

Letter to the Editor

Contribution of histamine metabolism to tachyphylaxis during the buildup phase of rush immunotherapy

To the Editor:

Rapid clinical tolerance has been described early after reaching the maintenance dose of rush and ultrarush specific venom immunotherapy (VIT). However, there is not much knowledge about the mechanisms contributing to the early unresponsiveness of effector cells during VIT. A temporarily decreased release of mediators from effector cells immediately after rush VIT resulting in tachyphylaxis has been supposed to account for early onset of protection.¹ So far, a reduced allergen-induced release of mediators from basophils, such as histamine² and leukotrienes, has been shown after rush VIT compared with pretreatment values.^{1,3-5} This effect has been accredited to loss of stored mediators^{6,7} and the inhibition of mediator release of basophils and mast cells by a transient increase of intracellular cyclic AMP.¹

One of the main soluble factors liberated by effector cells on allergen challenge is histamine. Excessive release of histamine can cause symptoms such as hypotension, urticaria, pruritus, flushing, headache, arrhythmia, dyspnea, and gastrointestinal symptoms, which, in addition to the local reactions at the injection site, represent typical side effects observable during the buildup phase of VIT. Histamine is synthesized by L-histidine decarboxylase (HDC) and can be metabolized in 2 ways: oxidative deamination by diamine oxidase (DAO), a secretory enzyme that scavenges extracellular histamine, or ring methylation by histamine-N-methyltransferase (HNMT), a cytosolic protein that catabolizes intracellular histamine. Enhanced histamine levels might lead to a substrate inhibition of DAO and HNMT by histamine metabolites. Therefore it is important to rule out whether histamine levels are controlled and maintained in a tolerable range by mechanisms of histamine metabolism or whether a negative feedback caused by high histamine release profoundly reduces DAO and HNMT activity during VIT.

We applied VIT to 27 patients (age range, 19-67 years; mean age, 47.2 ± 12.6 years; 17 female and 10 male patients; mean total serum IgE level, 151.0 ± 165.1 kU/L; mean tryptase level, 4.3 ± 1.7 μ g/L, excluding 1 patient with mastocytosis) sensitized to honeybee venom ($n = 5$; mean specific serum IgE level, 7.2 ± 17.5 kU/L) and wasp venom ($n = 22$; mean specific serum IgE level, 16.5 ± 25.28 kU/L) according to the rush protocol (ALK-lyophilisiert SQ; ALK-Scherax, Hamburg, Germany; Table I). All patients had a history of a systemic anaphylactic reaction to a Hymenoptera sting evaluated with a standard questionnaire (Table II).⁸ One patient had an additional indolent systemic mastocytosis (serum tryptase, 238 μ g/L). All patients received an H1 receptor antagonist, 10 mg of cetirizine, from day 2 to day 5 up to 3 times daily, as required. After informed consent, peripheral venous

blood was obtained immediately before VIT and after the last injection of each day during the buildup phase (Table I). One patient had a transient dizziness, a lump in the throat, tachycardia, and hypertension after the application of 6 and 8 μ g of wasp venom on day 3, which was regressive after the intravenous application of 2 mg of clemastine. Except for this patient, none of the patients had any moderate or severe side effects, despite transient local erythema, swelling, and pruritus at the injection site.

