Histamine Affects Interleukin-4, Interleukin-5, and Interferon-γ Production by Human T Cell Clones from the Airways and Blood

Frans H. Krouwels, Bernard E. A. Hol, René Lutter, Ben Bruinier, Aalt Bast, Henk M. Jansen, and Theo A. Out

Department of Pulmonology, Clinical and Laboratory Immunology Unit, Academic Medical Center, University of Amsterdam; Department of Pharmacochemistry, Free University; and Laboratory for Experimental and Clinical Immunology, Amsterdam, The Netherlands

High levels of histamine can be found in the airways of asthma patients. This study describes the effects of histamine on anti-CD3-induced production of IL-4, IL-5, and IFN-γ by T cell clones from subjects with allergic asthma and healthy subjects. T cell clones were obtained from bronchoalveolar lavage (BAL) fluid and blood. The number of clones tested, and the percentage of clones in which histamine inhibited or enhanced cytokine production by more than 25%, were as follows: IL-4, 47, 8.5%, and 4.3%; IL-5, 43, 14%, and 30%; and IFN-γ, 52, 40%, and 15%. Inhibition of IL-5 and IFN-γ production was reversed by IL-2. The enhancement of IFN-γ production was associated with an enhancement of both IL-2 production and proliferation. In 21% of the clones a combined effect consisting of inhibition of IFN-γ production and enhancement of IL-5 production was found. This response was reversed by H_2-receptor antagonists and was significantly associated with a histamine-induced increase in intracellular levels of cAMP. The role of cAMP in mediating the histamine effects was supported by the observations that the β_2-agonist salbutamol had effects similar to histamine and that high concentrations of PGE_2 mimicked the inhibitory effects of histamine. Clones from BAL fluid and blood showed similar responses, as did clones from patients with asthma and from control subjects. The enhancement of IFN-γ production by histamine, however, was found only in clones from healthy subjects. The results warrant further investigations on the role of cAMP in the regulation of cytokine production. **Krouwels, F. H., B. E. A. Hol, R. Lutter, B. Bruinier, A. Bast, H. M. Jansen, and T. A. Out. 1998. Histamine affects interleukin-4, interleukin-5, and interferon-γ production by human T cell clones from the airways and blood. Am. J. Respir. Cell Mol. Biol. 18:721–730.**

The airways of patients with allergic asthma show signs of chronic inflammation with the presence of high numbers of activated T lymphocytes (1, 2). Cytokines produced by these T cells are involved in the local inflammation (3–5). The cytokines interleukin-4 (IL-4, which induces IgE synthesis and the expression of adhesion molecules), interferon-γ (IFN-γ, counteracting the effects of IL-4 on IgE synthesis), and IL-5 (promoting eosinophilia) are presumed to play an especially important role. *In vivo*, mRNA for these cytokines is expressed in a high percentage of T cells from the airways of patients, as compared with healthy individuals (4).

Airway-resident T cells are exposed to mediators that are potentially capable of modulating T cell functions. Activated mast cells, present in the airways of patients with allergic asthma, can release large quantities of histamine into the epithelial lining fluid, resulting in levels up to 10^{-4} M (5, 6). T cells are equipped with receptors for histamine (7, 8) and studies on lymphocytes from peripheral blood have shown that T cell functions can be modulated by histamine (9, 10). These effects are attributed to the binding of histamine to H_2-receptors on the T cells, which leads to activation of adenylate cyclase, increased intracellular cAMP (cAMPi) levels, and the subsequent activation of cAMP-dependent protein kinase A (11).

We set out to obtain information on the effects of histamine on the production of T cell cytokines involved in the inflammation as described. We were particularly interested in the effects on human T cell clones from the airways. T lymphocytes in the airways are presumed to repre-

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Address correspondence to: Dr. T. A. Out, B1-236, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 100 DE Amsterdam, The Netherlands. E-mail: t.a.out@amc.uva.nl

Abbreviations: activator protein 1, AP-1; bronchoalveolar lavage, BAL; bronchoalveolar lavage from patients with asthma, A-BAL; bronchoalveolar lavage from controls, C-BAL; intracellular cAMP, cAMPi; nuclear factor of activated T cells, NF-AT; peripheral blood, PB; prostaglandin E_2, PGE_2.

sent a selection of peripheral blood (PB) T cells, recruited to the airways by specific homing (12). They show the phenotype of differentiated and activated cells (13–15). It has been shown that receptors for autacoids are nonrandomly distributed on lymphocytes (16), resulting in variations in the response to histamine within the lymphocyte population (17, 18). By using clones, we studied direct effects of histamine on T cells, that is, effects not influenced by the composition of the cell populations or by interactions between subsets (19). In addition, clones with various cytokine production profiles were selected for analysis.

In an earlier study on the proliferation of T cell clones and IL-2 production, we had found that T cell clones showed various phenotypes with respect to their response to histamine (20). Inhibition by histamine of proliferation, IL-2 production, and IL-2 receptor expression was found in several clones. In others, proliferation and IL-2 production were enhanced or not affected. In addition, the clones responded heterogeneously with respect to elevation of cAMPi levels by histamine.

Powerful cAMP-elevating agents such as prostaglandin E_2 ($PGE)_2$ and forskolin inhibit the production of IL-2 and IFN-γ and enhance IL-5 and, in some cases, IL-4 production (21–24). Histamine may similarly affect cytokine production, which is of particular interest in relation to allergic asthma. However, its potency to enhance cAMPi levels is much lower. Enhancement of IL-5 production by histamine was described in a murine helper T cell type 2 (Th2) T cell clone (25) and in PB T cell lines from patients with atopic asthma (26). It is not known whether this can occur in T cells from the airways, and in normal human T cells. For this reason, we extended our study on airway- and blood-derived T cell clones by investigating the effects of histamine on the production of cytokines presumed to be relevant in allergic asthma: IL-4, IL-5, and IFN-γ. We have compared the effects of histamine with the effects of PGE_2. In addition, the effects of the β_2-agonist salbutamol were analyzed. This drug is frequently used by asthma patients and it is an activator of adenylate cyclase (27).

