

Histamine Upregulates Th1 and Downregulates Th2 Responses due to Different Patterns of Surface Histamine 1 and 2 Receptor Expression

M. Jutel^a S. Klunker^b M. Akdis^b J. Małolepszy^b Oliver A.R. Thomet^b
T. Żak-Nejmark^a K. Blaser^b C.A. Akdis^b

^aWrocław University of Medicine, Wrocław, Poland;

^bSwiss Institute of Allergy and Asthma Research, Davos, Switzerland

Key Words

T cell subsets · Receptors · Histamine

Abstract

Histamine, which acts via G protein-coupled receptors, is an important mediator of immediate hypersensitivity and is also able to influence the nature of T cell responses. We demonstrated that TH1 and Th2 cells express distinct surface histamine receptor patterns and that Th1-type responses are enhanced by histamine, whereas Th2-type responses are negatively regulated, due to different intracellular signals generated by histamine stimulation. These findings account for negative feedback regulation in a wide variety of pathologies.

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Introduction

The existence of subsets of T helper lymphocytes which exert different effector functions provides a prerequisite for understanding the pathogenesis of many immune disorders such as allergy, autoimmune diseases, graft rejection and antitumor responses [1, 2]. For this reason, an understanding of the differential regulation of

Th1 and Th2 cell subsets as well as finding the corresponding cell surface markers is of great importance. This issue is currently under intensive investigation. However, there are still many open questions.

Histamine is a chemical messenger involved in various conditions, including inflammatory reactions, neurotransmission and numerous brain functions, regulation of gastrointestinal and circulatory functions, embryonic growth and wound healing [3, 4]. It is the major factor in anaphylaxis. The pharmacological effects of histamine are mediated through three types of membrane receptors, H1, H2 and H3, which belong to the G protein-coupled receptor (GPCR) family. Histamine receptors possess all structural features of GPCRs, including seven putative transmembrane domains, NH₂-terminal glycosylation sites and phosphorylation sites for protein kinase A and protein kinase C [5, 6]. H1 and H2 receptors (H1R; H2R) are expressed on many cell types, including lymphoid cells. H3 receptor (H3R) is expressed mainly on histaminergic neurons. Some immunoregulatory effects of histamine have already been demonstrated; however, their relevance is not fully understood [3].

We investigated the expression and functional properties of H1Rs and H2Rs on Th1 and Th2 cells and the effect of histamine on antigen-specific responses in these cells.

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Correspondence to: Dr. Marek Jutel
Department of Internal Medicine and Allergy
Wrocław University of Medicine, Traugutta 57
PL–50-417 Wrocław (Poland)
Tel. +48 71 344 2264, Fax +48 71 370 0129, E-Mail mjutel@diinet.wroc.pl

Materials and Methods

Th1 and Th2 cells were obtained by differentiation of cord blood-derived CD45RA⁺ T cells, as described in detail previously [7]. For flow cytometric analysis, 5×10^4 cells were stained either with fluorescein-conjugated histamine (HF) (Molecular Probes, Eugene, Oreg., USA) alone or together with histamine dihydrochloride (RBI Research Biochemicals, Natick, Mass., USA) or with the H1R antagonist tripeleennamine hydrochloride (RBI). Mouse IgG was used as negative control. HF, histamine dihydrochloride and tripeleennamine hydrochloride were used at optimized concentrations of 10^{-5} M, 10^{-3} M and 10^{-6} M, respectively. For the semiquantitative RT-PCR analysis of H1R and H2R, total RNA was isolated using Trizol reagent (Life Technologies, Basel, Switzerland), following the instructions of the manufacturer. One-step RT-PCR was performed using the One Tube Titan RT-PCR System (Roche Diagnostics, Basel, Switzerland). Primers used to amplify specific gene products were: H1R forward primer, 5'-ttt ctc tct ttt ctg tgg gtt att c-3'; H1R reverse primer, 5'-ctt ggg ggt ttg gga tgg tga ctt c-3'; H2R forward primer, 5'-ttc atc gtg tcc ttg gct atc-3'; H2R reverse primer, 5'-ctg aga ggc gtt gga ctt c-3'; β -actin forward primer, 5'-atc tgg cac cac acc ttc tac aat gag ctg cg-3'; β -actin reverse primer, 5'-cgt cat act cct gct tgc tga tcc aca tct gc-3'. Fragment size for the H1R PCR and H2R PCR products was 387 and 804 bp, respectively. β -Actin was used as the housekeeping gene. Primers amplified an 839-bp PCR product. Reverse transcription of RNA was performed at 50°C for 30 min, followed by 94°C for 2 min. Conditions for PCR were: 10 cycles at 94°C for 30 s, 55°C for H1R (62°C for H2R) for 30 s (annealing), 68°C for 45 s (elongation), followed by 25 cycles at 94°C for 30 s and 55°C for H1R (62°C for H2R) for 30 s (annealing) and 68°C for 45 s + 5s per step (elongation). The elongation time was prolonged in 1 cycle to 7 min (68°C). PCR products were identified by 1% agarose gel electrophoresis. Intracellular ionized calcium concentration was measured using a spectrofluorometric assay as previously described [8]. Peripheral blood mononuclear cells (PBMC), Th1 or Th2 cells (4×10^6), labeled with fura-2/AM, were stimulated with histamine (5×10^{-3} M) or with biotinylated monoclonal antibody to CD3 (Sigma Chemical Co., St. Louis, Mo., USA) (7 μ g/ml) followed by avidin (Sigma) (1 μ g/ml). Tripeleennamine was used at a concentration of 5×10^{-6} M. Intracellular cAMP levels were determined using the BiotrakTM cAMP enzyme immunoassay system (Amersham Pharmacia, Little Chalfont, UK), according to the manufacturer's instructions. For proliferation and cytokine secretion assays, Th1 and Th2 cells were incubated for 60 min with histamine dihydrochloride (10^{-7} – 10^{-3} M), either alone or with the H2R antagonist ranitidine dihydrochloride (RBI) (10^{-6} M) or the H1R antagonist tripeleennamine hydrochloride (10^{-6} M). Also, 1×10^5 cells (PBMC, Th1 or Th2 cells) were incubated with increasing concentrations of histamine (10^{-3} – 10^{-7} M) for 120 min. Thereafter, the cells were stimulated with 10 μ g/ml solid phase-bound anti-CD3 monoclonal antibodies. Cytokine levels were measured by ELISA after 48 h [9, 10]. [³H]-Thymidine incorporation was determined after 48 h. PBMC were stimulated with either Der p 1 (10 μ g/ml), phospholipase A2 (PLA) (10 μ g/ml) or purified protein derivate of *M. tuberculosis* (PPD) (20 μ g/ml). [³H]-Thymidine incorporation was measured in antigen-specific cultures after 5 days.

