Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy

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Background: H1 antihistamines increase safety during allergen-specific immunotherapy and might influence the outcome because of immunoregulatory effects.
Objective: We sought to analyze the influence of 5 mg of levocetirizine (LC) on the safety, efficacy, and immunologic effects of ultrarush honeybee venom immunotherapy (BVIT).
Method: In a double-blind, placebo-controlled study 54 patients with honeybee venom allergy received LC or placebo from 2 days before BVIT to day 21. Side effects during dose increase and systemic allergic reactions (SARs) to a sting challenge after 120 days were analyzed. Allergen-specific immune response was investigated in skin, serum, and allergen-stimulated T-cell cultures.
Results: Side effects were significantly more frequent in patients receiving placebo. Four patients receiving placebo dropped out because of side effects. SARs to the sting challenge occurred in 8 patients (6 in the LC group and 2 in the placebo group). Seven SARs were only cutaneous, and 1 in the placebo group was also respiratory. Difference of SARs caused by the sting challenge was insignificant. Specific IgG levels increased significantly in both groups. Major allergen phospholipase A_2-stimulated T cells from both groups showed a slightly decreased proliferation. The decrease in IFN-γ and IL-13 levels with placebo was not prominent with LC, whereas IL-10 levels showed a significant increase in the LC group only. Decreased histamine receptor (HR)1/HR2 ratio in allergen-specific T cells on day 21 in the placebo group was prevented by LC.
Conclusions: LC reduces side effects during dose increase without influencing the efficacy of BVIT. LC modulates the natural course of allergen-specific immune response and affects the expression of HRs and cytokine production by allergen-specific T cells. (J Allergy Clin Immunol 2008;122:1001-7.)

Key words: *Venom immunotherapy, antihistamine preventive medication, T cells, cytokines, histamine receptors*

Hymenoptera venom allergy is a major cause for severe and potentially fatal anaphylaxis.[1] Immunotherapy with hymenoptera venoms was shown to be highly effective.[2] However, in patients with honeybee venom (BV) allergy, it might cause systemic allergic side effects in up to 20% to 40%, mainly during the dose-increase phase. For this reason, preventive medication with antihistamines is often used during the initial phase of honeybee venom immunotherapy (BVIT) and was shown to significantly reduce large local and generalized cutaneous reactions in several double-blind, placebo-controlled trials.[3-5] Preventive medication with antihistamines was also effective in reducing side effects from immunotherapy with tree and grass pollen.[6,7] The antihistamines used were terfenadine, loratadine, cetirizine, and fexofenadine. Thus reduction of side effects seems to be a histamine receptor (HR) 1–mediated class effect of antihistamines.

The mechanism by which immunotherapy induces protection is associated with changes in the fine balance between allergen-specific regulatory T cells and T_H2 cells, T_H1 cells, or both.[8] Histamine, originally considered a mediator of acute inflammatory and immediate hypersensitivity responses, has also been demonstrated to regulate antigen-specific T_H1, T_H2, and regulatory T cells, as well as related antibody isotype responses. Histamine enhances T_H1-type responses by triggering HR1, whereas both T_H1- and T_H2-type responses are negatively regulated by HR2.

There is some evidence that the expression of HRs is altered during immunotherapy.[9-11] The question of whether preventive medication with H1 antihistamines during allergen immunotherapy could influence the immune response to this treatment for better or worse arose. Previously, the long-term efficacy of BVIT, as indicated by the reaction to a field sting or a sting challenge, has only been analyzed retrospectively in the 52 patients of the first double-blind, placebo-controlled trial on H1 antihistamine preventive medication during allergen immunotherapy.[12] The results of this retrospective study suggested an increased efficacy in patients with antihistamine preventive medication

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Supported by Swiss National Science Foundation grant nos. 32.118226 and 32-112306, the Global Allergy and Asthma European Network (GA2LEN), and UCB Pharma AG.
Disclosure of potential conflict of interest: U. R. Müller has received a research grant from UCB. M. Jutel has served as a clinical investigator for Schering-Plough and AllergoPharma Joachim Ganzer and as an investigator for AllergoPharma Joachim Ganzer. M. Akdis has had research collaboration with Allergopharma, Stallergenes, ALK-Abelló, SARM, and UCB Pharma and has served as Section Board Member for the European Academy of Allergology and Clinical Immunology. C. Akdis has had research collaboration with Allergopharma, Stallergenes, ALK-Abelló, SARM, and UCB Pharma and has served as the Vice President of the European Academy of Allergology and Clinical Immunology and as a committee member for the Global Allergy and Asthma European Network. The rest of the authors have declared that they have no conflict of interest.
Received for publication April 28, 2008; revised July 9, 2008; accepted for publication August 11, 2008.
Available online October 10, 2008.
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0091-6749/$34.00

doi:10.1016/j.jaci.2008.08.007

Abbreviations used
BV: Honeybee venom
BVIT: Honeybee venom immunotherapy
HR: Histamine receptor
LC: Levocetirizine
PLA: Phospholipase A_2, a major allergen of honeybee venom
SAR: Systemic allergic reaction
SE: Side effect
STEPC: Skin test end point concentration
TT: Tetanus toxoid

during early BVIT compared with efficacy in those without. We therefore performed a prospective, double-blind, placebo-controlled trial, which is presented here. This study includes a sting challenge after 4 months of BVIT, as well as extensive investigation of allergen-specific skin tests and antibody and T-cell response, including proliferation, cytokine secretion, and HR expression.

