

Histamine enhances TGF- β 1-mediated suppression of Th2 responses

S. KUNZMANN, P.-Y. MANTEL, J. G. WOHLFAHRT, M. AKDIS, K. BLASER, AND C. B. SCHMIDT-WEBER¹

Swiss Institute of Allergy and Asthma Research (SIAF), CH-7270 Davos, Switzerland

ABSTRACT Susceptibility of T cells to TGF- β 1 produced by regulatory T cells has an important impact on the induction and maintenance of peripheral tolerance and therefore on the development of autoimmunity, cancer, and allergy. Histamine not only mediates the deleterious effects of allergic reactions, it can also modulate the Th1/Th2 cell balance. We demonstrate that histamine dose-dependently enhanced TGF- β 1-mediated suppression and TGF- β 1 responsiveness of CD4⁺ T cells. This effect was mediated by the histamine 2 receptor (H2R), as demonstrated by receptor-specific agonists and antagonists. Furthermore, the histamine effect on TGF- β 1 responsiveness was cAMP/PKA dependent. This pathway is activated by the H2R, which is preferentially expressed on Th2 cells. Thus a higher additive effect of histamine on TGF- β 1 responsiveness was found in Th2 cells compared with Th1 cells. In fact, findings are confirmed by analysis of cytokine regulation, since activation of the H2R/cAMP pathway promoted TGF- β 1-mediated IL-4 inhibition but was ineffective in suppressing IFN- γ . These results demonstrate that histamine supports TGF- β 1 susceptibility of T cells. Moreover, Th2 cells are more affected by histamine-enhanced TGF- β 1 suppression, which is particularly important for the regulation of allergen-specific T cells in allergic immune responses.—S. Kunzmann, P.-Y. Mantel, J.G. Wohlfahrt, M. Akdis, K. Blaser, C. B. Schmidt-Weber *FASEB J.* 17, 1089–1095 (2003)

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After T cells encounter allergen by antigen-presenting cells, the balance of effector and regulatory cells determines the extent and type of the immune reaction. This process is mainly regulated by suppressive cytokines interleukin 10 (IL-10) and transforming growth factor β 1 (TGF- β 1) produced by CD4⁺ CD25⁺ regulatory T cells (1, 2). TGF- β 1 and IL-10 are also involved in the induction of peripheral tolerance during specific immune therapy (3). TGF- β 1 exerts its effect via the Smad signaling pathway (4). TGF- β 1 binds to the TGF- β receptor(R)-II, which recruits and phosphorylates TGF- β RI. The activated TGF- β RI phosphorylates Smad2 and Smad3, which form heterodimeric complexes with the common partner Smad4. This complex translocates into the nucleus, where it activates TGF- β 1 target genes.

Allergy is characterized by an unbalanced peripheral tolerance against harmless antigens, which leads to uncontrolled activation of Th2-like cells secreting IL-4, which promotes immunoglobulin E (IgE) production by B cells. However, at the moment of allergen exposure the T cells are coincidentally exposed to histamine released by IgE-triggered mast cells (5). IgE binds via Fc ϵ RI to mast cells, which upon allergen binding triggers the release of various inflammatory mediators, of which histamine represents a major mediator in type I allergic reactions (6, 7). Histamine mediates immediate-type hypersensitivity with increased vascular permeability, smooth muscle contraction, vasodilatation, flushing, mucus secretion, and pruritus (5, 8).

Histamine modulates cellular activity by binding with one of at least four G-protein-coupled histamine receptors (HR1-HR4; reviewed in refs 9, 10). H1R stimulation results in activation of phospholipase C and subsequently increases the second messengers inositol-1,4,5-tri-phosphate (IP₃) and intracellular calcium. HR1 signaling is involved mainly in allergic reactions related to smooth muscle contraction and vascular permeability (11). In contrast, H2R is coupled to the adenylate cyclase pathway mediating gastric acid secretion. It is further involved in cell growth and differentiation, smooth muscle relaxation of airway, and vascular and immune response (12). The H3R is also coupled to adenylate cyclase and triggers mitogen-activated protein kinase signaling pathways (13). The function of the recently described H4R is currently unclear (14, 15).

Recent investigations demonstrated an additional role for histamine as a negative regulator of T cells (16), in particular, on Th2 cells (17). Until now, it was not known whether histamine interacts with TGF- β 1-mediated suppression of T cells.

