

Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2

Natalija Novak, MD,^a Nihal Mete, MD,^{b,c} Caroline Bussmann, MD,^a Laura Maintz, MD,^a Thomas Bieber, MD, PhD,^a Mübcecel Akdis, MD, PhD,^b Judith Zumkehr, MSc,^b Marek Jutel, MD,^d and Cezmi Akdis, MD^b Bonn, Germany, Davos, Switzerland, Izmir, Turkey, and Wrocław, Poland

Background: Early desensitization of FcεRI-bearing mast cells and basophils has been demonstrated in allergen-specific immunotherapy and drug desensitization. However, its mechanisms have not been elucidated in detail. Histamine is one of the main mediators released on FcεRI triggering of basophils and mast cells, and it exerts its functions through histamine receptors (HRs).

Objectives: We sought to investigate HR expression on basophils of patients undergoing venom immunotherapy (VIT) and its effect on allergen, IgE, and FcεRI cross-linking-mediated basophil function and mediator release.

Methods: Basophils were purified from the peripheral blood of patients undergoing VIT and control subjects and were studied functionally by using real-time PCR, flow cytometry and ELISA assays.

Results: Rapid upregulation of H2R within the first 6 hours of the build-up phase of VIT was observed. H2R strongly suppressed FcεRI-induced activation and mediator release of basophils, including histamine and sulfidoleukotrienes, as well as cytokine production *in vitro*.

Conclusion: Immunosilencing of FcεRI-activated basophils by means of selective suppression mediated by H2R might be highly relevant for the very early induction of allergen tolerance

and the so-called desensitization effect of VIT. (J Allergy Clin Immunol 2012;■■■■:■■■-■■■.)

Key words: Human, basophils, histamine receptor 2, ultrarush immunotherapy, tolerance

Specific venom immunotherapy (VIT) for the treatment of honeybee and wasp venom allergy is highly effective in protecting sensitized patients from further systemic anaphylactic reactions to stings.¹ Rapid clinical tolerance has been described within a few hours in patients undergoing rush and ultrarush VIT.² Currently, VIT represents one of the most established methods for long-term treatment of allergic sensitizations, which otherwise might lead to severe anaphylactic reactions caused by overactivation, degranulation, and excessive release of mediators by mast cells and basophils. Consequently, knowledge about mechanisms involved in the early and late induction of allergen-specific tolerance is of major interest to improve treatment strategies for allergen-specific immunotherapy (SIT), to overcome unwanted side effects, and to identify markers for therapeutic efficacy. Many of the primary effects of SIT are exerted on effector cells. However, there is surprisingly little information about the mechanisms by which SIT modifies and suppresses immune responses of basophils and mast cells, particularly during repetitive administration of increasing allergen doses within a few hours during the build-up phase. Exhaustion of stored mediators of effector cells caused by repetitive stimulation and release (ie, tachyphylaxis) is regarded as a key mechanism responsible for protection at early time points of SIT.³ However, it is generally thought that these mechanisms exceed the borders of allergen SIT and are relevant for the mechanisms of rapid desensitization against certain drugs, which has been performed for penicillin at earlier times and is performed for cancer chemotherapy nowadays.⁴

One of the main soluble factors liberated by effector cells after allergen challenge is histamine, which mediates its effects through histamine receptors (HRs). Thus far, 4 different human HR types have been identified as H1R to H4R.⁵ Both the expression pattern of HRs and the modifications in the intensity of expression of a single HR type are decisive for the nature of the immune response.⁶⁻⁸ H1R possesses much proinflammatory and cell-activating properties, whereas H2R has been shown to be coupled to adenylate cyclase and phosphoinositide second messenger systems and to be associated with tolerogenic immune responses. This assumption evolves mainly from H2R-mediated suppressive effects on different T-cell subtypes^{9,10} and upregulation of both T_H1 and T_H2 cytokines in H2R-deficient mice.¹¹ Furthermore, H2R has been demonstrated to augment TGF-β-mediated suppression of T_H2 cellular responses.¹² In addition, expression of H2R on allergen-specific T cells increases significantly within 7 days in the beginning of the beekeeping season

From ^athe Department of Dermatology and Allergy, University of Bonn; ^bthe Swiss Institute of Allergy and Asthma Research (SIAF), Davos; ^cEge University Medical Faculty, Internal Medicine, Allergy and Clinical Immunology, Izmir; and ^dWrocław University Medical Faculty, Wrocław.

Supported by grants from the Deutsche Forschungsgemeinschaft (DFG NO454/1-4; SFB704 TPA4), a BONFOR grant of the University of Bonn, Swiss National Science Foundation Grant 32-118226, and the Christine Kühne Center for Allergy Research and Education (CK-CARE). N.N. was supported by a Heisenberg-Professorship of the DFG NO454/5-2.