METHODS

Study protocol

Fifty-four adult patients aged 18 to 65 years with a history of moderate-to-severe systemic allergic reactions (SARs) to honeybee stings grade II to IV,[13,14] positive intracutaneous skin tests to BV of less than or equal to 10^{-4} g/L, and BV-specific serum IgE (sIgE) levels of 0.7 kU/L or greater in the Immuno-CAP FEIA were included in the study. Exclusion criteria were pregnancy, breast-feeding, severe systemic and psychiatric disease, intake of β-blockers, angiotensin-converting enzyme inhibitors, and treatment with antihistamines within a week and systemic corticosteroids within a month before the start of the study.

Twenty-seven patients each were randomly assigned to preventive medication with 1 tablet daily of either 5 mg of levocetirizine (LC) or placebo from day −2 to day 21 of an ultrarush BVIT protocol.[15,16] During the ultrarush protocol on day 0, patients received 6 injections of BV starting at 0.1 μg, with a top dose of 50 μg and a cumulative dose of 111.1 μg. During the dose-increase (ultrarush) phase of BVIT, patients were monitored for blood pressure, pulse, electrocardiography, and peak flow in the intensive care unit, and a venous access with infusion of sodium chloride 0.9% was established before the first injection. On day 7, they received 2 injections of 50 μg, and on day 21, they received 1 injection of 100 μg. After the dose-increase phase, further injections of the maintenance dose of 100 μg of BV were administered on days 50, 80, and 110. Skin tests with BV, BV sIgE, and BV sIgG were repeated on day 110, and whole blood for PBMC cultures was taken on days −3 to −7, 21, and 110.

Primary end points were as follows: (1) occurrence of SARs and need for rescue medication after BVIT injections during the preventive treatment phase on days 0 to 21 and (2) occurrence of SARs and need for rescue medication after the sting challenge on day 120.

Secondary end points were as follows: (1) intracutaneous skin tests, (2) BV sIgE and sIgG serum antibodies, (3) phospholipase A_2 (PLA)–specific T-cell proliferation, (4) cytokine secretion in PLA-specific T-cell cultures, and (5) HR1 and HR2 receptor expression in PLA-specific T cells from before to day 110 of BVIT.

Written informed consent was obtained from each patient. The study protocol was approved by the Ethical Committee of the Canton of Bern, Switzerland.

Assessment of allergic reactions

SARs after BVIT injections or after the challenge were classified as purely subjective, such as itch, heat sensation, headache, and dizziness (grade 1); cutaneous, such as flush, urticaria, erythema, and angioedema (grade 2); gastrointestinal, such as abdominal cramps, vomiting, and diarrhea (grade 3); respiratory, such as dyspnea, wheezing, and decrease in peak flow of greater than 10% (grade 4); and cardiovascular, such as tachycardia, arrhythmia, decrease in blood pressure of greater than 20 mm Hg, collapse, and unconsciousness (grade 5).

Rescue medications were 0.3 mg of epinephrine administered subcutaneously for grade 2 and grade 3 reactions and 0.3 mg of epinephrine administered subcutaneously or intramuscularly, 2 mg of clemastine, and 125 mg of methylprednisolone administered intravenously for grade 4 reactions. Additional volume substitution and epinephrine administered by means of infusion were recommended for grade 5 reactions.

Sting challenge

The sting challenge with a live honeybee was performed on day 120 in the intensive care unit, with constant monitoring of pulse, blood pressure, electrocardiography, and repeated peak flow measurements before and after the challenge.[17] Intravenous access with an infusion of sodium chloride 0.9% was established before the challenge and remained for 2 hours. The sting was applied on the volar side of the forearm, and the stinger was left in the skin for 1 minute. Honeybees were kindly provided by the Swiss Institute for Agricultural Research in Bern.

Skin tests, BV sIgE, and BV sIgG serum antibodies

Lyophilized BV (Pharmalgen) for skin tests and venom immunotherapy was obtained from ALK-Abelló (Hørsholm, Denmark). Skin test end point concentration (STEPC) was determined by means of intracutaneous injection of 0.02 mL of serial dilutions of BV at 10^{-8}, 10^{-6}, and 10^{-4} g/L, as described earlier.[17] The lowest concentration resulting in a wheal reaction of 5 mm or greater in diameter with erythema is defined as STEPC. BV sIgE, sIgG, and tryptase levels were determined by means of Immuno CAP FEIA (Phadia, Uppsala Sweden).

Immunologic analyses

Material. Recombinant PLA (Api m 1) of BV *(Apis mellifera)* was used. Purified protein derivative of *Mycobacterium bovis* and tetanus toxoid (TT) were used as control antigens. None of the allergens contained detectable amounts of LPS, and all were more than 99% pure.