MATERIALS AND METHODS

Reagents

Recombinant (r) human TGF- β 1 was obtained from R&D Systems (Abingdon, UK); rIL-2, rIFN- γ , rIL-4, and neutraliz-

¹ Correspondence: Swiss Institute of Allergy and Asthma Research, Obere Strasse 22, CH-7270 Davos, Switzerland. E-mail: csweber@siaf.unizh.ch

ing anti-IL-4 (8F12 and 3H4) were gifts from Dr. C Heusser, Novartis (Basel, Switzerland); human rIL-12 and neutralizing anti-IL-12 mAb were bought from PharMingen (San Diego, CA, USA); and phytohemagglutinin was from Sigma (St. Louis, MO, USA). The mouse anti-human CD28 mAb (clone 15E8) was purchased from the Netherlands Red Cross blood transfusion service (Amsterdam, Netherlands); the mouse anti-human CD3 mAb (clone CRL 8001) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The pGL3ti (CAGA)₁₂ vector was described previously (18). Histamine, db-cAMP, and Tripolidine were purchased from Sigma and HTMT, Amathamine, and Imetit from Tocris (Wangen, Switzerland). Forskolin, MDL-12, and H89 were obtained from Calbiochem (Bad Soden, Germany).

Isolation of CD4⁺ T cells

Peripheral blood mononuclear cells were isolated from blood of healthy volunteers by Ficoll (Biochrom KG, Berlin, Germany) density gradient centrifugation. The interphase cells were washed three times and CD4⁺ T cells were purified using anti-CD4-Dynal magnetic beads and Detach-a-Bead antibodies (both from Dynal, Hamburg, Germany). The purity of CD4⁺ T cells was initially tested by flow cytometry and was >95%.

Cell cultures

Human CD4⁺ T cell cultures were performed in serum-free AIM-V medium (Life Technologies, Basel, Switzerland) in a humidified atmosphere containing 5% CO₂ at 37°C. CD45RA⁺ cells were negatively isolated from purified CD4⁺ T cells by using the MACS[®]-system (Milteny) as described (19). To remove memory T cells, CD4⁺ T cells were incubated with MACS microbead-conjugated anti-CD45RO. Freshly isolated CD4⁺CD45RA⁺ memory effector T cells were resuspended in AIM-V medium. The cells were cultured in 24-well plates at a cell density of 3 × 10⁶/well. They were stimulated with a combination of anti-CD2 mAb (4B2 and 6G4 each 0.5 µg/mL), anti-CD3 mAb (1 µg/mL), and anti-CD28 mAb (1 µg/mL) as well as IL-2 (20 ng/mL). For Th1 differentiation, human IL-12 (10 ng/mL) and neutralizing anti-IL-4 mAb (10 µg/mL) were added to individual wells. For Th2 differentiation, IL-4 (25 ng/mL) and neutralizing anti-IL-12 (10 µg/mL) were used. The growing cell cultures were expanded with fresh culture medium containing 25 ng/mL human IL-2. Th1 and Th2 cells were expanded in RPMI 1640 supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 1% MEM nonessential amino acids and vitamins, 100 U/mL penicillin, 100 µg/mL streptomycin, 50 µM 2β-mercaptoethanol (all from Life Technologies), and 10% fetal bovine serum (Sera Laboratory, Sussex, UK). After 12 days the cells were harvested, washed, and restimulated using the same procedure. After two cycles of stimulation, cytokine patterns of differentiated cells were verified by ELISA.

T cell proliferation assay

The cultures were set up in 200 µL AIM-V medium in 96-well flat-bottom microtiter plates (Costar, Corning, NY, USA). Samples in triplicate containing 10⁵ T cells were incubated in 96 flat-bottom-wells, which were previously coated with 1 µg/mL anti-CD3 mAb. Cells were cultured for 3 days, pulsed for 16 h with 1 µCi [³H]-thymidine (Hartmann, Braunschweig, Germany), and harvested on glass fiber filters using an automated multisample harvester (LKB, Pharmacia-Wallac, Turku, Finland). Filters were transferred in sample bags

with liquid scintillation fluid and analyzed using a β-scintillation counter (Pharmacia-Wallac).