Disclosure of potential conflict of interest: N. Novak has received research support from the German Research Council and is on the speakers' bureau for ALK-Abelló, Bencard Allergy Therapeutics, and Astellas Pharma. M. Akdis receives research support from the Swiss National Foundation and the European Union. J. Zumkehr is employed by CSL Behring. M. Jutel receives research support from the Polish National Science Centre. C. Akdis receives research support from Novartis, PREDICTA, the Swiss National Science Foundation, MeDALL, the Global Allergy and Asthma European Network, and the Christine Kühne Center for Allergy Research and Education; has consulted for Actellion, Avantis, Stallergenes, and Allergopharma; is President of the European Academy of Allergy and Clinical Immunology; is a fellow and interest group member of the American Academy of Allergy, Asthma & Immunology; is an ex-committee member of the Global Allergy and Asthma European Network; and is director of the Christine Kühne Center for Allergy Research and Education. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 28, 2011; revised March 23, 2012; accepted for publication April 26, 2012.

Corresponding author: Natalija Novak, MD, Department of Dermatology and Allergy, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany. E-mail: Natalija.Novak@ukb.uni-bonn.de.

0091-6749/\$36.00

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doi:10.1016/j.jaci.2012.04.039

Abbreviations used

cAMP: Cyclic AMP
 HR: Histamine receptor
 SIT: Allergen-specific immunotherapy
 VIT: Venom immunotherapy

in sensitized beekeepers who are stung but have effective tolerance against bee venom allergens.¹⁰ As a correlate of this finding, H2R attenuates histamine-mediated proliferation of allergen-specific T cells and increases their IL-10 production *in vitro*.^{10,13} These data imply that H2R might convey suppressive protolerogenic functions in protection against allergic diseases. H4R has significant homology (approximately 35% to 48%) with H3R, but in contrast to H3R, it is expressed by peripheral blood cells, including basophils,^{5,14} whereas expression of H3R is restricted to cells of the nervous system. H4R increases intracellular Ca^{2+} flux and mitogen-activated protein kinase signaling and inhibits the cyclic AMP (cAMP) pathway.⁵ Functions of H4R on different cells elucidated thus far are various and include promotion of chemotaxis, release of chemokines, modulation of adhesion molecule expression, and induction of apoptosis.¹⁵⁻¹⁷ However, the role of HR2 on basophil function has not been examined in detail. Therefore we investigated HR expression on basophils of patients undergoing VIT and its effect on allergen, IgE, and FcεRI cross-linking-mediated basophil function and mediator release in very early phases of allergen tolerance, the so-called desensitization phase.

METHODS**Characterization of patients**

VIT was performed on 42 patients (age range, 18-72 years; mean age, 47 ± 11.8 years; 23 female and 19 male patients; mean total serum IgE level, 164 ± 186.3 kU/L; mean tryptase level, 4 μg/L) sensitized to honeybee (n = 6; mean specific serum IgE level, 5 ± 14.1 kU/L) and wasp (n = 36; mean specific serum IgE level, 18 ± 23.8 kU/L) venom according to the rush protocol (ALK-lyophilisiert SQ; ALK-Scherax, Hamburg, Germany).³ All patients had a history of a systemic anaphylactic reactions to a Hymenoptera sting. Clinical symptoms and the severity of anaphylactic reactions to Hymenoptera sting were evaluated with a standard questionnaire. All patients received an H1R antagonist (1 tablet of 10 mg of cetirizine) every morning from days 2 to 5 up to 3 times daily, if required. After informed consent, peripheral venous blood was obtained immediately before VIT and 6 hours later on day 0 and after the last injection every following day during the build-up phase. The protocol was approved by the ethics committee of the University of Bonn.

None of the patients had any moderate or severe side effects despite transient local erythema or swelling at the injection site. For functional experiments, basophils of 41 control subjects were isolated. Because of blood volume limitations, we were unable to run all assays on the same subjects. Therefore for each experimental setting, a different group of subjects was used in the chronologic order in which the experimental subsections were performed.

Reagents

Monomeric human myeloma IgG was obtained from Calbiochem-Novabiochem Corp (San Diego, Calif). Monoclonal antibody against IgE (mIgG_{2a}, G7-18) and Fast Immune anti-CD63-fluorescein isothiocyanate/CD123PE/HLA-DR-peridinin chlorophyll protein antibody cocktail, anti-IL-8 (mIgG_{2b}, G265-8) phycoerythrin-labeled antibodies, and respective isotype controls were from BD Biosciences PharMingen (Heidelberg, Germany), and phycoerythrin-conjugated mAb against CD203c (mIgG₁, 97A6) was from Immunotech (Marseille, France). Dimaprit dihydrochloride,

histamine, clobenpropit, clozapine, and forskolin were from Sigma-Aldrich (Taufkirchen, Germany).