T-cell proliferation and cytokine detection. Allergen-specific T-cell proliferative response was determined by means of stimulation of 2×10^5 PBMCs for 5 days with 0.3 μmol/L PLA, 1 μg/mL TT, and purified protein derivative of *Mycobacterium bovis* in 200 μL of medium in 96-well flat-bottom tissue-culture plates in triplicate in RPMI 1640 medium supplemented as previously described.[18] Antigen-specific responding T cells were expanded until day 12 and restimulated with anti-CD2/CD3/CD28 mAbs for RNA expression. Solid-phase sandwich ELISAs for IFN-γ, IL-10, and IL-13 were performed in supernatants obtained after 5 days.[19]

Quantitative real-time PCR. T cells were lysed with RNeasy lysis buffer, and the RNA was isolated with the RNeasy mini kit (Qiagen, Hamburg, Germany) and eluted in 30 μL of double-distilled H_2O. Reverse transcription was performed with TaqMan reverse transcription reagents with random hexamers (Applied Biosystems, Rotkreuz, Switzerland). The PCR primers and probes were designed based on sequences reported in GenBank. Primers were as follows: EF-1α forward primer 5′CTG AAC CAT CCA GGC CAA AT 3′, EF-1α reverse primer 5′GCC GTG TGG CAA TCC AAT 3′, HR1 forward primer 5′-TCT CGA ACG GAC TCA GAT ACC A-3′, HR1 reverse primer 5′-CCT GTG TTA GAC CCA CTC CTC AA-3′, HR1 probe FAM-ACA GAG ACA GCA CCA GGC AAA GGC AA-TAMRA; HR2 forward primer 5′-GCT GGG CTA TGC CAA CTC A-3′, HR2 reverse primer 5′-GGT GCG GAA GTC TCT GTT CAG-3′, and HR2 probe FAM-CCC TGA ACC CCA TCC TGT ATG CTG C-TAMRA (all were from Microsynth AG, Balgach, Switzerland). cDNAs were amplified with SYBR-PCR Mastermix (Applied Biosystems) according to the recommendations of the manufacturer in a total volume of 25 μL in an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Relative quantification was performed as previously described.[20] All amplifications were carried out in duplicates.

TABLE I. Characterization of the study groups

Parameter	LC	Placebo	*P* value
No.	27	27	
Male/female sex	20/7	19/8	NS
Age (y), mean ± SD	40.6 ± 10.8	38.4 ± 9.4	NS
Age (y), range	18–58	17–57	
Atopy (positive skin prick test response to common inhalants)	16	12	NS
Severity (Mueller grade[12]) at the time of the first admission			
Grade 2	5	1	
Grade 3	12	10	
Grade 4	10	16	NS
STEPC MV (−log g/L), SD	5.78 ± 2.00	6.04 ± 1.55	NS
sIgE BV (kU/L), geometric MV ± SD	0.998 ± 0.076	0.95 ± 0.06	NS
sIgG BV (kU/L), geometric MV ± SD	0.860 ± 0.384	1.22 ± 0.30	NS
Baseline tryptase >11.4 ng/mL	1	0	
Beekeepers	4	5	

SD, Standard deviation; *NS,* not significant; *MV*, mean value.

TABLE II. Side effects of BVIT during and after premedication

Side effects of BVIT	Premedication with LC	Premedication with placebo	Odds ratio (95% CI)	*P* value
No. of patients	27	27		
Side effects during premedication (days 0-21)				
Total no. of injections	261	252		
Patients with subjective side effects	2	9	0.16 (0.03-0.8)	.04
Patients with objective side effects	5	9	0.45 (0.13-1.6)	NS
Episodes of subjective side effects	2/261	11/252	0.17 (0.04-0.77)	.01
Episodes of objective side effects	7/261	19/252	0.34 (0.14-0.82)	.01
Episodes with rescue medication	6/261	16/252	0.35 (0.13-0.9)	.03
Dropouts	0	4	0.1 (0.005-1.9)	NS
Side effects after premedication (days 50-110)	2	2		
Total no. of injections	81	72		
Objective side effects/injections	2/81	2/72	0.89 (0.12 - 6)	NS

NS, Not significant.

Statistical analysis

The proportions of venom immunotherapy injections and sting challenges with and without subsequent systemic allergic symptoms and the need for rescue medication were compared between the LC and placebo groups. The odds ratio and 2-sided *P* value were analyzed by using the Fisher exact test, and the corresponding 95% CIs were calculated by using the approximation of Woolf. The size of the groups was chosen based on previous studies on the safety of BVIT, which resulted in significant differences between groups.[3,5] Assuming an incidence of 2.5% SARs per injection, the study had an 80% power to detect a difference in risk of 5.5%. Based on the previous retrospective study,[12] we estimated the risk of SARs to re-exposure during BVIT with early premedication at 5%. Thus the actual study had an 80% power to detect a difference in risk of 30%.

Within groups, changes of skin reactivity, sIgE and sIgG levels, proliferation of PBMCs after PLA stimulation, HR expression, and interleukins in paired conditions before and after 21 and 110 days of BVIT were analyzed by using the Wilcoxon matched pairs test. Differences between the whole LC and placebo groups were analyzed by using the Mann-Whitney *U* test.

RESULTS

Patient groups

The 2 groups consisting of patients with preventive medication of either LC or placebo were comparable with regard to age, sex, atopy, skin sensitivity before treatment, and serum test results for specific IgE and IgG. The severity of sting reactions was comparable. Nine patients were beekeepers: 4 in the LC and 5 in the placebo groups. One patient of the LC group had increased baseline serum tryptase levels (Table I).