Serum tryptase levels determined with the UniCAP System (Pharmacia Diagnostics, Uppsala, Sweden) changed only slightly during the first 5 days of VIT, with a decrease after the first day and an increase until the end of the buildup phase (Fig 1, A). Because tryptase is a specific serum marker used to evaluate mast cell activation, these data support the hypothesis that there is only a very mild modulation of the activation state of mast cells and basophils during VIT because of rapid development of unresponsiveness of effector cells. Plasma levels of histamine evaluated by means of ELISA (BioSource Europe S.A., Nivelles, Belgium) remained relatively stable during the first 2 days of the buildup phase with a first peak and a 2-fold increase compared with the baseline level at day 3 and a second peak and a 3-fold increase at day 5 (Fig 1, B). This increase of plasma histamine levels might result from the enhancement of the cumulative amount of allergen applied at days 3 and 5 during this treatment schedule (Table I). Concomitantly, the mRNA level of HDC, which is responsible for intracellular decarboxylation of histidine to histamine, of PBMCs isolated during VIT and evaluated by means of real-time PCR (Applied Biosystems, Darmstadt, Germany), decreased about 60% during the first days of VIT and increased again to baseline levels at day 5 (Fig 2, A). Reduction of HDC mRNA implies decreased intracellular histamine *de novo* synthesis of PBMCs as a compensatory mechanism to avoid excessive histamine supply during repetitive allergen challenge.

DAO serum activity, evaluated by using radioextraction assays in which tritium-labeled putrescine dihydrochloride has been used as a substrate (Immunodiagnostik AG, Bensheim, Germany), showed a significant peak at the second day compared with the first day but returned to baseline levels afterward (Fig 2, B), suggesting that DAO serum activity remains relatively stable during the whole build-up phase of VIT and is not profoundly inhibited by histamine metabolites during this treatment schedule. The DAO activities of the patient with mastocytosis were slightly lower than the DAO activities of the other patients but followed the course of the other patients, and deviations were within the SEM. Parallel to the reduction of the HDC mRNA level of PBMCs during VIT, the mRNA level of HNMT, which metabolizes intracellular histamine, increased significantly from day 2. Interestingly, HNMT mRNA expression peaked in a manner similar to the plasma level of histamine (Fig 2, C), which implies that accelerated intracellular histamine degradation caused by high extracellular histamine

TABLE I. Dosing schedule of the buildup phase of VIT

Day:	1	1	1	1	1	2	2	2	2	3	3	4	4	5	5
Concentration (μ g/mL)	0.1	0.1	0.1	1	1	1	10	10	10	10	100	100	100	100	100
Applied dose (mL)	0.2	0.4	0.8	0.2	0.4	0.8	0.2	0.4	0.6	0.8	0.1	0.4	0.6	0.8	1

TABLE II. Clinical parameters of the study population

	Reaction after insect sting	No.	Percentage
I	Pruritus	17	63.0
	Flush	19	70.4
	Urticaria	16	59.3
	Angioedema	11	40.7
II	Nausea	6	22.2
	Rhinorrhea	2	7.4
	Dyspnea	13	48.2
	Hypotension (>20 mm Hg)	14	51.9
III	Vomitus	6	22.2
	Laryngeal edema	12	44.4
	Bronchospasm	2	7.4
	Anaphylactic shock/unconsciousness	3	11.1
IV	Bronchial arrest	0	0
	Cardiac arrest	1	3.7

Severity of anaphylactic reactions is based on the work of Ring and Messmer.⁸

levels might represent a second way to reduce the amount of histamine released by effector cells during this critical phase of VIT.

HDC mRNA and protein are highly expressed on basophils,^{9,10} and HNMT mRNA is highly expressed on monocytes.^{9,11} We observed an increase in circulating monocyte numbers (0.52 ± 0.13 to 0.72 ± 0.28 G/L and $8.48\% \pm 1.97\%$ to $9.4\% \pm 1.93\%$) and a decrease in circulating basophil numbers (0.03 ± 0.01 to 0.01 ± 0.02 G/L and $0.46\% \pm 0.23\%$ to $0.16\% \pm 0.15\%$) in 5 patients from the first day compared with the fifth day of rush VIT after reaching the maintenance dose. Thus, the downregulation of HDC mRNA and upregulation of HNMT mRNA on PBMCs of patients undergoing rush VIT might result from both changes in the mRNA level and changed numbers of basophils/monocytes in the fraction of PBMCs.