For more information on isolation of basophils, see the **Methods** section in this article's Online Repository at www.jacionline.org.

Cell-surface and intracellular phenotyping of basophils by means of flow cytometry

Extracellular and intracellular staining of basophils was performed as reported in detail elsewhere.¹⁸ Measurement of CD63 surface expression was performed with the help of a flow cast using heparin blood according to the manufacturers protocol (BD Biosciences PharMingen).

Receptor ligation

Purified basophils ($0.5-1 \times 10^5$ mL) were resuspended in HEPES-buffered Tyrode solution, containing 1 mmol/L CaCl_2 , warmed for 15 minutes at 37°C, and left unstimulated or incubated with 2.5 μg/mL human myeloma IgE for 30 minutes. After 2 washing steps, basophils were incubated for 30 minutes with 1 μg/mL anti-IgE mAb. For selected experiments, basophils were preincubated for 60 or 120 minutes with histamine, dimaprit, clobenpropit, or forskolin (10^{-8} to 10^{-4} mol/L, Sigma-Aldrich) before FcεRI stimulation.

Measurement of histamine, sulfidoleukotrienes, IL-4, and IL-8

Histamine levels in cell-culture supernatants were analyzed by using ELISA (Neogen Corp, Scotland, United Kingdom), according to the manufacturer's instructions. Sulfidoleukotriene levels were evaluated with the Leukotriene C4 EIA Kit, according to the manufacturer's instructions (Cayman Chemical Co, Ann Arbor, Mich).

IL-4 and IL-8 were detected in supernatants by using the Flex Set detecting kit from BD Biosciences and the BDArray (BD Biosciences, Heidelberg, Germany), according to the manufacturer's instructions.

For more information on real-time PCR, see the **Methods** section in this article's Online Repository.

Statistical analysis

Statistical analysis was performed with SPSS 17.0 software for Windows (SPSS, Inc, Chicago, Ill). Quantitative values were compared between different conditions using the Wilcoxon signed-rank test. Results are presented as means ± SEMs, respectively. Any *P* values are 2-sided and subject to a global significance level of 5%.

RESULTS**Rapidly increased H2R and H4R mRNA expression in basophils during the build-up phase of rush immunotherapy**

There is insufficient knowledge about the mechanisms of very early induction of allergen tolerance and suppression of effector cell activation and mediator release, although it is commonly observed in repetitive and increasing doses of allergen administration during VIT.¹⁹ Histamine represents the essential mediator released from mast cells and basophils during allergen exposure. Accordingly, we measured H1R, H2R, and H4R mRNA expression in PBMCs and H2R and H4R mRNA expression of purified basophils during the build-up phase of ultrarush VIT. A significant upregulation of basophil H2R and H4R mRNA expression was observed as early as 6 hours after the first injections, with further increased expression of H2R and H4R mRNA until day 4 (Fig 1). In addition, we observed a significant increase in H2R mRNA expression in PBMCs at days 3 and 4 (see Fig E1 in this article's Online Repository at www.jacionline.org). H4R mRNA expression increased slightly in PBMCs during the first

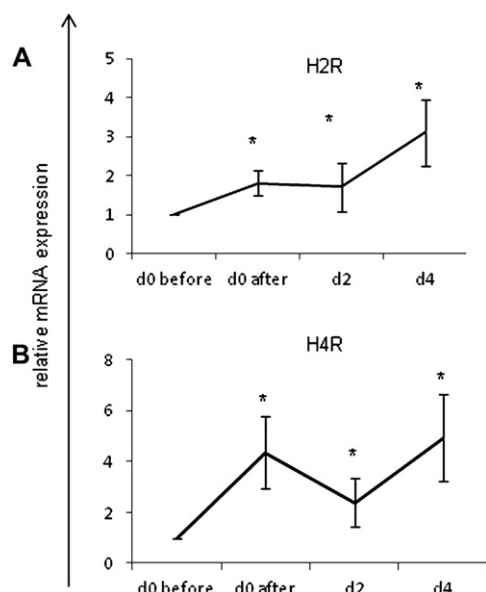


FIG 1. Basophil H2R/H4R mRNA expression is significantly upregulated during the first 6 hours of ultrarush immunotherapy. H2R (**A**) and H4R (**B**) mRNA expression levels of purified basophils are shown normalized to mRNA expression of 18s as a housekeeping gene on day 0 before VIT, 6 hours after the beginning of VIT, and on days 2 and 4 ($n = 7$). $*P < .05$.

days of allergen application, whereas no significant changes were detected for H1R mRNA expression of PBMCs. From these data, we conclude that circulating basophils upregulate H2R and H4R mRNA expression within a few hours of VIT. It is important to emphasize here that cells were analyzed immediately after purification without any cultures, representing the *in vivo* situation.