Side effects of BVIT

For more information, see Tables E1 and E2 in this article's Online Repository at www.jacionline.org. As shown in Table II during the phase of preventive medication, subjective systemic side effects, such as heat sensation, uneasiness, itch, and headache, were recorded in 2 patients with 2 episodes in the LC group and 9 patients with 11 episodes in the placebo group ($P = .04$). Objective SARs (>grade 2) were mostly cutaneous (grade 2) but occasionally also gastrointestinal (grade 3) or respiratory (grade 4). No cardiovascular side effects occurred. SARs during the preventive medication phase from days 0 to 21 were observed significantly more often in patients of the placebo group, with 19 SARs (13 on day 0, 4 on day 7, and 2 on day 21), after 252 injections than in the LC group, with 7 SARs (3 on day 0, 3 on day 7, and 1 on day 21) after 261 injections ($P = .03$). The difference was mainly found in cutaneous (12 in the placebo group vs 3 in the LC group) and gastrointestinal reactions (4 in the placebo group vs 0 in the LC group), although not in respiratory reactions (3 in the placebo group vs 4 in the LC group). Rescue medication had to be used significantly more often in patients receiving placebo than in those receiving LC ($P < .01$). Four patients of the placebo group dropped out because of side effects (ie, 2 on day 0 the other

TABLE III. SARs to sting challenge

Allergic reactions to sting challenge	LC	Placebo	Odds ratio (95% CI)	*P* value
No. with re-exposure	27	23*		
Subjective symptoms only	2	3	0.53 (0.08-3.51)	NS
Objective symptoms	6	2	3 (0.5-16)	NS
Subjective and objective symptoms	8	5	1.5 (0.42-5.51)	NS
Rescue medication used	3	2	0.76 (0.12-5)	NS

NS, Not significant.
*Four patients of the placebo group retired from the study because of side effects to immunotherapy injections.

2 on day 50 and day 80), all of them because of repeated SARs. There were no dropouts in the LC group. No significant difference in SARs was observed between groups (2 in the placebo group vs 2 in the LC group) during BVIT injections on days 50, 80, and 110.

Sting challenge response

All patients received a sting challenge on day 120, except the 4 dropouts. No severe SARs to re-exposure were observed. Two patients in the LC group had only subjective and 6 had objective allergic symptoms compared with 3 patients with only subjective and 2 with objective symptoms in the placebo group (Table III). All patients in the LC group had cutaneous reactions only, 1 patient in the placebo group had cutaneous symptoms, and another had both cutaneous and respiratory symptoms. Two patients in the placebo group and 3 in the LC group needed rescue medication. There was no significant difference between groups with regard to either objective symptoms or the use of rescue medication.

Skin tests and immunologic analyses

There was no significant change in STEPC and sIgE values between before BVIT and day 110 of BVIT in either group. sIgG levels, however, increased highly significantly in both the LC and placebo groups (Fig 1, *A*). Between the 2 groups, there was no significant difference in either STEPC, sIgE, or sIgG values before BVIT and on day 110.

Allergen-specific T-cell tolerance was suggested as an essential immunoregulatory mechanism during the course of venom- or aeroallergen-specific immunotherapy. Accordingly, we investigated BV major allergen PLA−stimulated or control antigen TT−stimulated T-cell proliferation. In patients receiving LC, significantly reduced allergen-specific PBMC proliferation was demonstrated after 110 days of BVIT. In patients receiving placebo, a tendency for suppressed proliferation was observed (Fig 1, *B*).

A significant decrease in IFN-γ levels was observed after 21 days of BVIT in the placebo group compared with the LC group (Fig 2, *A*). A tendency for decreased IFN-γ levels was found in both groups ($P = .056$ in the LC group and $P = .059$ in the placebo group) on day 110. A decrease in IL-13 secretion was observed in both groups but was significantly stronger in the placebo group on day 110 ($P = .016$). A significant increase in IL-10 production was demonstrated after 21 days of BVIT in the LC group (Fig 2, *C*) but not in the placebo group. No significant difference in IL-10 secretion was observed between the LC and placebo groups during the whole course of BVIT. There was no change in TT-stimulated parallel cultures (data not shown).

Changes in HR1 and HR2 expression in response to BVIT and the influence of H1 antihistamines was one of the major questions of the present study. Accordingly, the effect of BVIT on HR expression in PLA-stimulated T cells was further investigated (Fig 3). Although there was no statistically significant change in HR1 or HR2 expression during the course of BVIT in both groups, the ratio of HR1 versus HR2 mRNAs during venom immunotherapy decreased significantly after 21 days in the placebo group compared with the LC group ($P = .013$). This indicates the induction of HR2 dominance in allergen-specific T cells in the early course of BVIT. LC prevented this effect.

DISCUSSION

This is the first prospective, double-blind, placebo-controlled study aiming at the demonstration of H1 antihistamine preventive medication during the dose-increase phase on the clinical efficacy and mechanisms of BVIT. As in previous studies with other H1 antihistamines,[3-5] preventive treatment with LC significantly reduced systemic allergic side effects of BVIT during the dose-increase phase, especially cutaneous reactions, such as urticaria, flush, or angioedema.[3-5] It thus justifies its use in BVIT during dose increase, as recommended in international guidelines.[2,21] In both groups gastrointestinal and respiratory reactions were much less often observed, and cardiovascular side effects were observed not at all. An influence of H1 antihistamine preventive treatment on these more severe side effects cannot be definitely excluded because of a possible type II error. The need for rescue medication was also significantly reduced by H1 antihistamine preventive medication. In the present study and in our previous studies,[3,5] the preventive medication was only administered during the dose increase, when most of the allergic side effects of BVIT occur.