RT-PCR

Total RNA was isolated from human CD4⁺ T lymphocytes using the RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The RNA was eluted in 40 µL water and subjected to reverse transcription. Approximately 5 µg total RNA (12 µL) was reverse transcribed by addition of 500 µg/mL oligo (dT)₁₂ primer (Roche, Basel, Switzerland), RNase Inhibitor (Roche; 10 U/µL), dNTP (5 mM each dNTP; Qiagen), and Omniscript transcriptase (Qiagen; 0.2 U/µL) for 1 h at 37°C. The cDNA was denatured at 90°C for 5 min and used for PCR amplification. PCR reactions were performed with Taq-polymerase (Qiagen). RT-PCR was performed using the following primers: IL-4 forward 5×CTG CTT CCC CCT CTG TTC TTC C 3×, IL-4 reverse 5× TCT GGT TGG CTT CCT TCA CAG G 3×, IFN-γ forward 5×CTG TTA CTG CCA GGA CCC ATA TG 3×, IFN-γ reverse 5× GAA CCA TTA CTG GGA TGC TCT TCG 3×, GAPDH forward 5× CTT CGC TCT CTG CTC CTC CT 3×, and GAPDH reverse 5× GCT GAT GAT CTT GAG GCT GTT G 3×. The primer for IL-4 and IFN-γ were used as described before (20). PCR products were loaded next to a standard (1kbpplus, Life Technologies) and analyzed on 1% agarose gels. Image analysis was performed using a fluorescent imager analyzer FLA 3000 (Fuji, Dielsdorf, Switzerland) and quantified using AIDA software (Raytest, Urdorf, Switzerland).

Transfections and reporter gene assays

CD4⁺ T cells were purified as described above and rested in serum-free AIM-V medium (Life Technologies) overnight. An amount of 2 µg TGF-β1-sensitive pGL3ti (CAGA)₁₂-Luciferase reporter gene was added to 3 × 10⁶ CD4⁺ T cells that had been washed in PBS and resuspended in 100 µL of NucleofectorTM solution for T cells (Amara Biosystems, Cologne, Germany), electroporated using the U-15 program of the NucleofectorTM (Amara), and immediately transferred into prewarmed AIM-V medium. Transfected cells were seeded into 24-well plates, and TGF-β1 (1 ng/mL) and/or histamine and/or histamine receptor agonists were added to the cells. If using histamine receptor antagonists or enzyme inhibitors, the cells were preincubated with them for 30 min before giving histamine. Twenty-four h after transfection, luciferase activity in cell lysates was measured by the dual luciferase assay system (Promega Biotech Inc., Madison, WI, USA) according to the manufacturer's instructions in a Berthold Lumat LB 9507 luminometer (Bad Wildbach, Germany). Data were normalized by the activity of Renilla luciferase under the control of thymidine kinase promoter of pRL-TK. All values were obtained from experiments performed in triplicate and repeated at least three times.

RESULTS

Histamine and TGF-β1 cooperatively inhibit CD4⁺ T cell proliferation

TGF-β1 is known to inhibit T cell proliferation (21). However, it was unclear whether TGF-β1 signaling is still operative in the presence of histamine. Therefore, CD4⁺ T cells were stimulated with anti-CD3 mAb in the presence or absence of histamine and/or TGF-β1 un-

der serum-free conditions. The addition of histamine resulted in dose-dependently decreased proliferation ($67\% \pm 2.86$; **Fig. 1**, left panel) as measured by ^3H -thymidine incorporation. TGF- β 1 (1 ng/mL) inhibited anti-CD3 mAb-induced proliferation to $70\% \pm 2.49$ (Fig. 1, right panel). TGF- β 1 and histamine cooperatively inhibited T cell proliferation below levels of TGF- β 1 or histamine alone in a dose-dependent fashion ($97\% \pm 0.57$; Fig. 1, right panel) and without inducing cell death (data not shown). This shows that histamine and TGF- β 1 cooperatively inhibit CD4 $^+$ T cell proliferation.

Effect of histamine on TGF- β 1 signaling

TGF- β 1 transmits intracellular signals via the SMAD pathway (4), which can be modulated by T cell activation (21), cytokines (22, 23), and possibly by G-protein-coupled receptor signaling. Since T cells are exposed to histamine, particularly in allergic reactions, we tested T cell ability to respond to TGF- β 1 in the presence of histamine using a TGF- β 1-responsive reporter gene construct that was transiently transfected into human primary CD4 $^+$ T cells before addition of TGF- β 1 and/or histamine. Histamine enhanced the reporter gene activity ($2.9\text{-fold} \pm 0.12$; **Fig. 2A**). The effect was consistently observed for multiple donors (Fig. 2B; $n=13$, $P \leq 0.01$). To analyze the mechanism of the histamine synergy with TGF- β 1, the H1R agonist Betahistidine was investigated in the range of 10^{-3} to 10^{-9} M. Betahistidine did not change the reporter gene activity (Fig. 2C) and the H1R antagonist Triprolidine at 10^{-3} to 10^{-9} M did not block histamine-enhanced