Decreased circulating basophil numbers during the first few days of SIT

Because one possible mechanism of tachyphylaxis and tolerance induction could be selective deletion of cells by means of apoptosis, we next evaluated the absolute number of basophils in the peripheral blood. Circulating basophil numbers started to decrease 6 hours after the first injection, and this reached statistical significance at day 4 (Fig 2, A). For comparison, the number of eosinophils remained unchanged (Fig 2, B). 7-Aminoactinomycin and Annexin-V staining of basophils from patients' blood showed that this reduction was partially based on decreased basophil viability *in vivo* (Fig 2, C).

Inhibition of basophil activation through H2R

The next question was whether stimulation of H2R with H2R agonists or direct activation of cAMP signaling suppress FcεRI-mediated basophil activation and mediator release. We investigated CD63 and CD203c expression on basophils, which represent activation- and degranulation-related basophil markers, within 30 to 60 minutes of IgE cross-linking. We found dose-dependent reduction of FcεRI cross-linking-mediated CD203c expression by purified basophils stimulated with an H2R agonist or the cAMP inducer forskolin (Fig 3, A). Furthermore, FcεRI cross-linking-induced activation and upregulation of CD63 expression of basophils from whole blood samples was profoundly reduced by means of preincubation of the blood

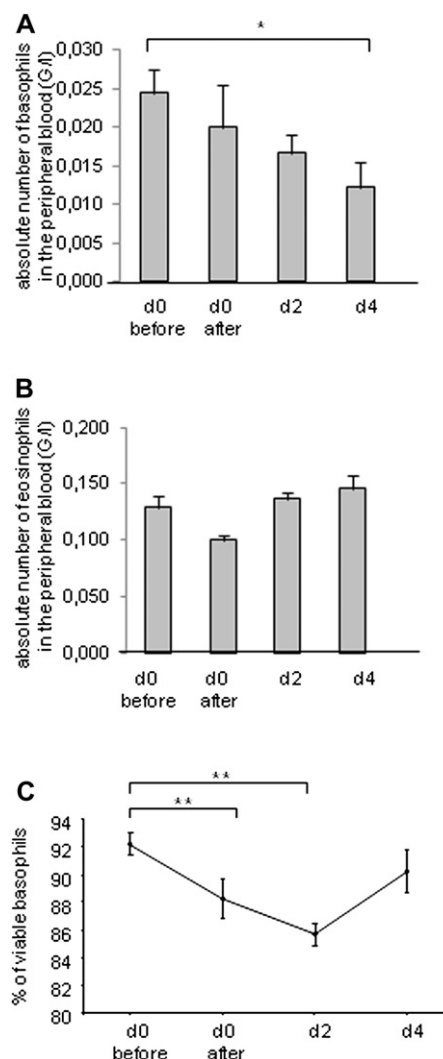


FIG 2. Absolute number of viable basophils in peripheral blood decreases during the build-up phase of ultrarush immunotherapy. **A** and **B**, The absolute number of basophils (Fig 2, A) and eosinophils (Fig 2, B) in the peripheral blood (differential blood count) of 13 patients (mean $\pm$ SEM) is depicted. **C**, Percentages of nonapoptotic, nonnecrotic, viable basophils in the peripheral blood of 7 patients during the build-up phase of ultrarush VIT. Values are presented as means $\pm$ SEMs. $*P < .05$ and $**P < .01$.

with the H2R agonist dimaprit or the cAMP inducer forskolin before FcεRI triggering (Fig 3, B and C). Interestingly, a strong additive effect on basophil suppression (58% reduction) was achieved by using preincubation of basophils with dimaprit and forskolin together.

H2R attenuates IgE, FcεRI cross-linking-induced release of soluble mediators, and IL-4 and IL-8 production of basophils

Release of soluble mediators and proinflammatory cytokines after FcεRI aggregation is a characteristic feature of basophils.²⁰ Therefore we wanted to evaluate the effect of H2R ligation on mediator release of basophils and their production of cytokines. We found significant downregulation (88%) of the intracellular IL-8 expression (data not shown) and IL-4 and IL-8 protein release after activation of basophils through FcεRI when they were