As indicated by the tolerance of a sting challenge by a live honeybee after 4 months of BVIT, efficacy was not significantly altered by H1 antihistamine preventive medication and was found in the previously reported range (75% to 90%).[2,17,22] All sting reactions were only cutaneous, except for 1 respiratory and cutaneous reaction in 1 patient in the placebo group. Although the rate of systemic reactions was slightly and insignificantly higher in patients receiving preventive treatment with LC compared with placebo, it must be noted that there were 4 dropouts because of repeated systemic allergic side effects in the placebo group, who were not challenged. It is well known that patients with systemic allergic side effects to venom immunotherapy injections are at a significantly higher risk to react to a re-sting than those who tolerate the treatment without side effects.[23,24] Even if it is assumed that all 4 dropouts had SARs to the sting challenge, the difference between groups would not be significant. Because the study is powered to detect a risk difference of 30% or greater, it does not exclude smaller differences. It has to be realized also that there are quite a few differences between our previous retrospective analysis,[12] suggesting increased efficacy of BVIT when used together with H1 antihistamine preventive medication and the present study.

First, in the previous study a rush protocol was used with a much higher cumulative dose of 425 μg over 5 days compared with the cumulative dose of 111.1 μg over 1 day in the ultrarush protocol of the present study. Although no difference in the efficacy of BVIT between the rush and ultrarush protocols was observed in previous retrospective comparative studies,[15,25] the 4-fold higher initial dose in the earlier study might have been responsible for a different effect on the immune and clinical response.

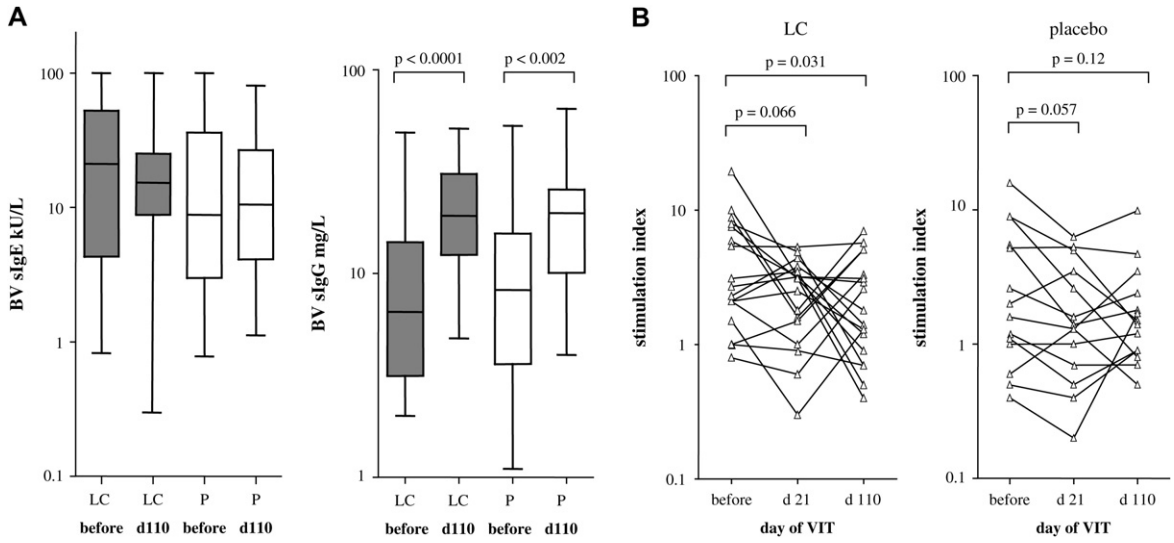

FIG 1. BV sIgE and sIgG antibodies and PLA-stimulated proliferation of PBMCs during ultrarush BVIT. **A**, sIgE levels did not change significantly, although there was a highly significant increase of sIgG levels in both groups. **B**, PLA-stimulated proliferation decreased in both groups, but the reduction reached significance only in the LC group on day 110. *P*, Placebo.

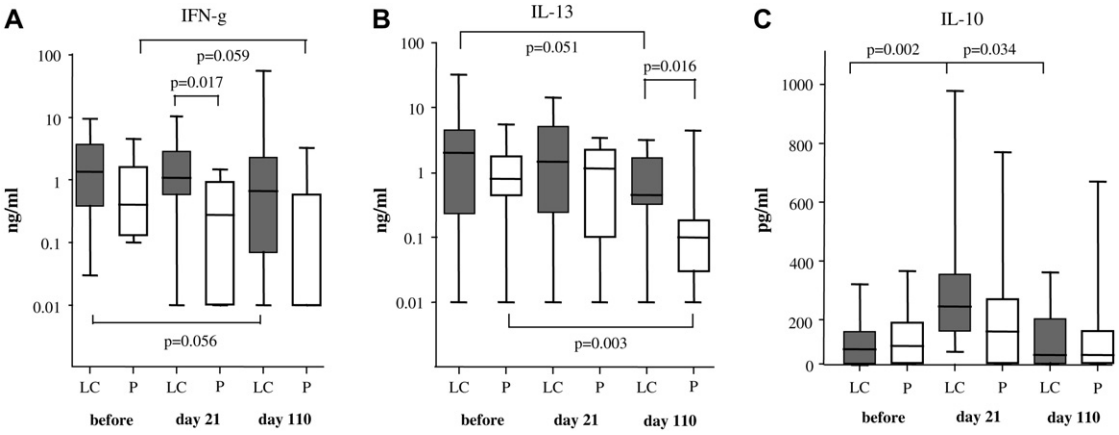

FIG 2. Cytokine secretion in PLA-stimulated PBMC cultures during BVIT. **A**, IFN-γ levels decreased significantly more in the placebo group on day 21. **B**, IL-13 levels decreased in both groups between before BVIT and day 110 but significantly more in the placebo group. **C**, IL-10 levels increased significantly on day 21 compared with those before BVIT in the LC group. *P*, Placebo.