Materials and Methods

Medium and Cell Culture

In the experiments on cytokine production and proliferation we used Iscove's modified Dulbecco's medium (GIBCO, Paisley, UK) supplemented with complement-inactivated pooled human serum (10% [vol/vol]; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service [CLB], Amsterdam, The Netherlands), 2×10^{-5} M 2-mercaptoethanol (Merck, Munich, Germany), 2 mM sodium pyruvate (Merck), penicillin (100 U/ml; Gist-brocades, Delft, The Netherlands), and streptomycin (100 μg/ml; GIBCO), and stimuli as indicated. For the preparation and propagation of T cell clones the cells were cultured in the same medium containing in addition irradiated peripheral blood mononuclear cells (PBMCs), IL-2 (20 U/ml, either as Lymfocult [Biotest, Dreieich, Germany], or as a supernatant of concanavalin A [ConA]-stimulated PBMCs), and phytohemagglutinin (PHA) (HA-16; Wellcome, Dartford, UK). All cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Histamine, Histamine Antagonists and Agonists, and Other cAMP-Elevating Agents

Histamine was purchased from Sigma (St. Louis, MO). *Histamine antagonists*: Triprolidine (H_1-antagonist) and famotidine (H_2-antagonist) were obtained from Sigma; ranitidine-HCl (H_2-antagonist) came from Glaxo (Middlesex, UK); thioperamide dimaleate (H_3-antagonist) was synthesized in the laboratory of A. Bast. Stock solutions of 10^{-3} M were made in culture medium (triprolidine) or ethanol (other antagonists) and stored at −20°C. In the experiments, a final concentration of 10^{-7} M (triprolidine, thioperamide) or 10^{-6} M (famotidine, ranitidine) in culture medium was used. *Histamine agonists*: 2-Pyridylethylamine (H_1-agonist) came from Janssen Chimica (Beerse, Belgium); impromidine (H_2-agonist) was obtained from Smith Kline & French Laboratories (Welwyn, Hertfordshire, UK); the H_3-agonist (*R*)-α-methylhistamine came from Research Biochemistry International (RBI, Natick, MA). The histamine agonists were used at a final concentration of 10^{-7} M. Salbutamol came from Glaxo and PGE_2 from Sigma.

Preparation of T Lymphocyte Clones

T cell clones were prepared from the bronchoalveolar lavage (BAL) fluid and peripheral blood of three healthy subjects and three patients with allergic asthma (28). Briefly, the macrophages were removed from the BAL cells either by centrifugation through a density gradient or by adherence to culture flasks. Peripheral blood mononuclear cells were isolated on a Ficoll density gradient. Lymphocytes were cloned by limiting dilution, seeding the cells at a density of 0.3 cell/well. In some cases CD2-positive T cells were directly isolated from the BAL with a fluorescence activated cell sorter, and seeded at one cell/well with an automated cell deposition unit. The cells were cultured as described previously.

Four groups of clones were analyzed; clones from BAL fluid (A-BAL) and peripheral blood (A-PB) from patients with allergic asthma ($n = 10$ and $n = 9$, respectively), and from healthy control subjects (C-BAL, $n = 15$ and C-PB, $n = 20$). Clones in each group originated from at least three individuals. The clones used in this study were randomly obtained from a large panel of clones. The criteria were as follows: viable cells, sufficient growth within 1 wk to obtain the number of cells to perform experiments in fivefold incubations with controls and at least two histamine concentrations, and production of at least one of the cytokines IL-4, IL-5, and IFN-γ when stimulated with coated anti-CD3. All clones tested were $CD4^+CD45RO^+$ $CD45RA^-$; they all expressed CD25, HLA-DR, VLA-4, and VLA-5, although to variable extents.

Study Design

$CD4^+$ clones with a Th0, Th1, or Th2-like cytokine production profile as previously assessed (29) were selected. The clones were thawed and expanded (30). Seven days later, the cells were collected, washed, and seeded at 1.0×10^6 cells/ml in anti-CD3-coated wells (CLBT3/3, coating concentration, 0.5 μg/ml, provided by R. A. W. van Lier,

CLB). Incubations were performed in quintuplicate, in 96-well round-bottom plates (Costar, Cambridge, MA). Histamine, prostaglandin, or salbutamol was added together with the stimulus unless stated otherwise. Supernatants were harvested and pooled after the indicated time and stored at −20°C. The proliferative response was assessed by measuring the incorporation of [^{3}H]thymidine 3 d after initiation of the stimulation.

In the histamine receptor-blocking experiments, clones were incubated with H_1-, H_2-, or H_3-receptor antagonists for 30 min at 37°C. Subsequently the cells were stimulated with anti-CD3 in the presence of the antagonists and histamine. Histamine agonists were added to the cells directly after they had been seeded in the anti-CD3-coated wells.

The study was approved by the internal review boards of the hospitals and informed consent was given by the subjects participating in the study.

Cytokine Assays

Cytokine concentrations were measured by ELISA. IFN-γ was measured using monoclonal antibody (MAb) MD2 as a capture antibody and biotinylated MAb MD1 as a detecting antibody (30). IL-5 was measured using MAb TRFK-5 anti-IL-5 and biotinylated MAb 7 anti-IL-5 (31). In both assays the plates were subsequently treated with streptavidin poly-HRP (1:10,000 [vol/vol]; CLB) in phosphate-buffered saline (PBS) containing 2% (vol/vol) cow's milk, and tetramethylbenzidine (Sigma) and H_2O_2. IL-4 was measured by ELISA (Pelikine, CLB). Lower limits of detection: IFN-γ, 50 pg/ml; IL-5, 250 pg/ml; IL-4, 2 pg/ml. The interassay coefficients of variation of the ELISAs over the assay range used were below 10%. Recombinant human IL-4 (rhIL-4) and rhIL-5 were obtained from Genzyme (Boston, MA).

IL-2 was determined using the CTLL-2 bioassay (32). CTLL-2 cells were cultured in the presence of rhIL-2 (Cetus, Emeryville, CA) containing medium or the supernatants to be tested. After 24 h, cells were pulsed with [^{3}H]thymidine (0.2 μCi/well; NEN-Du Pont, Boston, MA) and cultured for an additional 15 h. Cells were then harvested and incorporated radioactivity was determined. All conditions were performed in quintuplicate, and expressed as mean and SD. The incorporation of [^{3}H]thymidine in the CTLL-2 cells was not affected by histamine at concentrations of 10^{-7} to 10^{-3} M.