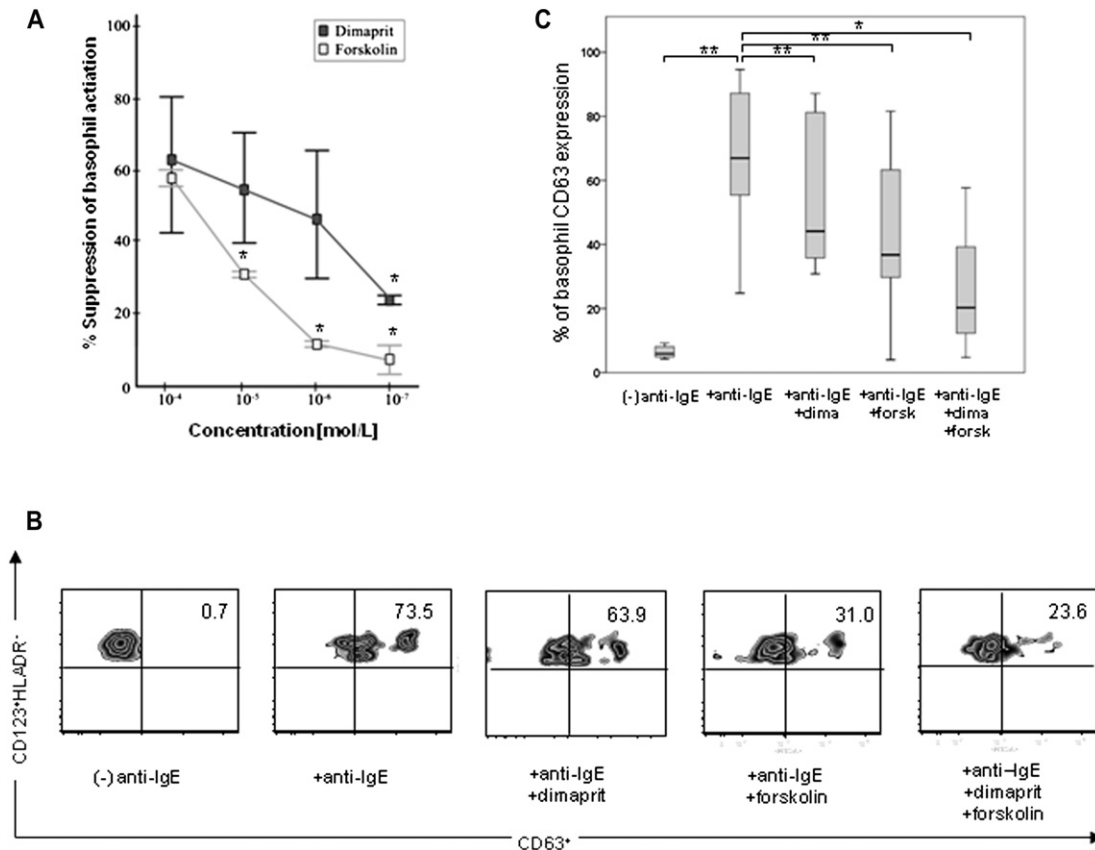


FIG 3. H2R suppresses IgE/FcεRI cross-linking-induced basophil activation. **A**, The mean $\pm$ SEM value of the percentage of suppression of basophil activation normalized to the CD203c surface expression of FcεRI-activated control cells and cells after preincubation with increasing amounts of dimaprit or forskolin for 6 experiments is depicted on the y-axis, whereas concentrations of dimaprit/forskolin are shown on the x-axis. **B**, Percentages of CD63 expression of CD123⁺/HLA-DR⁺ basophils from whole blood samples with and without FcεRI-cross linking by anti-IgE mAb after preincubation with 10^{-4} mol/L dimaprit (*dima*), 10^{-4} mol/L forskolin (*forsk*), or both are depicted. **C**, Mean $\pm$ SD values of the percentage of CD63-expressing basophils of 10 independent experiments are shown. * $P < .05$ and ** $P < .01$.

preincubated for 1 hour with the H2R agonist dimaprit or the cAMP inducer forskolin (Fig 4, A and B). In addition, we observed a dose-dependent reduction (up to 82%) of sulfidoleukotriene release of basophils after FcεRI aggregation in the presence of the H2R agonist dimaprit or the cAMP inducer forskolin (Fig 4, C).

Moreover, we observed a significantly lower histamine release of basophils stimulated through H2R with the ligand dimaprit before FcεRI activation (Fig 4, D). Together, these data provide clear evidence for a dominant function of H2R on the activation, degranulation, mediator release, and cytokine production of basophils and suggest a role for other cAMP inducing G protein-coupled receptors.

DISCUSSION

In this study we elucidated a novel function of H2R on human basophils, which might be of crucial importance for early H2R-mediated suppression of these cells, leading to the induction of rapid allergen tolerance in the periphery, namely the early desensitization effect in clinical settings. We provide comprehensive evidence for attenuated FcεRI-mediated activation and mediator release of basophils after selective stimulation of H2R. These data imply that H2R exerts immunosuppressive effects on

basophils. Here we provide substantial data representing the direct *in vivo* situation in subjects who receive repeated and increasing doses of allergens because analyses have been performed directly after purification of the cells without any further culture. We observed sequential upregulation of inhibitory H2R on basophils within 6 hours during the build-up phase of VIT, which might contribute to very early allergen tolerance induction and immunosilencing of effector cells during this critical treatment phase. It is essential that effector cell hyperresponsiveness be constrained in the very early hours after allergen exposure to prevent systemic anaphylaxis.