Second, terfenadine was used in the previous study, and its daily dose was 240 mg (ie, 2 times higher than the usual daily dose). In the present study we used 5 mg of LC daily (ie, the usual daily dose). A number of studies have documented at least an equivalent or superior activity of LC when compared with other second-generation antihistamines.[26-28] The higher antihistamine dose together with the much higher cumulative BV dose administered in the previous study probably resulted in stronger interference with HR1 in the presence of the allergen.

Third, in the present study no antihistamines were used as rescue medication for only cutaneous allergic side effects, whereas these were allowed in the previous trial. This seems to be the main reason for the high dropout rate in the current study's placebo group and might also have influenced the immune response because of the lower total H1 antihistamine dose in the present study compared with previous studies.

Fourth, re-exposure in the earlier retrospective study occurred either by field stings (in 17 patients) or by sting challenge (in 24 patients) after an average of 30 months. This is our usual procedure in patients receiving BVIT and helps us to decide whether this treatment can be stopped. In the present prospective study, the sting challenge was relatively early, after 4 months, and the skin sensitivity and sIgE values did not significantly decrease after this short period. However, a strong reduction both in skin sensitivity and sIgE levels is most often observed after 30 months of venom immunotherapy[29] and was also documented during the 3-year control period in the previous study. Prolonged venom immunotherapy is generally thought to be associated with increased protection, although this is not documented by prospective studies with repeated sting challenges during venom immunotherapy.[22] Ultrarush protocols have been widely used in Europe since the late 1980s.[2,15,16] Early side effects have been reported to be

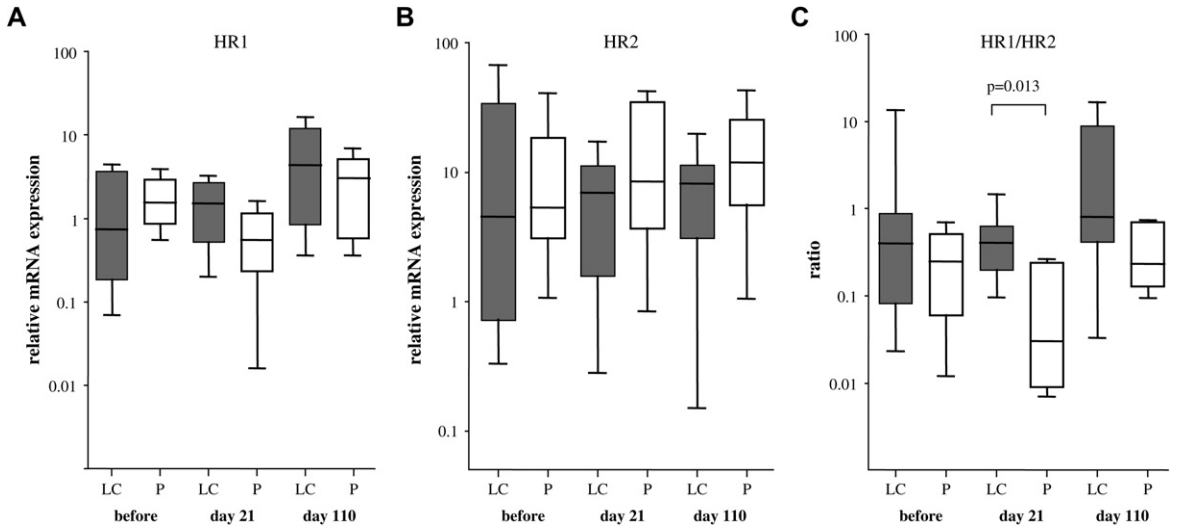

FIG 3. Effect of BVIT on HR expression in PLA-stimulated T cells. **A**, HR1 mRNA expression. **B**, HR2 mRNA expression. **C**, HR1/HR2 mRNA expression ratio. Although no significant difference in HR1 and HR2 expression is observed between groups, the HR1/HR2 ratio is significantly lower in the placebo group on day 21. *P*, Placebo.

comparable with conventional protocols; however, they provide the advantage of early protection from anaphylaxis.[2]

The present study demonstrates several immunologic effects of LC preventive medication during early BVIT, some of which can be considered a positive contribution to enhanced allergen-specific T-cell tolerance. These effects can be listed as a more efficient decrease in T-cell proliferation in parallel with more IL-10 level increases after 21 days of BVIT. The increased IL-10 levels in the LC group are associated with a somewhat more significant increase in sIgG levels. IL-10 has been previously demonstrated to play a role in the induction of IgG4.[19] In contrast, the more significant and unexpected decrease in IL-13 levels in the placebo group could be considered a disadvantage of H1 antihistamine use. Both HR agonists (histamine) and antagonists (LC in the present study) interfere with the peripheral tolerance induced during venom immunotherapy in several pathways. Histamine enhances T_H1-type responses by triggering HR1, whereas both T_H1- and T_H2-type responses are negatively regulated by HR2. Human $CD4^+$ T_H1 cells predominantly express HR1 and $CD4^+$ T_H2 cells predominantly express HR2, which results in their differential regulation by histamine.[30] The significantly decreased value of the HR1/HR2 ratio in the placebo group indicates HR2 dominance during venom immunotherapy. LC pretreatment prevented the decrease of HR1/HR2 expression ratio. HR2 has been shown to act as a negative feedback regulator of HR1-mediated effects by suppressing IL-4 and IL-13 production and T-cell proliferation.[30] HR2 contributes to allergen-specific T-cell tolerance in several ways. Histamine induces the production of IL-10 by dendritic cells through HR2.[31] In addition, histamine induces IL-10 production by T_H2 cells[32] and enhances the suppressive activity of TGF-β on T cells, again through HR2.[20] As observed in the placebo group, the natural response to dose increase in the ultrarush protocol seems to be a deviation toward HR2 in allergen-specific T cells. Surprisingly, LC inhibits this effect. These interesting and partly unexpected findings with regard to the involvement of HRs in the *in vivo* modulation of allergen-specific T-cell tolerance during BVIT remain to be further elucidated.

