (possibly induced by histamine or PGE$_2$) is overactive in a number of pathological conditions such as HIV infection and atopic diseases may lead to new intervention strategies designed to upregulate IL-12 production. The use of inhibitors of PGE$_2$ synthesis and histamine H$_2$ antagonists may be valuable to redirect Th2 to Th0/Th1 responses in these conditions and may be combined with vaccination or hyposensitization therapy.

Acknowledgments

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