Assays for Cell Proliferation

Proliferation assays were performed in parallel to the induction of cytokine production. Cells were incubated at 1×10^6 cells/ml in anti-CD3-coated wells for 72 h. During the last 4 h, the cells were exposed to [^{3}H]thymidine. The cells were then harvested and treated as described for the CTLL-2 assay.

Measurement of Intracellular cAMP

The measurement of cAMPi in the T cell clones was performed as described earlier (20). Briefly, cells were incubated at 37°C in PBS containing 0.4% (wt/vol) human albumin (CLB) and 0.5 mM 3-isobutyl-1-methyl-xanthine (Sigma). After 10 min, histamine (10^{-3} M) was added. Five minutes later the reaction was stopped. The cAMP in the supernatants was determined with the Rianen RIA assay (Du Pont, Wilmington, DE). We considered cells responsive to histamine when the amount of cAMPi increased by more than 30% (20). Forskolin and PGE_2 (both from Sigma) were used as a positive control for enhancement of cAMPi.

Statistical Analysis

Correlation between two variables was calculated using the Spearman rank correlation test. Linear regression analysis was applied to the response per dose of histamine. Differences between unpaired groups were analysed with the Mann–Whitney U test. Paired data were compared using the Wilcoxon matched pair signed rank test, and the Friedman test. The Fisher 2×2 exact test was used to analyze the distribution of responses between groups of clones. Probability values less than 0.05 were considered statistically significant.

Results

Characteristics of Cytokine Production and Histamine-Induced Changes

Figure 1 shows the time course of the production of IFN-γ, IL-4, and IL-5, and the effects of histamine for three Th0 clones derived from BAL fluid of healthy subjects. Maximal levels were obtained for IL-4 after 24 h and for IL-5 and IFN-γ after 45 h. These time points were used to evaluate cytokine production. Parallel experiments showed that the coefficients of variation in the cytokine production were below 10% (illustrated for clone 1 in Figure 1). For this reason, we considered a histamine-induced change of more than 25% significant in our further analysis.

In Figure 1 two types of response to histamine are illustrated: inhibition (clone 1) or enhancement (clone 2) of IFN-γ production; and inhibition (1 and 2) or enhancement (clone 3) of IL-5 production. In most cases, the production of IL-4 was hardly affected by histamine. The effects of histamine were dose dependent in the range of 10^{-7} to 10^{-3} M histamine, although at 10^{-7} M the effects were generally below 25% (not shown). After 72 h of culture the cytokine levels produced with histamine present were often close to control values. There was no induction of cytokine production when the cells were incubated with histamine in the absence of anti-CD3 stimulation.

The response of each clone to histamine remained stable for several weeks. However, when the clones were repeatedly frozen and restimulated, or when they were cultured continuously under standard conditions for more than 3 wk, the addition of histamine no longer affected the anti-CD3-induced cytokine production. In several experiments, we treated the T cell clones to obtain a low degree of activation by washing the cells 3 d before the experiment, and then incubating the cells in medium without IL-2 and PHA until the initiation of stimulation with anti-CD3 (33). Under these conditions, histamine had only a small effect on cytokine production or no effect at all.

When the cells were preincubated with histamine (10^{-5} or 10^{-3} M) for 1 h before they were stimulated with anti-CD3, the response was similar to that when histamine was added together with the stimulus. Also, when histamine was added 1 h after stimulation with anti-CD3 a similar re-