Until the present study, the knowledge about mechanisms leading to desensitization of effector cells and early induction of tolerance on the level of the primary mediators of allergic reactions during VIT has been limited. A temporarily decreased release of mediators from effector cells immediately after rush VIT has been supposed to account for early onset of protection.²¹⁻²³ Furthermore, sulfidoleukotriene and histamine release of basophils was reduced by addition of IL-10 or IFN- γ *in vitro*, levels of which increase in the sera of patients during the build-up phase of VIT, implying that an increase in these soluble factors in the cellular microenvironment might decrease effector cell functions *in vivo*.^{23,24} In addition, upregulation of intracellular cAMP has been shown in PBMCs during VIT.^{21,25} Because

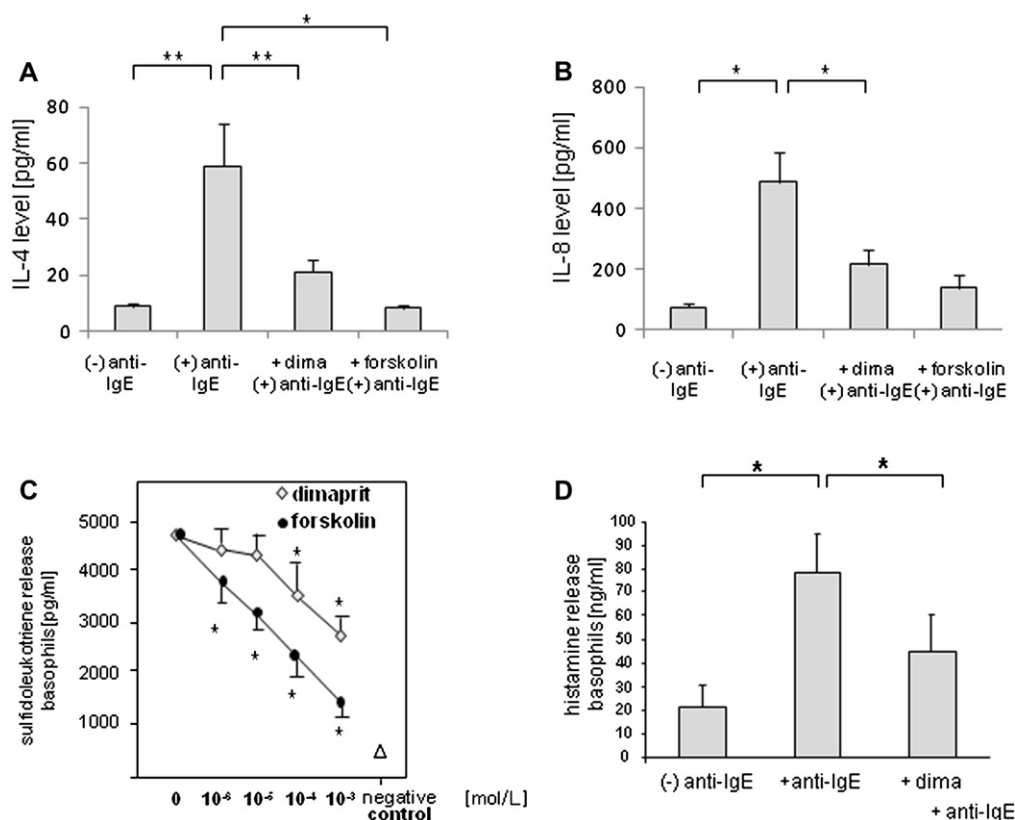


FIG 4. Basophil IL-4 and IL-8 release and sulfidoleukotriene release of basophils decreases after preincubation with dimaprit (*dima*) or forskolin. **A** and **B**, Mean $\pm$ SEM values of IL-4 (Fig 4, A) and IL-8 (Fig 4, B) levels detected by using Flex Set assays after 12 hours of 12 experiments ($n = 6$ experiments for preincubation with forskolin) are shown. **C**, A dose-dependent decrease in sulfidoleukotriene release depicted as mean $\pm$ SEM values on the y-axis for 6 experiments is shown. **D**, Mean $\pm$ SEM values of histamine released from basophils after 20 minutes of Fc ϵ RI stimulation with or without dimaprit preincubation for 6 experiments are depicted on the y-axis. * $P < .05$. ** $P < .01$.

one of the most prominent mediators released by effector cells during allergen challenge is histamine, levels of which increase profoundly in the plasma of patients undergoing VIT during the early build-up phase,³ an endogenous histamine- and HR-mediated feedback regulation leading to the attenuation of basophil activation would be a plausible and reasonable mechanism. This might represent one of the most important effects on basophils during the build-up phase of VIT, anticipating a natural requirement to control immediate-type (type I) hypersensitivity reactions. On the basis of the data demonstrated here, such a self-inactivation of basophils would be carefully regulated by the frequency of IgE and Fc ϵ RI-mediated effects of the allergen together with the concentration of basophil and mast cell mediators in the cellular microenvironment and might be mainly responsible for the relatively low rate of anaphylactic reactions in clinical practice. This seems to be an essential mechanism to regulate the thresholds of systemic anaphylaxis and probably takes place to prevent anaphylaxis in conditions in which allergens pass to the systemic circulation as a result of high doses of exposure, such as the in patients at the beginning of the pollen season, in cat owners, and perhaps also during blood transfusions.