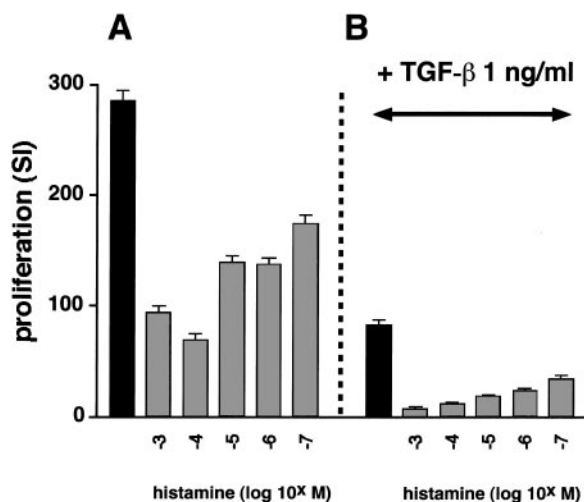


Figure 1. Histamine and TGF- β 1 cooperatively inhibit anti-CD3 mAb-induced CD4 $^+$ T cell proliferation. Human CD4 $^+$ T lymphocytes were stimulated with anti-CD3 mAb in serum-free AIM-V medium (solid bar, A) and/or in the presence of increasing histamine concentrations (gray bars, A) B) The same conditions, but with the addition of 1 ng/mL TGF- β 1. Cells were cultured for 3 days. The stimulation index (SI) is shown and error bars indicate the standard error of the mean. A representative experiment of 3 independent experiments is shown.

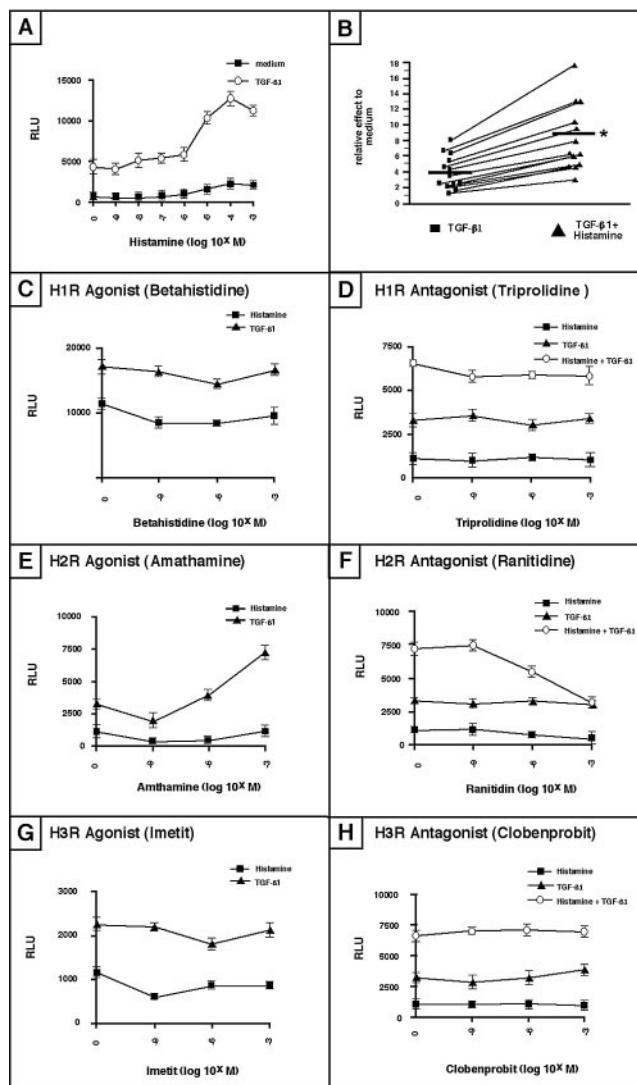


Figure 2. Histamine dose-dependently enhances Smad2/3 transactivating capacity in CD4 $^+$ T cells mediated by the H2R. Luminometric analysis of TGF- β 1-responsive (CAGA) $_{12}$ -Luc reporter (2 μg) transfected T cells (3×10^6) incubated with/without 1 ng/mL TGF- β 1, increasing concentrations of histamine, or both for 24 h as compared with medium (A). B) Statistical analysis of the enhancing effect of histamine on TGF- β 1 response. Relative increase of TGF- β 1 alone is compared with the relative increase by TGF- β 1 and histamine in 13 independent individuals. Lines indicate the mean of the data in each group. Asterisk indicates statistical significance ($P \leq 0.0023$) according to the Man Whitney U Test. Luminometric analysis of (CAGA) $_{12}$ -Luc reporter transfected T cells (3×10^6) incubated with 1 ng/mL TGF- β 1, histamine (10^{-4} M), or both for 24 h compared with medium and in the presence of different concentrations of the H1R agonist Betahistidine (C), the H1R antagonist Triprolidine (D), the H2R agonist Amathamine (E), the H2R antagonist Ranitidine (F), the H3R agonist Imetit (G), and the H3R antagonist Clobenprobit (H). If the antagonists were used, cells were preincubated for 30 min with the antagonists before histamine and TGF- β 1 was added. In all experiments firefly luciferase activity of (CAGA) $_{12}$ -Luc was normalized by Renilla luciferase activity of pRL-TK vector using dual luciferase reporter system. Activity is given as relative light units (RLU). All values were obtained from experiments performed in triplicate and the error bars indicated standard error of the mean. The figures are representative for 3 independent experiments.