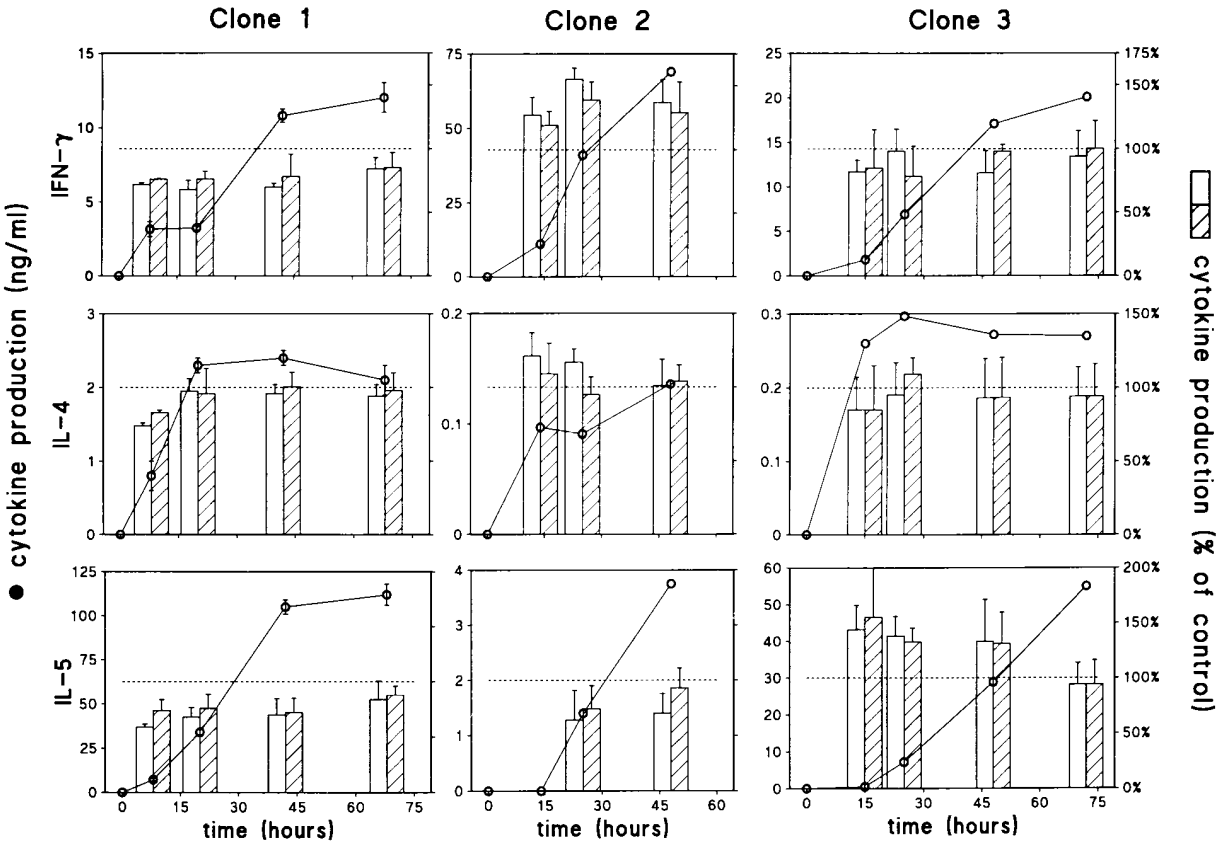

Figure 1. Time course of cytokine production by three $CD4^+$ T cell clones. Cells were stimulated with anti-CD3 and supernatants were collected for cytokine assays at the indicated time points. The *line graphs* represent the cytokine production (in ng/ml) at the various time points without histamine added, obtained from quintuplicate incubations. The *error bars* in the line graph for clone 1 indicate the range of cytokine production in a duplicate experiment that was run in parallel. This demonstrates the close agreement when two experiments are run under identical conditions. The *open* and *hatched bars* represent the mean and SD of the cytokine production in the presence of histamine (*open bars*, 10^{-3} M; *hatched bars*, 10^{-5} M histamine; $n = 3$) expressed as percentages of the control incubations performed on the same occasion during the same time interval and with the same batches of clonal cells. Here the *error bars* represent 1 standard deviation based on three experiments performed on three different occasions. The *dotted line* indicates a production of 100% (i.e., the results of the *line graph*). Histamine dose-dependent effects analyzed for the 24- and 45-h time points (linear regression analysis: slope relating the amounts of cytokine produced with the concentrations of histamine, statistically significant different from zero): clone 1, IFN-γ ($P < 0.03$) and IL-5 ($P < 0.02$); clone 2, IFN-γ ($P < 0.03$) and IL-5 ($P < 0.04$); clone 3, IL-5 ($P < 0.03$). Additional analysis for histamine-induced effects (Friedman test): clone 1, IL-4 ($P < 0.01$).

sponse was found. After a preincubation period of 24 or 48 h with histamine the effect of histamine was slightly greater. However, the variability in cytokine production also increased by this procedure. Thus, for further analysis we decided to study the effects of histamine in experiments where histamine was added together with the stimulus.

The Effect of Histamine on Clones from BAL Fluid and Blood from Subjects with Asthma and from Healthy Subjects

The effect of 10^{-5} M histamine on panels of T cell clones derived from the BAL fluid of patients with allergic asthma (A-BAL) and healthy controls (C-BAL) and from the blood of patients and controls (A-PB and C-PB) is shown in Figure 2. None of the clones had been frozen and thawed more than twice. All clones were tested 1 wk after thawing and restimulation.

Inhibition of the production of IFN-γ was found in 21 of the 52 clones (40%); these were found in all 4 groups (A-BAL, A-PB, C-BAL, and C-PB). The strongest inhibition was found in clones producing low amounts of IFN-γ; the amount of IFN-γ produced after anti-CD3 stimulation showed an inverse correlation with the percentage of inhibition of IFN-γ production (Spearman rho = −0.63; $P < 0.01$). Enhancement of IFN-γ production (8 clones) was found only in clones from healthy subjects. The production of IFN-γ was not affected by histamine in 23 of the 52 clones (44%).

The production of IL-4 was not significantly affected by histamine in 41 of the 47 clones (87%). In four clones, histamine inhibited IL-4 production (median IL-4 production 63% of the control) and in two clones, histamine enhanced IL-4 production (136 and 143% of the control). The latter clones were low IL-4 producers (163 and 104 pg/ml, respectively). Their IL-5 production was also enhanced by

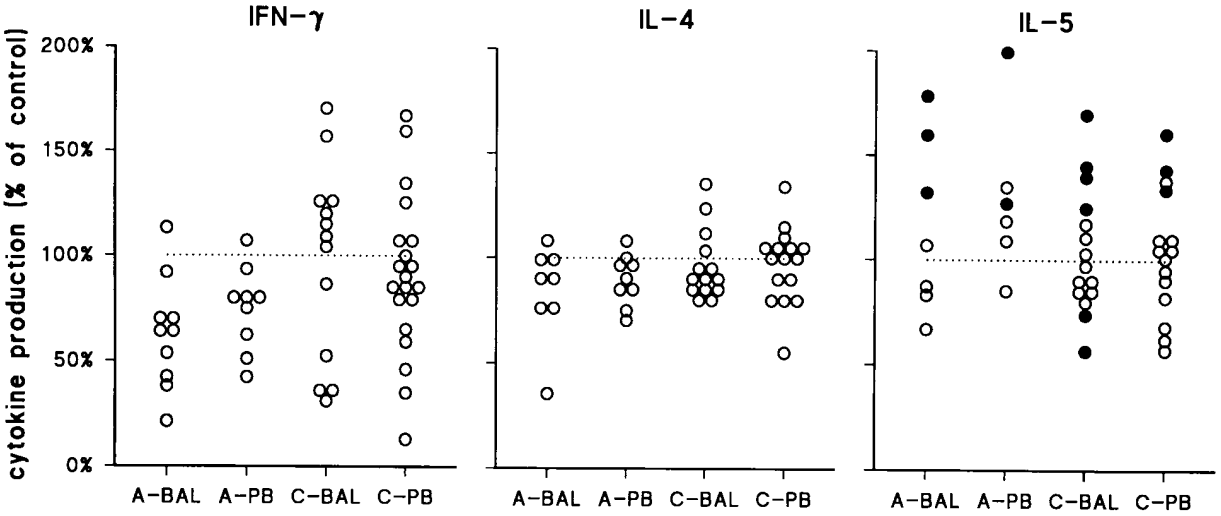

Figure 2. The effect of histamine on the production of IFN-γ, IL-4, and IL-5 by T cell clones. The concentration of the cytokine in the supernatant of cells stimulated with anti-CD3 in the presence of histamine (10^{-5} M) was expressed as a percentage of the control experiment without histamine. T cell clones were derived from the following: patients with asthma, bronchoalveolar lavage (A-BAL; n = 10, 8, and 7 for IFN-γ, IL-4, and IL-5, respectively); patients with asthma, peripheral blood (A-PB; n = 9, 9, and 6); healthy subjects, peripheral blood (C-PB; n = 20, 16, and 15); healthy subjects, bronchoalveolar lavage (C-BAL; n = 13, 12, and 14). For several clones the production of only one or two cytokines could be measured owing to the small volume of the supernatant. The *filled symbols* represent T cell clones that produced less than 3 ng/ml IL-5.

histamine. Their IFN-γ production was inhibited by histamine.