In a recent study[33] an unfavorable effect (ie, increased IgE and decreased IgG responses) was reported during BVIT under H1 antihistamine preventive treatment with clemastine in mice sensitized to BV. Although both the sensitization procedure and the immunotherapy protocol chosen were not really comparable with the situation in human subjects, the dose of antihistamine related to body weight was more than 100 times higher in these mice than the dose usually used in human subjects. That the dose of an antihistamine might have an influence on immune response in mice is suggested by another article,[34] in which a much lower preventive H1 antihistamine dose significantly suppressed the T_H2 response in mice sensitized to ovalbumin.

In conclusion, this study confirms a significant reduction of systemic allergic side effects and need for rescue medication by H1 antihistamine preventive medication during the dose-increase phase of immunotherapy with BV. On the other hand, an increased efficacy of BVIT, as suggested by a previous retrospective open analysis, could not be confirmed in this prospective, double-blind, placebo-controlled trial. Nevertheless, premedication with antihistamines in the early phase of immunotherapy remains very valuable by increasing safety and preventing dropouts, especially during BVIT. This study also demonstrates that continuous administration of an H1 antagonist effectively modulates HR expression in specific T cells. The clinical and immunologic effect of LC preventive medication results from regulation of the immune response through allergen-specific T-cell tolerance and activation of HR-dependent pathways.

Clinical implications: The use of H1 antihistamines decreases side effects of BVIT, but its distinct regulatory role on allergen-specific immune response should be considered and further investigated.

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Correction

With regard to the February 2007 article entitled "Macrophage inflammatory protein 3α deficiency in atopic dermatitis skin and role in innate immune response to vaccinia virus" (J Allergy Clin Immunol 2007;119:457-63), the authors wish to include Kevin Kisich as a coauthor on the basis of his contribution to the overall study design and generation of Figure 5. The author list should read as follows: Byung Eui Kim, MD, PhD, Donald Y. M. Leung, MD, PhD, Joanne E. Streib, BA, Kevin Kisich, PhD, Mark Boguniewicz, MD, Qutayba A. Hamid, MD, PhD, and Michael D. Howell, PhD.

In addition, the authors would like to correct the information regarding the source of MIP-3α used in the antiviral assay. In the Methods section, under "Antiviral assays," the first sentence in the second paragraph should read "Macrophage inflammatory protein 3α (gift from Dr J. Lubkowski, National Cancer Institute, National Institutes of Health, Bethesda, Md) was diluted to the proper concentrations in 0.01X tryptic soy broth containing 10 mmol/L sodium phosphate buffer, pH 7.4."

TABLE E1. Placebo group

				Side effects during BVIT						Sting challenge placebo		
				Side effects of BVIT								
Patient no.	Sex	Year of birth	Diagnosis	Day	Dose	Symptoms	Grade	Rescue medication	Comments	Symptoms at re-sting	Grade	Rescuemedication
1	Female	1969	BG 4	0	30 μg	Flush, erythema head, chest, tachycardia 73 → 107	3	Adrenaline0.3 mg sc	Dropout			
				0	30 μg	Urticaria, angioedema, dysphagia	3	Adrenaline sc, clemastine and prednisolone iv				
3	Female	1968	BG 3				0		Atopy		0	
8	Female	1963	BG 4	0	10 μg	Flush, itch, dizziness, abdominal pain, nausea	3	Clemastine iv	Dropout Atopy			
				0	10 μg	Itch, dizziness, orthostatic problems, nausea	3	Clemastine and prednisolone iv				
								Volume substitution				
9	Male	1962	BG 4				0		Atopy		0	
11	Male	1986	BG 3				0		Atopy Beekeeper		0	
12	Male	1968	BG 2	0-21	100 μg	Generalized itch	1, 1, 1		Dropout Beekeeper			
				50	100 μg	Generalized itch, dizziness	1					
				80	100 μg	Flush, generalized itch, collapse	4	Clemastine + prednisolone iv				
13	Male	1966	BG 3				0		Beekeeper	Oral and perioral itch	1	
14	Male	1973	BG 4				0		Atopy	Nausea	1	
15	Male	1963	BG 4	0	10 μg	Hypesthesia left arm	1		Atopy		0	
				7	100 μg	Dizziness, feeling weak	1					
17	Female	1946	BG 3	0	50 μg	Headache, itch	1				0	
19	Female	1962	BG 4				0				0	
20	Male	1966	BG 3	0	30 μg	Flush, urticaria, chest tightness	4	Clemastine and prednisolone iv			0	
				77	30 μg	Erythema trunk	22	Primatene inhalation				
				21	50 μg	erythema, itch	2					
					100 μg	Erythema trunk						
21	Male	1966	BG 3	7	100 μg	Generalized itch	1		Atopy Beekeeper		0	
22	Male	1964	BG 4				0				0	
25	Male	1964	BG 3	0	20 μg	Headache, itch neck chest	1		Beekeeper		0	
26	Male	1957	BG 4	0	50 μg	Abdominal distress, diarrhea	3		Dropout			
				7	100 μg	dizziness	1					