reporter gene activity (Fig. 2D). Similar results were obtained using the H3R agonist Imetit (Fig. 2G) and the antagonist Clobenpropit (Fig. 2H). In contrast, the H2R agonist Amathamine could mimic the effect of 10^{-4} M histamine dose-dependently starting at a concentration of 10^{-6} M (Fig. 2E). Likewise, the H2R antagonist Ranitidine prevented histamine enhancement of TGF- β 1 in a dose-dependent manner starting at 10^{-6} M (Fig. 2F). As a conclusion, histamine did not inhibit TGF- β 1-responsive reporter gene activity in primary CD4⁺ T lymphocytes but enhanced activity mediated by H2R by two- to threefold.

Histamine-enhanced TGF- β 1 signaling depends on the adenylate cyclase activity and protein kinase A (PKA)

The H2R is assumed to trigger the G α s protein, which in turn stimulates adenylate cyclase and the synthesis of cAMP (12). If the observed interaction of histamine with TGF- β 1 signaling is mediated by the H2R, the enhancing effect of histamine could be sensitive to an irreversible adenylate cyclase inhibitor, such as MDL 12 (330A, hydrochloride). MDL-12 dose-dependently inhibited histamine-enhanced Smad activation (Fig. 3A) while showing no effect on the TGF β 1 effect alone. Correspondingly, forskolin, an activator of adenylate cyclase, dose-dependently enhanced TGF- β 1-induced reporter gene activity (Fig. 3B). Cyclic AMP-mediated suppression is known to be mediated via the activity of PKA (24). Histamine-enhanced TGF- β 1-induced reporter gene activity is dose-dependently inhibited by the PKA inhibitor H89 (Fig. 3C) and reached the same levels as TGF- β 1 alone at a concentration of 10^{-4} M H89. It had no influence on the TGF- β 1 effect alone. Taken together, histamine enhances the TGF- β 1 signaling by a cAMP/PKA-dependent pathway.

TGF- β 1 and histamine are more effectively cooperating in Th2 than in Th1 cells

We have previously shown that H2R is expressed higher on Th2 cells whereas H1R is increased on Th1 cells (17). Since agonists showed that H2R enhances TGF- β 1 signaling, Th1 and Th2 cells were subjected to TGF- β 1-induced reporter gene assays. Only small differences were found between Th1 and Th2 cells when TGF- β 1 alone was added (3.0 ± 0.49 -fold in Th1 cells vs. 4.0 ± 0.40 -fold increase in Th2; Fig. 4). A big difference in TGF- β 1 signaling was observed if histamine was used together with TGF- β 1. Whereas Th1 cells showed a $8.4\text{-fold} \pm 0.81$ higher expression after addition of TGF- β 1 and histamine in comparison with the stimulated control cells (Fig. 4, left side), Th2 cells showed a $21.5\text{-fold} \pm 1.22$ increase (Fig. 4, right side). According to the different expression of the H1R and H2R on Th1 and Th2 cells, TGF- β 1 and histamine are more effectively cooperating in Th2 than in Th1 cells.