IL-5 production in response to histamine showed no difference between clones from the four groups. Enhancement of IL-5 production by histamine was found in 13 clones. Eleven of these belonged to the clones that produced relatively low amounts of IL-5 (< 3 ng/ml; Figure 2, *filled symbols*). Ten clones produced > 50 ng/ml IL-5. In three of these histamine inhibited, and in one it enhanced, IL-5 production. Only two clones of the 29 that produced < 3 ng/ml IL-5 showed histamine-enhanced IL-5 production. Thus, the response of low-IL-5 producers differed significantly from that of high-IL-5 producers (Fisher's exact test, $P < 0.0005$). Inhibition of IL-5 production by histamine was found in six clones.

The inhibition and stimulation of cytokine production by histamine were not restricted to one individual subject, or to one particular group of clones (asthma, healthy, BAL fluid, PB), with the exception that we did not observe the stimulation of IFN-γ production in clones from patients with asthma. There was no relation between Th0, Th1, or Th2 cytokine production profile and histamine effects.

The proliferation of the cells was assessed in parallel in 35 clones (not shown). Inhibition of proliferation by histamine was found in 10 clones (29%), and occurred in all four groups. The proliferation increased by more than 25% in one clone, and by 11% in two clones. These results are similar to our previous findings (20). No relation was found between histamine effects on proliferation or IL-2 production and the basal expression of the surface markers CD25, HLA-DR, VLA-4, and VLA-5.

Modulation of cytokine production by histamine may be independently regulated for each cytokine, but it may also be the result of a common mechanism. In the latter case a concerted modulation of cytokine production by histamine would be expected. We found that enhancement of IL-5 production by histamine was significantly related to inhibition of IFN-γ production: eight of 12 clones with enhanced IL-5 production showed inhibited IFN-γ production, whereas in 27 clones in which histamine had no effect or inhibited IL-5 production only eight clones showed inhibited IFN-γ production by histamine (Fisher's 2×2 exact test, $P = 0.035$). In 39 clones the response of all three cytokines could be analyzed; the main types of response were as follows: (*1*) no histamine-induced change in the production of either IFN-γ, IL-4, or IL-5: 26% of the clones; (*2*) inhibition of IFN-γ production, no change in IL-4 production, and enhancement of IL-5 production: 21% of the clones; (*3*) inhibition of IFN-γ production with no effect on either IL-4 or IL-5 production: 18% of the clones.

Relation with IL-2 Production

Histamine-induced inhibition of IFN-γ production may be due to inhibition of IL-2 production (10). In addition, IL-2 is required both for IL-4 and IL-5 production (34, 35) and for enhanced production of IL-4 and IL-5 by PGE_2 (36). We therefore measured the IL-2 concentration in the supernatants collected at 24 h and related this to the effects of histamine on the production of IFN-γ, IL-4, and IL-5.

In supernatants from 21 of the 33 clones tested, IL-2 was detected. The IL-2 levels were not correlated with levels of IFN-γ, IL-4, or IL-5, irrespective of the presence of histamine. Five of the six clones with inhibited IL-5 production did not produce detectable IL-2. No other restrictions in histamine effects on cytokine production were ob-

served, either in the non-IL-2-producing clones or in the clones that did produce IL-2.

The effect of histamine on IL-2 production was studied in 10 clones that produced more than 0.1 U/ml of IL-2. In seven clones, a dose-dependent inhibition was found with significant inhibition at 10^{-3} M only (IL-2 production in the presence of histamine: 20 to 74.5% of the control). This inhibition of IL-2 production was not significantly correlated with the effect of histamine on proliferation, or on the production of IFN-γ, IL-4, or IL-5. A dose-dependent enhancement of IL-2 production was repeatedly found in two clones: at 10^{-5} M 108 and 111%, and at 10^{-3} M 113 and 165% of the controls. In these clones, the production of IFN-γ and proliferation were also enhanced by histamine.

By measuring the IL-2 concentration in the supernatants, we may have missed the IL-2 consumed by binding to the IL-2 receptors. Therefore, we tested the effect of adding exogenous IL-2 (Table 1). Whereas rhIL-2 (90 U/ml) alone did not induce any detectable production of cytokines (not shown), it slightly enhanced the anti-CD3-induced cytokine production. The addition of rhIL-2 reversed the inhibition of IFN-γ and IL-5 production by histamine. This effect was independent of the cytokine production level without added IL-2, illustrated with the use of three different clones. In the presence of histamine plus IL-2 the production of IL-5 was even higher than without these additions. The addition of histamine and/or rhIL-2 did not significantly affect the production of IL-4 in these clones (not shown).

Effect of Histamine Antagonists

Enhancement of IL-5 production and inhibition of IFN-γ and IL-2 production may be the result of elevation of cAMPi (21–26). As the binding of histamine to H_2-receptors results in an increase in cAMP, we analyzed whether the effects of histamine on cytokine production could be mimicked and reversed by type-specific receptor agonists and antagonists, respectively. For our analysis, we selected

TABLE 1

*Addition of rhIL-2 to counteract inhibition of IFN-γ and IL-5 production by histamine**

	IFN-γ (*ng/ml*) Histamine		IL-5 (*ng/ml*) Histamine	
	(–)	(+)	(–)	(+)
Clone I				
Anti-CD3	74.9	54.7	9.0	6.1
Anti-CD3 + IL-2	77.4	77.0	11.7	10.7
Clone II				
Anti-CD3	10.8	8.0	25.2	18.2
Anti-CD3 + IL-2	11.8	10.9	35.0	32.7
Clone III				
Anti-CD3	0.40	0.26	6.8	5.0
Anti-CD3 + IL-2	0.45	0.44	7.2	8.1

* Cytokine concentrations in the supernatants from three different clones collected at 45 h after stimulation with anti-CD3 with added rhIL-2 (final concentration, 90 U/ml), either without (–) or with (+) the presence of 10^{-5} M histamine. For clones I and II, a representative experiment of two is shown. At 10^{-3} M histamine essentially the same results were obtained for all clones.