However, the mRNA level of the enzyme might differ from the protein level, and the intake of an H1 receptor antagonist might have influenced the laboratory data. Preventive medication with

H1 receptor antagonists has been shown to reduce side effects during dose increase of Hymenoptera VIT in several double-blind, placebo-controlled trials¹² and modifies the expression of histamine receptors and cytokine production by allergen-specific T cells without impairing the efficacy of VIT.¹²

In conclusion, we could show that counterregulatory mechanisms between histamine synthesis and degradation, together with the application of histamine receptor antagonists during the buildup phase, might attenuate side effects of histamine during VIT. They could thereby contribute to the development of unresponsiveness and early tolerance on the level of histamine-producing and histamine-releasing cells.^{3,4} Previously, a modified histamine metabolism has been found in allergic compared to nonallergic subjects. Increased HDC and decreased HNMT mRNA expression has been found in scrapings of nasal mucosa of patients with allergic rhinitis compared with levels seen in nonallergic subjects.¹³ The expression of HNMT mRNA correlated positively with the expression of HDC mRNA in nonallergic, but not allergic, patients.¹³ Thus, the redirection of a modified histamine metabolism in allergic subjects might contribute to the effects of specific immunotherapy.

Insight into the regulation of histamine metabolism during VIT needs to be transferred and expanded to other mast cell mediators, as well as types of treatment schedules, such as ultrarush immunotherapy. Furthermore, it would be important to rule out which of these mechanisms are deregulated in patients with severe side reactions during VIT, patients with mastocytosis, or patients with histamine intolerance. Modifications of histamine metabolism might be genetically predetermined and result in deficient function of histamine-degrading enzymes or might be caused by secondary factors that increase histamine plasma levels or block the activity of histamine-degrading enzymes, as well as the hyperresponsiveness of mast cells. Better knowledge about this tight regulation would allow selective therapeutic intervention to counteract side effects caused by mast cell mediators and help to actively support and accelerate the development of effector cell hyporesponsiveness and tachyphylaxis during VIT.