Results and Discussion

Differential H1R and H2R Expression on Th1 and Th2 Cells

The staining of Th1 and Th2 cells with FITC-labeled histamine demonstrated significantly stronger histamine binding to Th1 cells as compared to Th2 cells. The inhibition of histamine binding by the H1R-specific antagonist, tripeleennamine hydrochloride, in Th1 but not in Th2 cells indicated predominant H1R expression on Th1 cells. Furthermore, using semiquantitative RT-PCR, predominant H1R mRNA expression was detected in Th1 cells as compared to Th2 cells. In contrast, H2R mRNA was predominantly expressed on Th2 cells. Stimulation of PBMC with IL-12 resulted in enhanced expression of H1R, whereas IL-4 suppressed H1R mRNA expression by anti-CD3 stimulation. Thus, Th1 and Th2 cells are characterized by different surface histamine receptor patterns.

Distinct Histamine-Induced Signals in Th1 and Th2 Cells

The primary signal from H1R is delivered through calcium-dependent activation of phospholipase C with generation of IP₃ and DAG, while stimulation through H2R results in the induction of adenylyl cyclase and cAMP formation. Since H1Rs are overexpressed on Th1 cells, stimulation of Th1 but not Th2 cells with histamine resulted in significant Ca²⁺ flux. This effect was specifically blocked by tripeleennamine, demonstrating H1R-dominated signaling in Th1 cells. In contrast, Th2 but not Th1 cells showed dose-dependent increases in cAMP after histamine stimulation. This effect was fully abrogated by the specific H2R antagonist ranitidine, but not tripeleennamine, indicating H2R-dominated signaling in Th2 cells.

Histamine Stimulation Affects Proliferative and Cytokine Responses

Analyses of the influence of histamine on immune effector functions revealed enhanced anti-CD3-induced proliferation of Th1 cells, while Th2 cells were inhibited. In addition, histamine enhanced PPD-induced proliferation in PBMC cultures. In contrast, specific allergen-driven cells from house dust mite- and bee venom-allergic individuals were suppressed by histamine. Moreover, the secretion of the Th1 cytokine IFN- γ was enhanced in Th1 cells after histamine stimulation, whereas Th2 cytokines (IL-4, IL-13) were inhibited in Th2 cells. Studies on the mechanisms of allergen-specific immunotherapy (SIT) provide evidence for the interpretation of the current data. Successful SIT induces peripheral tolerance in Th2-type specific T