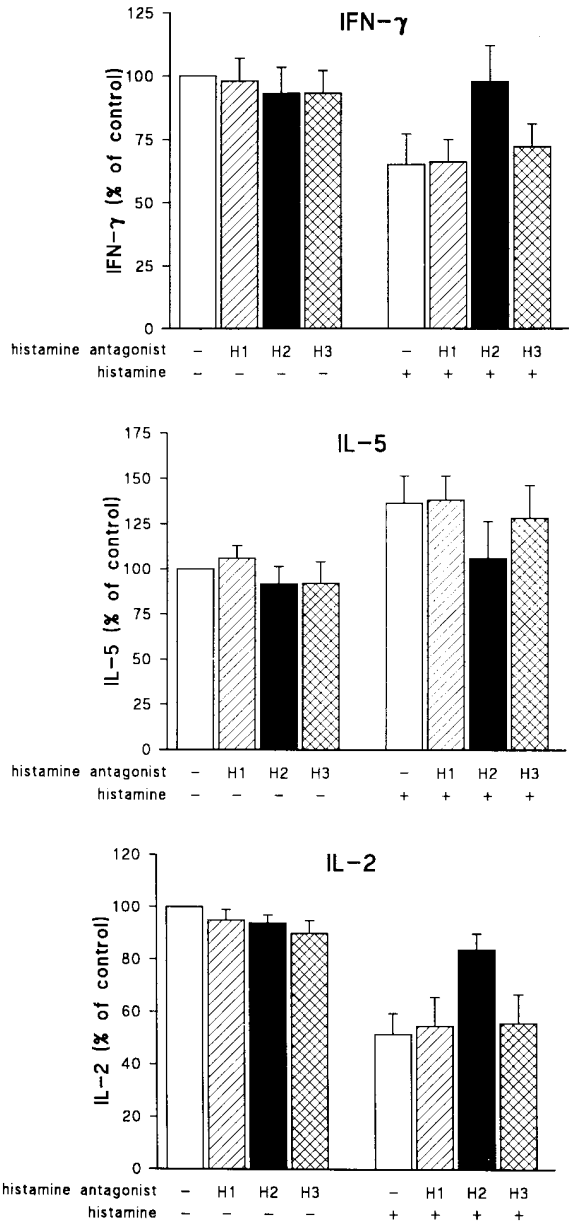

Figure 3. The involvement of H_1-, H_2-, and H_3-receptors in histamine-induced modulation of cytokine production. The cytokine production induced by anti-CD3 without any additions was set to 100%. Histamine was used at a concentration of 10^{-5} M. *Open bars:* Histamine without antagonists. *Hatched bars:* histamine (10^{-5} M) plus 10^{-5} M triprolidine (H_1-antagonist). *Solid bars:* histamine (10^{-5} M) plus 10^{-5} M ranitidine (H_2-antagonist). *Cross-hatched bars:* histamine (10^{-5} M) plus 10^{-5} M thioperamide (H_3-antagonist). Bars represent mean and 1 SD of three (IFN-γ and IL-2) or two (IL-5) experiments. At 10^{-3} M histamine essentially the same results were obtained. IFN-γ and IL-2: significant inhibition by histamine, $P < 0.03$; and in the presence of antagonist not significantly different from control without histamine. IL-5: significant stimulation by histamine, $P < 0.02$; and in the presence of antagonist not significantly different from control without histamine.

a clone showing inhibited production of IFN-γ and IL-2, and enhancement of IL-5 production by histamine. In addition, two clones were selected with inhibited IFN-γ production only, two with inhibited IL-2 production only, and one with enhanced IL-5 production only. IL-4 production by these clones was not affected by histamine.

The cells were preincubated with H_1-, H_2-, or H_3-antagonists and stimulated with anti-CD3 in the presence of the antagonists. In the absence of histamine, the antagonists did not significantly affect the cytokine production induced by anti-CD3 (Figure 3). Histamine-induced inhibition of the production of IFN-γ was blocked by the H_2-antagonists ranitidine (10^{-5} M) (and famotidine, 10^{-5} M, not shown). The enhancement of IL-5 production by histamine was strongly reduced by the H_2-antagonist ranitidine (10^{-5} M) (and by famotidine, not shown). Also, the inhibition of IL-2 production was largely reversed by ranitidine. The H_1-antagonist triprolidine and the H_3-antagonist thioperamide did not affect cytokine production. Histamine agonists and in particular the H_2-agonist impromidine, however, did not mimic the effects of histamine.

cAMP Responses

We demonstrated earlier that histamine has a heterogeneous effect on T cell clones with respect to cAMPi levels. In randomly chosen clones, we measured cAMPi in response to histamine. We subdivided the clones into those showing an increase in cAMPi by a factor 1.3 or more (20) on addition of histamine and those with a lower cAMPi increase (Table 2). In the first group, histamine inhibited IFN-γ and enhanced IL-5 production, which was significantly different from the effects of histamine in the latter.

PGE_2 induces much higher cAMPi than histamine (20). Therefore, clones with various types of response to histamine were also analyzed for their response to PGE_2. PGE_2 (10^{-6} M) inhibited IFN-γ production in each of 12 clones tested (median IFN-γ production, 42% of the control; range, 11–65%), including five clones in which histamine had no effect on IFN-γ production and two clones in which histamine enhanced IFN-γ production. However, in the latter two clones PGE_2 at 10^{-8} M increased IFN-γ production (109 and 170% of control). The effect of PGE_2 on IL-5 production was tested in six clones. PGE_2 inhibited IL-5 production (median, 35%; range, 12–40%) by the clones in which histamine had no effect or inhibited IL-5 production. In two clones in which histamine enhanced IL-5 production, PGE_2 also enhanced the production of IL-5 (to 160 and 186% of control). These clones produced high amounts of IL-2 (51 and 15 U/ml). The production of IL-4 was either inhibited by PGE_2 ($n = 8$; median IL-4 production, 34% of control; range, 22–67%), or not affected ($n = 3$; range, 76–100%; all three were high IL-2-producing clones).