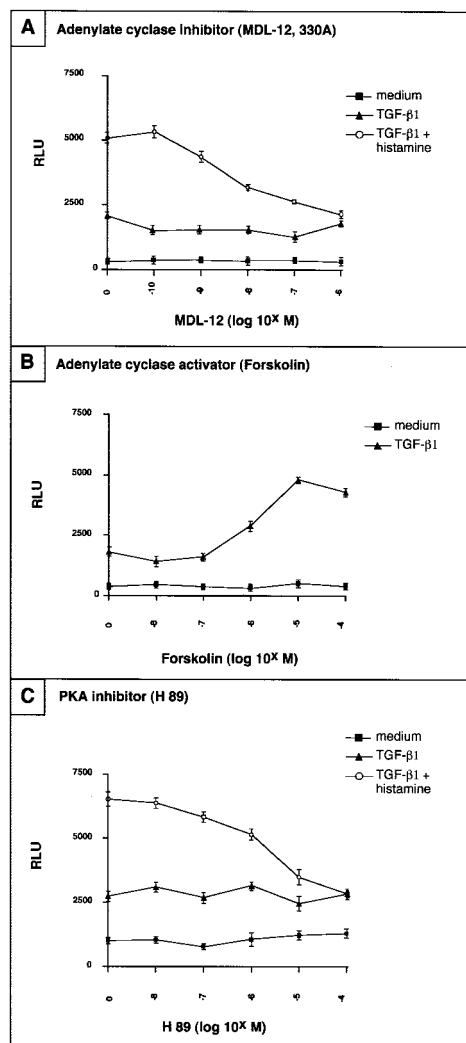


Figure 3. The effect of histamine on the TGF- β 1 response in luciferase assay is mediated by cAMP/PKA pathway. Lumino-metric analysis of the TGF- β 1-responsive (CAGA)₁₂-Luc reporter transfected T cells (3×10^6) incubated with TGF- β 1 (1 ng/mL), histamine (10^{-4} M), or both for 24 h compared with medium and to different concentrations of the adenylate cyclase inhibitor MDL-12 (A), the adenylate cyclase activator forskolin (B), or the PKA inhibitor (H89; C) in the presence of medium or histamine and/or TGF- β 1. The activity is given as relative light units (RLU). Data are representative for 3 independent experiments.

Histamine and TGF- β 1 cooperatively inhibit IL-4 but not IFN- γ mRNA expression

To analyze the synergism of histamine and TGF- β 1 on the key cytokines of Th1 and Th2 cells, IL-4 and IFN- γ expression was investigated in T cells after addition of histamine and TGF- β 1. Cells were incubated with histamine and/or TGF- β 1 for 2 h and reverse transcribed RNA was subjected to RT-PCR. Addition of histamine only suppressed the expression of Th2-associated IL-4 mRNA ($54\% \pm 2.45$); Th1-associated IFN- γ mRNA was not affected (Fig. 5A, B). In contrast, TGF- β 1 suppressed IL-4 and IFN- γ expression ($62\% \pm 1.63$ and

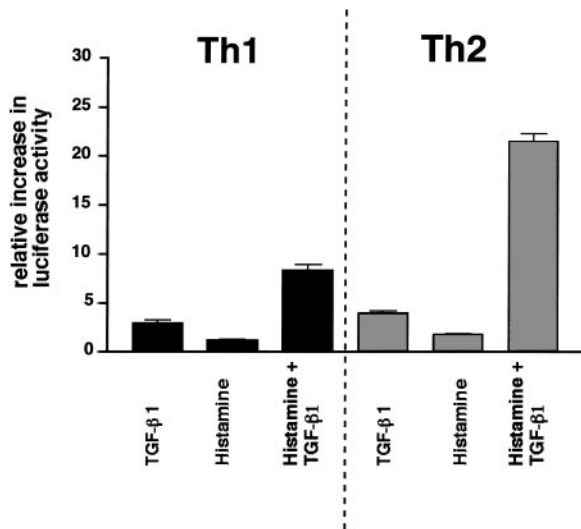


Figure 4. The effect of histamine on the TGF-β1 response in luciferase assay is higher in Th2 compared with Th1 cells. Luminometric analysis of the TGF-β1-responsive (CAGA)₁₂-Luc reporter transfected Th1 (left side) and Th2 (right side) cells (3×10^6) incubated with TGF-β1 (1 ng/mL), histamine (10^{-4} M), or double incubation with TGF-β1 and histamine for 24 h. The values are given as relative increase in luciferase activity compared with medium. The data are representative for 3 independent experiments.

IFN-γ, $67\% \pm 2.35$, respectively). Histamine only cooperated with TGF-β1 to suppress IL-4 ($20\% \pm 4.08$ in comparison to $62\% \pm 2.45$ TGF-β1 alone) to almost background levels, whereas histamine was ineffective on IFN-γ mRNA expression ($58\% \pm 1.22$ compared with $62\% \pm 2.35$ TGF-β1 alone). Thus, histamine and TGF-β1 cooperate in down-regulating of the Th2 cytokine IL-4 but not of IFN-γ.

To identify the histamine receptor responsible for down-regulation of IL-4, specific receptor agonists were used. Only the HR2 agonist amthamine, but not the HR1 agonist Betahistidine or the HR3 agonist Imetit, decreased IL-4 mRNA expression (Fig. 5C, D). Consistent with the ineffectiveness of histamine on IFN-γ, none of the agonists influenced mRNA expression of this gene. Since the H2R is known to mobilize adenylate cyclase, we tested whether pharmacological activation (forskolin) of this enzyme leads to reduced IL-4 expression. Forskolin inhibited IL-4 mRNA expression in a dose-dependent fashion but IFN-γ remained unaffected (Fig. 5E, F).

