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] Mübeccel Akdis, MD, PhD,[b] Judith Zumkehr, MSc,[b] Marek Jutel, MD,[d] and Cezmi Akdis, MD[b] *Bonn, Germany, Davos, Switzerland, Izmir, Turkey, and Wroclaw, 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;130:1153-8.)

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

From [a]the Department of Dermatology and Allergy, University of Bonn; [b]the Swiss Institute of Allergy and Asthma Research (SIAF), Davos; [c]Ege University Medical Faculty, Internal Medicine, Allergy and Clinical Immunology, Izmir; and [d]Wroclaw University Medical Faculty, Wroclaw.

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 NO4545/5-2.

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.

Available online June 12, 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

doi:10.1016/j.jaci.2012.04.039

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

suppressive effects on different T-cell subtypes[5,9,10] 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 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 IgE 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 ($mIgG_1$, 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 manufacturer's protocol (BD Biosciences PharMingen).

Receptor ligation

Purified basophils (0.5-1 × 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, III). 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