Effect of Salbutamol

The β_2-agonist salbutamol is a bronchodilator frequently used by patients with asthma. Similar to histamine, β-agonists can enhance cAMPi in T cells by activating adenylate cyclase (17, 37). We therefore compared the effects of salbutamol with those of histamine (Figure 4), in T cell clones selected for a range of histamine-induced effects.

In all clones, salbutamol and histamine affected cytokine production and proliferation in a similar way. At 10^{-6} M salbutamol the quantitative response of the modulation of cytokine production by salbutamol was near that induced by 10^{-5} M histamine. This was shown by a close correlation between the responses to both agents: IFN-γ, $n = 12$, rho = 0.96, $P < 0.0001$; IL-4, $n = 8$, rho = 0.74, $P < 0.025$; IL-5, $n = 16$, rho = 0.77, $P < 0.005$; proliferation, $n = 15$, rho = 0.72, $P < 0.005$. When clones were exposed to both agents concomitantly no additive effects were found.

Discussion

In the present study, we investigated whether physiological concentrations of histamine can affect anti-CD3-induced production of IFN-γ, IL-4, and IL-5 in panels of $CD4^+$ T cell clones from the airways and blood of patients with allergic asthma and healthy individuals. In addition, we related the modulation of production of those cytokines by histamine with histamine effects on IL-2 production and on cAMPi levels. We also compared histamine effects with those of PGE_2 and the β_2-agonist salbutamol.

Histamine affected the production of IFN-γ and IL-5 in a dose-dependent fashion in the range from 10^{-7} to 10^{-3} M. The production of IL-4 was not affected by histamine in most clones tested. The effects of histamine on IFN-γ and IL-5 were not uniform despite identical procedures in the generation of the clones and their pretreatment: we found inhibition, no effect, and enhancement of production. The main patterns of response to histamine were as follows: (*1*) no change in the production of either IFN-γ, IL-4, or IL-5: 26% of the clones; (*2*) inhibition of IFN-γ and enhancement of IL-5 production: 21% of the clones; (*3*) only inhibition of IFN-γ production: 18% of the clones. These heterogeneous responses were found in clones from the BAL fluid and blood both from patients with allergic asthma and from healthy subjects. However, there was one exception. The enhancement of IFN-γ production by histamine

TABLE 2

*Relation between histamine effects on intracellular cAMP and cytokine production**

	IFN-γ	IL-5
Rise in cAMPi < 1.3	98.8%† ($n = 6$, 73–119%)	81.0% ($n = 4$, 65–90%)
Rise in cAMPi > 1.3	48.3% ($n = 5$, 22–86%)‡	128.1 ($n = 3$, 126–132.3%)‡

* The effect of histamine (10^{-5} M) on the cytokine production by clones showing no change in cAMPi and clones showing increased cAMPi levels after incubation with histamine. In the latter group, histamine inhibited the production of IFN-γ and enhanced the production of IL-5.

† Cytokine production in the presence of histamine as percentage of control.

‡ Significant difference between low- and high-cAMP responding clones ($P < 0.05$, Mann–Whitney U test).

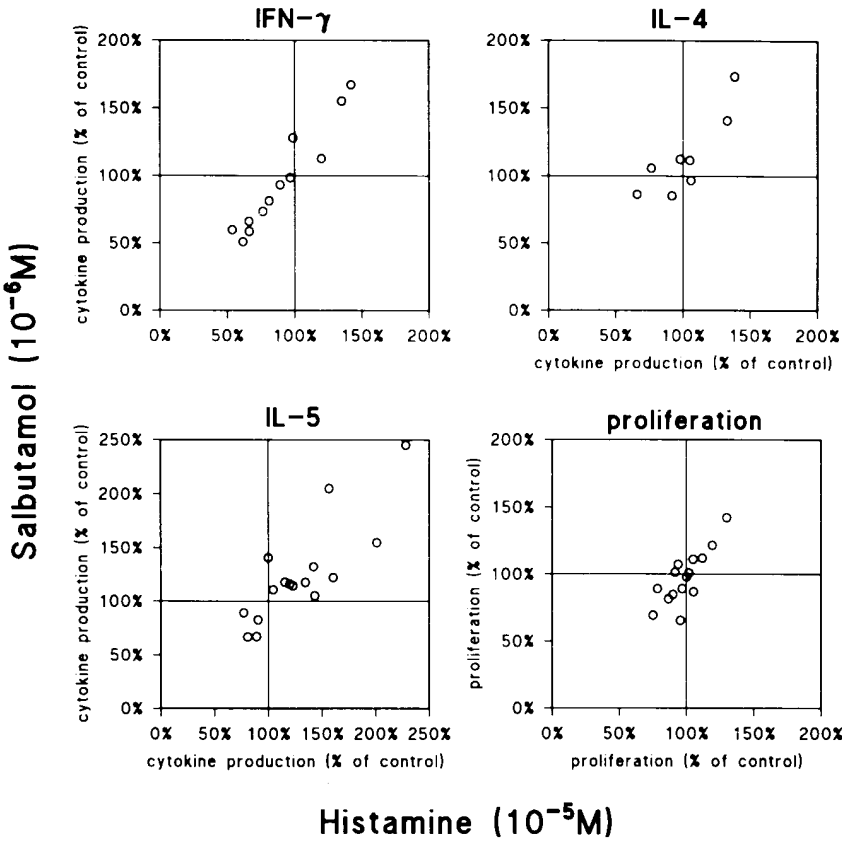

Figure 4. Effects of salbutamol and histamine on the production of IFN-γ, IL-4, and IL-5 and on the proliferation of T cell clones. The cells were stimulated with anti-CD3 with or without salbutamol (10^{-6} M) or histamine (10^{-5} M). The proliferation and the concentration of cytokines in the supernatants in the presence of the agents are expressed as a percentage of the control.

was found only in clones from healthy subjects. The inhibition of IFN-γ production by histamine and the enhancement of IL-5 production were associated with triggering of H_2-receptors and elevation of cAMPi. Salbutamol affected cytokine production and proliferation in a way similar to histamine.