Since adenylate cyclase is preferentially activated by H2R in human Th2 cells (17, 25), we tested whether IFN-γ is responsive to cAMP using the stable, membrane-permeable cAMP analog dibutyryl-cAMP (db-cAMP). T cells treated with db-cAMP were dose-dependently inhibited in IL-4 but not in IFN-γ mRNA expression (Fig. 5G, H). It could be shown that histamine inhibited mRNA expression of the Th2 cytokine IL-4 in primary CD4⁺ T lymphocytes mediated by the H2R and adenylate cyclase while having no influence on the expression of the Th1 cytokine IFN-γ.

DISCUSSION

TGF-β1 plays an important role in regulating immune responses, especially in induction and maintenance of peripheral tolerance (2, 26, 27). However, TGF-β1-mediated suppression is not a constitutive parameter of

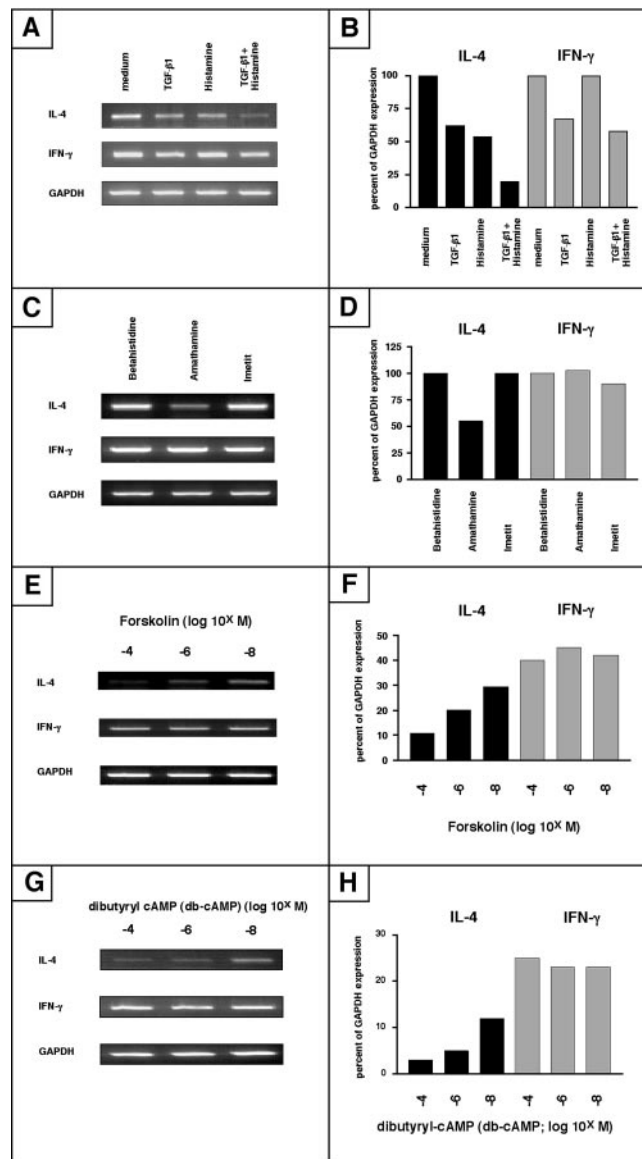


Figure 5. TGF-β1 and histamine cooperatively inhibit IL-4 but not IFN-γ mRNA expression mediated by the H2R and a cAMP-dependent pathway. Cultured human CD4⁺ T lymphocytes were incubated with histamine (10^{-4} M) and/or TGF-β1 (1 ng/mL) for 2 h. RT-PCR for IL-4, INF-γ and GAPDH were performed (A, B). Right panels show electrophoretic visualization of the amplicons and the left panel GAPDH normalized quantification of the PCR products. C, D) Cultured T cells were incubated for 2 h either with the HR1 agonist Betahistidine (10^{-4} M), the HR2 agonist Amthamine (10^{-4} M), or the HR3 agonist Imetit (10^{-4} M). The lower panels show cells that were incubated with the adenylate cyclase activator forskolin in increasing concentrations for 2 h (E, F) or with different concentrations of dibutyryl cAMP (db-cAMP) for 2 h (G, H). The figures are representative for 3 independent experiments.