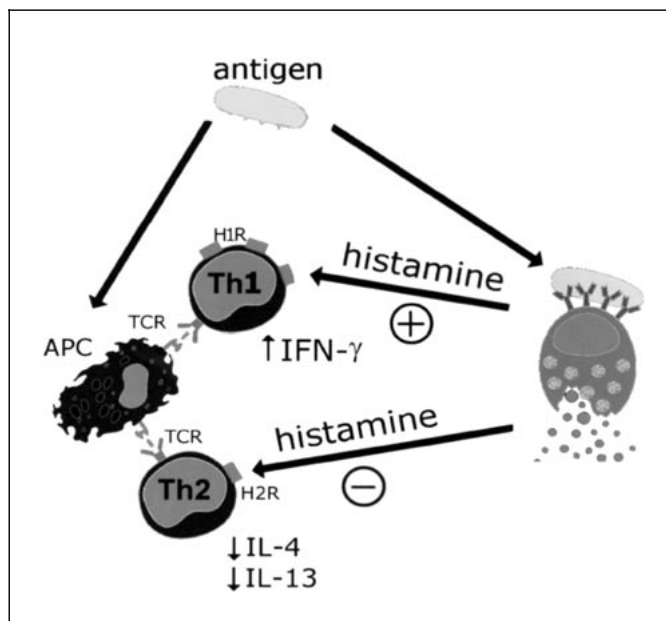


Fig. 1. Histamine counterregulates Th1 and Th2 responses. Th1-type responses are enhanced by histamine, whereas Th2-type responses are negatively regulated, due to distinct histamine receptor patterns and different intracellular signals generated by histamine stimulation. TCR = T cell receptor; APC = antigen presenting cell.

cells, which in turn reconstitute a Th1/Th0-type cytokine profile [11–14]. Since mast cells and basophils are targets of SIT [15, 16], histamine released by high doses of allergen during SIT can redirect the immune response from a dominating Th2-type towards a Th1-type pattern. Moreover, histamine produced by neoplastic cells enhances Th1-type cellular immunity directed against tumors [17].

The above results support the concept that histamine enhances Th1-type responses by interaction with H1R, whereas Th2-type responses are negatively regulated by H2R triggering. It can be concluded that histamine triggers negative feedback regulatory mechanisms in allergic inflammation and specific immunotherapy (fig. 1). Histamine has not been considered an important immunoregulator to date. Since we clearly showed the impact of this mediator on T lymphocyte regulatory mechanisms, these data contribute to the understanding of immune regulation in general and provide new therapeutic strategies aiming at the restoration of homeostasis with a balance between mediator secretion and receptor expression.

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References

- Mosmann TR, Sad S: The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996;17:138–146.
- Romagnani S: Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994;12:227–257.
- Falus A, Meretey K: Histamine: An early messenger in inflammatory and immune reactions. *Immunol Today* 1992;13:154–156.
- Leurs R, Smit MJ, Timmerman H: Molecular pharmacological aspects of histamine receptors. *Pharmacol Ther* 1995;66:413–463.
- Yamashita M, Fukui H, Sugama K, Horio Y, Ito S, Mizuguchi H, Wada H: Expression cloning of a cDNA encoding the bovine histamine H1 receptor. *Proc Natl Acad Sci USA* 1991;88:11515–11519.
- Gantz I, DelValle J, Wang LD, Tashiro T, Munzert G, Guo YJ, Konda Y, Yamada T: Molecular basis for the interaction of histamine with the histamine H2 receptor. *J Biol Chem* 1992;267:20840–20843.
- Sallusto F, Mackay CR, Lanzavecchia A: Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 1997;277:2005–2007.
- Faith A, Akdis CA, Akdis M, Simon HU, Blaser K: Defective TCR stimulation in anergized type 2 T helper cells correlates with abrogated p56(lck) and ZAP-70 tyrosine kinase activities. *J Immunol* 1997;159:53–60.
- Akdis M, Simon HU, Weigl L, Kreyden O, Blaser K, Akdis CA: Skin homing (cutaneous lymphocyte-associated antigen-positive) CD8+ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. *J Immunol* 1999;163:466–475.
- Akdis M, Akdis CA, Weigl L, Disch R, Blaser K: Skin-homing, CLA+ memory T cells are activated in atopic dermatitis and regulate IgE by an IL-13-dominated cytokine pattern: IgG4 counter-regulation by CLA- memory T cells. *J Immunol* 1997;159:4611–4619.
- Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR: Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-γ secretion in specific allergen-stimulated T cell cultures. *J Immunol* 1995;154:4187–4194.
- Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Muller U, Blaser K: Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. *J Clin Invest* 1996;98:1676–1683.
- Akdis CA, Blaser K: IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: Two key steps in specific immunotherapy. *FASEB J* 1999;13:603–609.
- Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K: Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998;102:98–106.
- Jutel M, Muller UR, Fricker M, Rihs S, Pichler W, Dahinden C: Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. *Clin Exp Allergy* 1996;26:1112–1118.
- Jutel M, Skrbic D, Pichler WJ, Muller UR: Ultra rush bee venom immunotherapy does not reduce cutaneous weal responses to bee venom and codeine phosphate. *Clin Exp Allergy* 1995;25:1205–1210.
- Bartholeyns J, Bouclier M: Involvement of histamine in growth of mouse and rat tumors: Antitumoral properties of monofluoromethyl-histidine, an enzyme-activated irreversible inhibitor of histidine decarboxylase. *Cancer Res* 1984;44:639–645.

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