We propose 2 mechanisms related to classical models of tolerance induction to be responsible for early control of basophil activation in patients undergoing VIT: (1) reduction of total basophil numbers caused by increased “deletion” of the cells and (2) allergen-mediated upregulation of H2R on basophils leading

to effective “suppression.”²⁶ In this context we could clearly show that H2R-induced suppression of basophil activation and mediator release was most likely mediated by the cAMP pathway because both stimulation with an H2R agonist and with a direct cAMP inducer were able to achieve these inhibitory effects. The cAMP pathway is one of the best-characterized second messenger pathways.²⁷ H2R belongs to the G α /s protein-coupled receptors, and histamine binding induces intracellular signals, such as adenylate cyclase activation, leading to the production of cAMP.⁹ Several years ago, cAMP activation was shown to regulate the function of human basophils and lung mast cells and to inhibit mediator release of these cells in a dose-dependent fashion.²⁵ Currently, we know that this adenylate cyclase-mediated second messenger system is also associated with other G protein-coupled receptors.⁵ For example, induction of the cAMP pathway has been linked to TGF- β -mediated inhibition of IL-4 secretion of CD4⁺ T cells, which is further enhanced by H2R ligation and concomitant cAMP induction.²⁸ Therefore H2R-mediated adenylate cyclase induction and the respective downstream signaling are very likely to occur in response to H2R ligation on basophils as well.

Whether the novel H2R-mediated mechanisms on basophils described here are limited to effector cells in the blood or are also of relevance for mast cells in peripheral organs, such as the skin or the mucosa of the airways, remains to be investigated.

The question arises of whether cotreatment of patients with severe side effects, particularly during the build-up phase of VIT, with H2R agonists or cAMP inducers might accelerate the establishment of the early desensitization effect because of effector cell silencing. Furthermore, because it has been shown very recently that concomitant treatment with H1R antagonists reduces the frequency of side effects without any effect on the efficacy of VIT,²⁹ it would be important to rule out whether treatment with H1R antagonists promotes or reduces the protective effects driven by H2R on basophils. Additionally, on the basis of this novel concept of suppressive function of H2R on effector cells, it would be important to figure out whether genetic modifications of H2R expression or function might lead to a (partial) loss of these in some settings, perhaps even life-threatening self-protecting effects, which promote the manifestation of effector cell hyperresponsiveness in patients with some types of allergic diseases. Such a mechanism might be of relevance for patients with severe anaphylaxis of unknown cause or any kind of exceptionally intense intolerance reactions.

Taken together, we introduce a novel mechanism that might explain basophil silencing based on histamine/HR-mediated tolerogenic functions, which are most likely responsible for the early induction of tachyphylaxis under some physiologic conditions and might contribute to both the safety and efficacy of SIT, particularly during the build-up phase. A detailed understanding of these pathways would allow therapeutic intervention to promote effector cell silencing, particularly in patients with severe side effects or increased risk for anaphylactic reactions during VIT, as well as in patients with diseases in which unwanted effector cell hyperresponsiveness represents a severe and intractable pathogenetic factor.

Clinical implications: H2R-mediated basophil suppression might contribute to early protective mechanisms during the build-up phase of VIT.

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METHODS

Isolation of basophils

Blood was taken from volunteers at the Department of Dermatology and Allergy of the University of Bonn. PBMCs were isolated as described previously.^{E1} Enrichment of basophils was performed as described elsewhere.^{E2} Briefly, 45 mL of K3-EDTA blood was mixed with 6 mL of HetaSep (STEMCELL Technologies, Vancouver, British Columbia, Canada) split into 2 tubes and centrifuged to separate red blood cells from leukocytes. The upper phase containing leukocytes was washed with Dulbecco PBS with 1 mM of EDTA and 2% FBS and centrifuged again. Afterward, the pellet was resuspended in purification buffer and transferred to another tube, and Human Basophil Enrichment Cocktail (EasySep Human Basophil Enrichment Kit, STEMCELL Technologies) was added. After incubation, purification buffer and EasySep Magnetic Nanoparticles containing mAbs to the cell-surface antigens CD2, CD3, CD4, CD15, CD16, CD19, CD24, CD34, CD36, CD45RA, and glycophorin A were added to the antibody cell suspension, followed by another incubation period. Cells were transferred to an EasySep magnet and incubated again. Alternatively, basophils were isolated from PBMCs with the Basophil Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) and the AUTOMACS technique, according to the manufacturer's instructions. Unbound cells were collected, washed, and resuspended in HEPES-buffered Tyrode solution without calcium. The purity and viability of the cells was determined by means of flow cytometric staining with a FACS-Canto (BD Biosciences) and analyzed with FACSDiva (BD Biosciences) or Epics XL Flow Cytometer (Beckman Coulter AG) and FlowJo (TreeStar, Inc, Ashland, Ore) software.