We observed that the effects of histamine depended on the activation state of the T cell clones. When the clones were brought to a resting state by cultivating the cells without stimulus and IL-2 (33) the effects of histamine were much less, possibly owing to downregulation of histamine receptors (38). We also found that preincubation of the cells with ConA plus IL-2 resulted in a more pronounced effect of histamine. This was similar to the findings by Shibata and coworkers (39). In addition, the long-term culture of the cells gradually decreased the influence of histamine, eventually causing it to disappear. We have not analyzed whether this phenomenon was related to the presence of histamine receptors or to changes in intracellular signaling mechanisms. To minimize those influences, we have standardized all experiments with respect to the number of restimulation events and the timing of the experiments in relation to restimulation.

There was a close correlation between the effects of histamine and salbutamol, both relatively weak inducers of cAMPi (17, 37). As most of the effects of histamine were also seen after adding the potent cAMPi-elevating agent PGE_2 (20, 38, 40), we have concluded that variations in the levels of cAMPi are important regulatory events in the modulation of IFN-γ and IL-5 production by T cell clones. With respect to inhibition of proliferation of T lymphocytes and to inhibition of IL-2 and IFN-γ production, our findings confirm earlier reports (10, 18, 20, 24, 26, 37).

The β_2-agonist terbutaline was described as raising cAMP in mouse Th1 T cell clones and not in Th2 T cell clones (41). In our panel of clones we did not observe such dichotomy.

Our studies show that IL-5 production by human T cells can be enhanced by histamine and salbutamol, and confirm the results obtained with histamine in a mouse cell line (25). Enhancement of IL-5 production by PGE_2 had been previously described in human T cells (22), and an enhancement of IL-5 production by histamine has been shown in T cell lines from patients with atopic asthma (26). Remarkably, we observed enhancement of IFN-γ production by histamine in several T cell clones. It was found in clones from healthy persons only and was associated with histamine-enhanced IL-2 production and proliferation. Further research is necessary to confirm that such enhancement is absent in T cell clones from patients with asthma in a larger panel of T cell clones from more patients. The nature and involvement of histamine receptors in this effect have not been addressed as this study sought primarily to examine the potency of histamine in modulating cytokine production in panels of clones.

The important finding in this study is that histamine and salbutamol may both enhance and inhibit the production of a particular cytokine, depending on the T cell clone studied and the conditions applied. Earlier, Li and Fox made a similar observation concerning the effects of PGE_2

on the production of IL-3/granulocyte-macrophage colony-stimulating factor (42). It suggests that the second-messenger cAMPi may have counteracting effects on the expression of various cytokine genes. These effects may be in signaling pathways, in inducting transcription of cytokine genes, and/or in posttranscriptional regulation. Studies on the regulation of the expression of the IL-5 gene give support to the hypothesis that cAMPi may have positive as well as negative regulatory effects on the transcription of the IL-5 gene. A positive effect of cAMPi may proceed via the nuclear factors that bind to the IL-5P region of the IL-5 promoter (23, 43) and/or the conserved lymphokine element 0 (44). In the former the NF-IL-5P and the AP-1 proteins c-Fos and c-Jun are involved (43), and in the latter the AP-1 proteins c-Fos and JunB are involved (44). The IL-5 promoter contains additional binding sites for NF-AT and AP-1 and it may be hypothesized that the AP-1 proteins c-Fos and c-Jun are involved in these sites. Inhibitory effects of cAMPi may be explained, then, by the induction of high levels of JunB by cAMPi (45, 46). JunB may compete with c-Jun, thereby diminishing active AP-1 complexes composed of c-Fos and c-Jun (45). In our experiments, we observed in particular a strong enhancement of IL-5 production by cAMPi-increasing agents when the anti-CD3-induced production of IL-5 was relatively low. It can be hypothesized that when the production of IL-5 is higher, pathways and factors are active for IL-5 transcription that are inhibited by cAMPi (46). Under such conditions exogenous IL-2 may neutralize an inhibitory effect of cAMPi by increasing the level of NF-AT and of AP-1 composed of c-Fos and c-Jun (47). The interpretation of our findings via these mechanisms is purely hypothetical and requires additional experiments using various stimuli and specific inhibitors of signal transduction pathways.

Similarly counteracting mechanisms induced by cAMPi may also be supposed for the regulation of the expression of the IFN-γ gene. Its promoter region contains elements that can bind cAMP response element-binding protein (CREB) and activating transcription factor (ATF) via which cAMPi may stimulate the expression of the IFN-γ gene (48). In addition, binding regions that carry similarities to the AP-1-binding site have been demonstrated (48). AP-1 proteins often form complexes with NF-AT in binding to the promoter and these may form a regulatory point for inhibition of the IFN-γ gene expression by cAMPi-dependent mechanisms. In such mechanisms, the potential effects of IL-2 in increasing NF-AT may explain the effects of IL-2 on the IFN-γ production (49, 50), as well as the observation that IL-2 reversed the inhibition of IFN-γ production by histamine, as demonstrated in this and other reports (10). In addition, IFN-γ mRNA stabilization by cAMPi has been described (51). The expression of IFN-γ, however, is likely to proceed via still more complex regulatory events than the ones discussed here (52, 53).

This study demonstrates that histamine and salbutamol can affect the production of IFN-γ, IL-4, and IL-5 by airway- and blood-derived T cell clones in various ways. We propose that most of the effects are mediated via regulation by cAMPi. The direct effects of histamine on T cells can be either inhibitory or stimulatory. We did not observe differences in this respect between subgroups and subsets of clones—BAL- and blood-derived clones, and Th0, Th1, Th2 clones—neither did we observe a differentiation with regard to membrane surface markers. The lack of enhancement of IFN-γ production in asthma clones deserves further investigation. Our results may imply that in a normal polyclonal population of T lymphocytes, there is no net direct effect of histamine in T cell cytokine production, and that the physiological role of histamine in T cell cytokine production via direct interaction with T cells may be limited. cAMPi-regulating agents have important effects on antigen-presenting cells (54) and then, in concert with direct effects on T lymphocytes, may direct immunological reactions. In the local environment of the lungs of patients with allergic asthma an already skewed T cell cytokine profile toward Th2 cytokine pattern may occur. It will be of interest to analyze to what extent histamine and PGE_2 in the concentrations released from activated mast cells further catalyze the local reactions toward the Th2 type by inhibiting IFN-γ production and enhancing IL-5 production by local T cells.

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