T cells, but depends on the activation status of the T cell (21) and multiple environmental factors, like cytokines (22, 23). Histamine is known to inhibit TCR signaling and IL-2 expression (17, 28, 29). Since TGF- β 1 responsiveness depends on activation of T cells, it appeared that histamine can modulate TGF- β 1 signaling. The current results demonstrate that histamine and TGF- β 1 synergize in TGF- β 1 signaling, suppression of proliferation, and cytokine expression of T cells.

In fact, cooperative inhibition of T cell proliferation by TGF- β 1 and histamine was stronger than with the single stimuli, reaching background levels without inducing cell death. Since the histamine concentrations necessary for cooperative suppression are within the range found in plasma during an acute allergic reaction (10^{-4} to 10^{-3} M) (30, 31), antihistamine drugs may interfere with TGF- β 1-mediated tolerance. To evaluate the potential of clinically used H1R antagonists, the interaction of histamine with the TGF- β 1 pathway was analyzed. Histamine alone increased the TGF- β 1 responsiveness of T cells. It was previously shown that nonlymphoid cells respond to histamine with enhanced TGF- β 1-induced IL-11 expression in an H1R- and Ca^{2+} -dependent fashion (32). However, H1R and H3R antagonists could not abolish the histamine-enhanced TGF- β 1 signaling in human T cells. Neither the H1 nor the H3 agonists influenced the TGF- β 1-responsiveness of T cells. In contrast, histamine-enhanced TGF- β 1 signaling could be mimicked using an H2R agonist and inhibited by using an H2R antagonist. Activation of H2R by histamine results in adenylate cyclase activation, which elevates intracellular cAMP levels. In turn, cAMP binds to the regulatory subunit of PKA and induces the release of catalytic subunits (12). The adenylate cyclase inhibitor MDL-12 and the inhibitor of the PKA H89 reversed the histamine effect of histamine in the TGF- β 1-specific luciferase assay. These experiments support that histamine-enhanced TGF- β 1 responsiveness depends on H2R activation.

We recently demonstrated that Th2 cells express H2R more abundantly than H1R (17). It was further shown that the adenylate cyclase is preferentially expressed by Th2 cells (25). We therefore hypothesized that Th2 cells are more susceptible to TGF- β 1 enhancement by histamine. In fact, the additive effect of histamine was much stronger in Th2 differentiated T cells than in Th1 cells, confirming the important role of H2R activation.

The effect of histamine on TGF- β 1-mediated suppression is also reflected by regulation of the Th1 and Th2 cytokines. Whereas TGF- β 1 moderately inhibited IL-4 and IFN- γ , histamine suppressed only IL-4 and not IFN- γ gene expression. The H2R agonist Amathamine, but not the H1R (Betahistidine) or H3R (Imetit) agonists, suppressed IL-4, whereas IFN- γ expression was not affected by these drugs, supporting the role of the H2R signal and cAMP pathway in the regulation of Th2 cytokines. Since the adenylate cyclase inducing forskolin and cell-permeable cAMP both inhibited dose-dependent IL-4 but not IFN- γ , it becomes obvious that

the histamine effect on T cells is based on the cAMP pathway. These results confirm the observation that cAMP metabolism participates in the regulation of cell growth and differentiation and support the general concept that the adenylate cyclase-cAMP/PKA pathway conveys an "off" signal for lymphocytes (33–36). The molecular mechanisms underlying the role of cAMP in differential regulation of Th1 and Th2 cytokines are still unclear. The current results show that cAMP is, independent of Th1/Th2 differences in HR expression, selectively suppressing the IL-4 promoter activity, since cell-permeable cAMP is also selective for the IL-4 gene. Thus, known differences in H2R and adenylate cyclase expression cannot account for this difference (17, 25).

The present study clearly demonstrates that histamine can interfere with TGF- β signaling in healthy donors. This raises the question of whether frequently used antihistamine drugs such as H1R antagonists (e.g., Dimetinden) for the treatment of allergy symptoms, in particular seasonal allergic rhinitis, modulate disease by promoting TGF- β -dominated functions in allergic donors. Although this question cannot be answered on the basis of the current results, it is interesting to note that anti-H1R antagonists were used as adjuvant therapy to promote the development of peripheral tolerance (37, 38). Treatment of sensitized mice with anti-H1R antagonists prevented the development of airway hyperresponsiveness in both the primary sensitization and in challenge, as well as in the adoptive transfer experiments (39).

The described molecular interaction between histamine and TGF- β 1 signaling is important in regulating T cells and peripheral tolerance. This knowledge is likely to improve antihistamine therapy and the design of future strategies for antihistamine treatment. **[F]**

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