Real-time PCR

mRNA was isolated with the RNeasy Mini kit (Qiagen, Hilden, Germany) and the Nucleo Spin RNA XS Kit (Macherey-Nagel GmbH, Düren, Germany) and was subjected to cDNA synthesis with TaqMan reverse transcription reagents by using random hexamers, according to the manufacturer's

instructions (Applied Biosystems, Darmstadt, Germany). The prepared cDNA was amplified with the TaqMan Assay Master Mix (Applied Biosystems), according to the recommendations of the manufacturer, in an ABI Prism 7300 Sequence Detection System (Applied Biosystems). Primers were as follows: H1R forward primer, 5'-TCT CGA ACG GAC TCA GAT ACC A-3'; H1R reverse primer, 5'-CCT GTG TTA GAC CCA CTC CTC AA-3'; H1R probe, FAM-ACA GAG ACA GCA CCA GGC AAA GGC AA-TAMRA; H2R forward primer, 5'-GCT GGG CTA TGC CAA CTC A-3'; H2R reverse primer, 5'-GGT GCG GAA GTC TCT GTT CAG-3'; and H2R probe, FAM-CCC TGA ACC CCA TCC TGT ATG CTG C-TAMRA; H4R forward primer, 5'-GGG TCT TGA AGATTG TTA CTC TGA TG-3'; H4R reverse primer, 5'-CTA GAATCATTTG GCC CAT TCA CT-3'; and H4R probe, FAM-GCC AGC ACC CAA ACG GCC A-TAMRA (all from Microsynth AG, Balgach, Switzerland). The endogenous control (18s) was from Applied Biosystems. Relative quantification and calculation of the range of confidence was performed by using the comparative $\Delta\Delta CT$ methods.^{E3} All analyses were conducted in duplicates, and experimental samples were quantitated by using sample dilutions, ensuring that the cycle threshold values were in the linear proportion of the standard curve.

REFERENCES

- E1. Novak N, Allam JP, Hagemann T, Jenneck C, Laffer S, Valenta R, et al. Characterization of FcεRI-bearing CD123 blood dendritic cell antigen-2 plasmacytoid dendritic cells in atopic dermatitis. *J Allergy Clin Immunol* 2004;114:364-70.
- E2. Gibbs BF, Papenfuss K, Falcone FH. A rapid two-step procedure for the purification of human peripheral blood basophils to near homogeneity. *Clin Exp Allergy* 2008;38:480-5.
- E3. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008;3:1101-8.

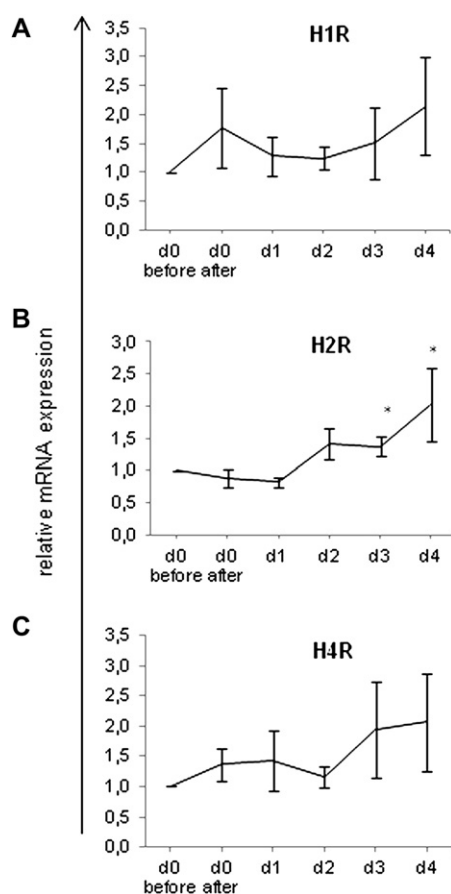


FIG E1. PBMC H2R mRNA expression is significantly upregulated during the first 5 days of ultrarush immunotherapy. H1R (A), H2R (B), and H4R (C) mRNA expression levels (mean $\pm$ SEM values of 12 experiments for PBMCs) are illustrated normalized to expression of 18s as a housekeeping gene. * $P < .05$.