				50 –80	100 μg	Dizziness, diarrhea, psychological problems	1					
29	Male	1956	BG 4				0		Atopy		0	
32	Male	1976	BG 4				0		Atopy		0	
33	Male	1948	BG 4				0				0	
35	Male	1952	BG 3	0	50 μg	Headache	1				0	
39	Male	1980	BG 3	0	50 μg	Generalized itch, erythema trunk	2	Primatene inhalation		Laryngeal tightness, no hoarseness, normal peak flow	1	
				7	50 μg	Chest tightness, flush	2	Primatene inhalation				
				21	100 μg	Urticaria neck, back arms	2					
43	Male	1972	BG 3	0	50 μg	Hoarseness, urticaria neck, trunk	2	Adrenaline 0.3 mg sc no effect→ clemastine			0	
46	Male	1968	BG 4	0	20 μg	Erythema neck, generalized itch	2	Adrenaline 0.3 mg sc		Dyspnea, oral itch, decrease in peak flow >20%	4	Primatene inhalation
				0	30 μg	Generalized urticaria	2	Primatene inhalation				
				7	50 μg	Generalized urticaria	2					
47	Female	1982	BG 4	0	30 μg	Dyspnea, wheezing	4	Primatene inhalation	Atopy			
				0	50 μg	Dyspnea, wheezing	4	Salbutamol inhalation				
48	Male	1958	BG 4	7	50 μg	Headache	1		Atopy		0	
50	Male	1963	BG 4				0			In both arms, single urticarial wheals	2	I-cetirizine, 5 mg; prednisone, 50 mg po
51	Male	1963	BG 4	0	50 μg	Generalized itch	1		Atopy		0	

Primatene Mist, Armstrong Pharmaceuticals, Inc, Rancho Cucamonga, Calif. *BG*, Grade of allergic bee-sting reaction[14]; *sc*, subcutaneous; *iv*, intravenous; *po*, by mouth.

TABLE E2. LC group

				Side effects during BVIT								
				Side effects during BVIT days 0–d21						Reaction to re-sting		
Patient no.	Sex	Year of birth	Diagnosis	Day	Dose	Symptoms	Grade	Rescue medication	Comments	Symptoms at re-sting	Grade	Rescuemedication
2	Male	1960	BG 3				0		Atopy		0	
4	Male	1965	BG 4				0		Atopy		0	
5	Male	1971	BG 3				0			Generalized itch, flush head and neck, conjunctivitis	2	
6	Female	1983	BG 3	0	10 μg	Dyspnea, wheezing	4	Primatene inhalation			0	
				7	50 μg	Cough, hoarseness, erythema head trunk	4	Primatene inhalation				
7	Female	1970	BG 3				0		Atopy		0	
10	Female	1957	BG 4				0				0	
16	Male	1949	BG 4	0	50 μg	Urticaria, cough	2	Primatene inhalation, iv clemastine, 2 mg, + prednisolone, 50 mg	Atopy	Itch chin and neck	1	
18	Male	1970	BG 3				0				0	
23	Female	1966	BG 3				0		Atopy Beekeeper		0	
24	Male	1967	BG 3	7	50 μg	Cough, rhinitis, decrease in peak flow 560 → 420	4	Primatene inhalation	Atopy Beekeeper	Cough, rhinitis	2	LC, 10 mg po
				21	100 μg	Rhinitis, cough	2					
27	Female	1953	BG 3				0		Beekeeper	Flush face, generalized itch	2	
28	Male	1955	BG 4				0				0	
30	Male	1967	BG 4				0		Atopy		0	
31	Male	1956	BG 3	0	50 μg	Flush, heat sensation	2	Primatene inhalation	Atopy	Flush head and neck	2	
34	Male	1952	BG 2				0		Atopy		0	
36	Male	1959	BG 4				0		Atopy		0	
37	Male	1974	BG 2				0				0	
38	Male	1965	BG 2				0			Erythema face, trunkUrticarial wheals trunk	2	LC, 10 mg po
40	Female	1987	BG 4	21	100 μg	Feeling dizzy, no decrease in blood pressure	1		Atopy	Heat sensation, itch on back	1	
41	Male	1967	BG 3	0	20 μg	Tightness in throat, no decrease in peak flow	1		Atopy		0	
42	Male	1967	BG 2				0				0	
44	Male	1985	BG 3				0		Atopy	Urticaria neck, chest Headache	2	LC, 10 mg po
45	Male	1958	BG 4				0				0	

49	Male	1955	BG 3				0				0
52	Male	1959	BG 2				0		Atopy Beekeeper		0
53	Male	1947	BG 4				0				0
54	Male	1949	BG 4	7	50 μg	Dizziness, dyspnea, decrease in blood pressure 125 → 95 mm Hg	4	Adrenaline, 0.3 mg sc; volume substitution	Atopy Systemic mastocytosis	Dizziness, heat sensation	1

Primatene Mist, Armstrong Pharmaceuticals, Inc, Rancho Cucamonga, Calif. *BG*, Grade of allergic bee-sting reaction[14]; *iv*, intravenous; *sc*, subcutaneous.