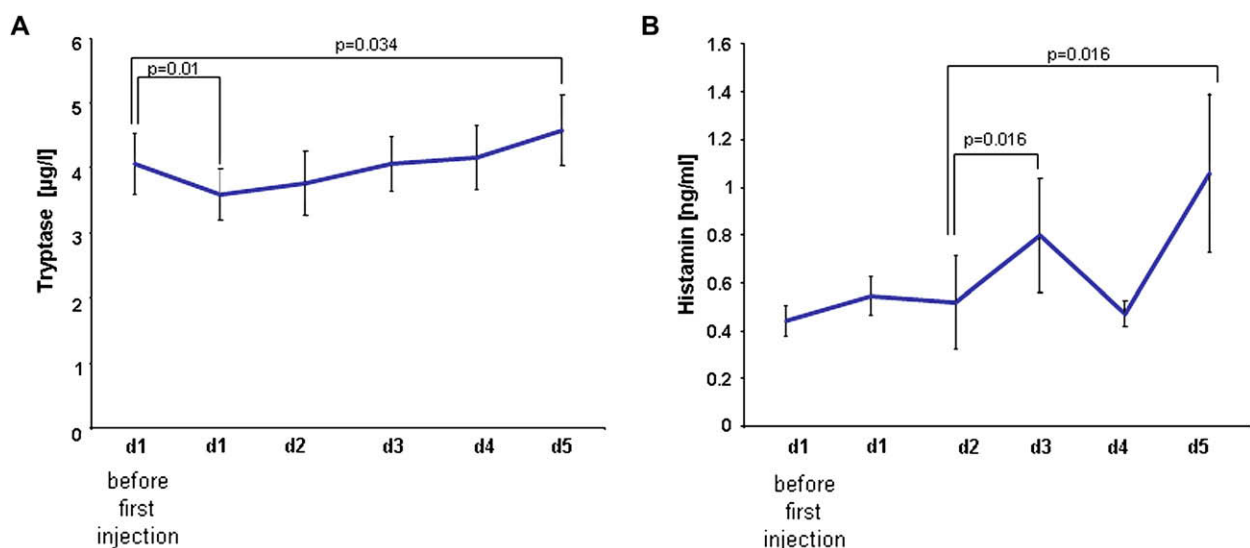


FIG 1. Kinetics of the mast cell mediators tryptase ($n = 11$; **A**) in the sera and histamine ($n = 13$; **B**) in the plasma of patients before the first and immediately after the last subcutaneous application of Hymenoptera venom on each day (d) during the build-up phase of VIT.

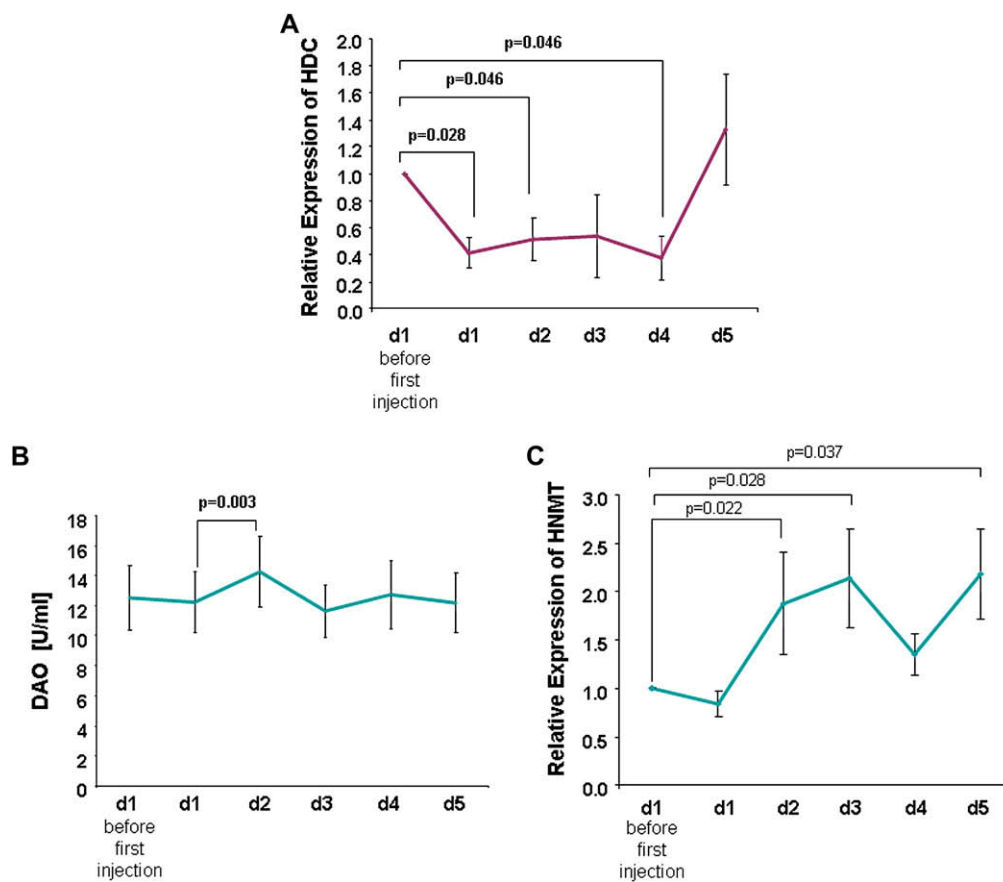


FIG 2. Kinetics of the histidine decarboxylase (HDC) mRNA level of PBMCs ($n = 6$; **A**) and the histamine-degrading enzymes diamine oxidase (DAO) ($n = 16$; **B**) and histamine-N-methyltransferase (HNMT) ($n = 10$; **C**) before the first and after the last application of Hymenoptera venom on each day (d) of the buildup phase of VIT.

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