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Initiating yellow jacket venom immunotherapy with a 100-μg dose: A challenge?

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Clinical Implications

- This study gives a first suggestion that updosing schedules of yellow jacket venom immunotherapy might be simplified significantly in the future.

TO THE EDITOR:

Yellow jacket venom immunotherapy (YJ-VIT) is highly effective in reducing the occurrence and severity of systemic reactions to YJ stings and improving quality of life.[1] However, VIT may also induce systemic adverse reactions. It is a common belief that updosing of venom at the start of VIT reduces the occurrence of systemic adverse reactions, although this effect has never been demonstrated.

Remarkably, in the first published study testing immunotherapy with Hymenoptera venom extracts in 1978, untreated patients tolerated 1, 10, and 100 μg of purified venom at 30-minute intervals, whereas 58% reacted systemically to an immediate subsequent sting challenge.[2] This suggests that the composition of Hymenoptera venom extracted for purposes of immunotherapy could be different from the venom injected by a living insect. We hypothesized that venom extract is modified in such a way that the maintenance dose can be applied as the starting dose in selected patients. Elimination of updosing would reduce costs, increase patient convenience, and might shorten time to reach clinical protection.

We designed a randomized controlled pilot study in which a 4-monthly 100 μg Pharmalgen (ALK-Abelló, Hørsholm, Denmark) injection schedule was compared with updosing according to a cluster protocol in terms of adverse reactions, clinical efficacy, and immunological effects (Table I). Randomization was computerized, stratified, and balanced for age, sex, and severity of field sting reaction (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). Participants had a recent history of a systemic reaction grade I-IV[3] following a YJ field sting and YJ venom–specific (s)IgE level of greater than 0.35 kUA/L in serum. Exclusion criteria included near-fatal sting reactions or mastocytosis (for full list of exclusion criteria, see this article's Online Repository at www.jaci-inpractice.org). The clinical efficacy of both protocols was assessed by a sting challenge 4 weeks after the last injection and compared with sting challenge outcomes in nontreated patients (comparator group, selected and stung concurrently with the other study patients, same selection criteria applied).

The trial was registered at the European Clinical Trials Database (EudraCT no. 2013-001990-26) and was approved by the National Research Ethics Service and the local Institutional Review Board. Participants provided written informed consent.

Sting challenges were performed on the volar side of the arm according to European guidelines with *Vespula vulgaris* or *Vespula germanica* caught in the wild on the morning of the challenge.[4] Local sting reactions were measured after 15 minutes.

During injections and sting challenges patients were under constant medical supervision and adverse reactions were documented according to predefined criteria including condition of the skin, heart rate, blood pressure, and respiratory rate. Suspect systemic reactions were objectified by the course of serum tryptase and the urinary histamine metabolites, comparing baseline values to values 1 and 2 hours after the first symptoms. Symptoms of late-phase reactions were systematically evaluated using a questionnaire.

Immunological changes during VIT were evaluated by the allergen-blocking capacity of patients' sera before VIT and before the sting challenge, using the cell-free enzyme-linked immunosorbent-facilitated antigen-binding assay (see this article's Online Repository at www.jaci-inpractice.org).[5]

Ten patients were randomized to the 100-μg protocol, 8 to the cluster protocol. The comparator group consisted of 20 patients. All participants completed the study regimen. Subjects did not differ in demographic characteristics, grade of the field sting reaction, interval between the field sting and start of VIT or sting challenge, YJ venom-sIgE or mast cell parameters, except for a slightly higher methylimidazole acetic acid level in the comparator group compared with the cluster protocol group (1.4 vs 1.0 mmol/mol creatinine; $P = .027$; see Table E1) although all mast cell parameters were within normal range.

None of the subjects developed a systemic reaction to VIT. Fatigue and flu-like symptoms developed repeatedly in 4 patients of each protocol, several hours after the injection. On the 100-μg protocol, large local reactions appeared in 8 out of 10 patients after the first YJ venom injection, in 7 after the second, in 3 after the third, and in 1 after the fourth injection. During the course of VIT, a reduction in local reaction size to the injections was observed. With the cluster protocol, 6 out of 8 patients developed a large local reaction on the first day, and 1 patient after each injection until the fifth week of updosing.

None of the subjects treated with VIT reacted systemically to the sting challenge. In the comparator group, 4 out of 20 patients (20%) had a clinically systemic reaction to the sting challenge that could be biochemically supported by an increase and subsequent decrease in mast cell parameters (3 grade I and 1 grade III; see Table E2 in this article's Online Repository at www.jaci-inpractice.org). Two other subjects in the comparator group showed signs of a systemic reaction with tachycardia and flushing of the head, neck, and chest, but the mast cell parameters showed no significant change.

Ves v 5-sIgE antibodies were detectable in all sera and increased equally during the 100-μg and cluster protocol (Δ1.39 and 1.20 KUA/L, respectively; $P = .573$; Figure 1, *A*). Ves v 5-sIgG_4 antibody concentrations were also equally increased (Δ1.23 and

TABLE I. Injection scheme 100-μg and cluster protocol

Injection scheme 100-μg protocol			Injection scheme cluster protocol		
Week	Injection	Dose (μg)	Week	Injection	Dose (μg)
1	1	100	1	1	0.0001
				2	0.001
				3	0.01
				4	0.1
				5	1
				6	10
4	2	100	2	7	10
8	3	100	3	8	20
12	4	100	4	9	40
			5	10	60
			6	11	80
			7	12	100
			12	13	100
Final cumulative dose		400			421

1.87 mgA/L, respectively; $P = .534$; Figure 1, *B*). The latter resulted in a comparable decrease in the Ves v 5-sIgE/-sIgG$_4$ ratio, seen in both schedules (Δ12.25 and 15.26, respectively; $P = .344$; Figure 1, *C*). All sera showed an increased ability to inhibit allergen-IgE complex formation after VIT (Figure 1, *D*). No difference was found in the decrease in allergen-IgE complex binding to CD23 between the patients during the 100-μg and cluster protocol (47.8% and 37.8%, respectively; $P = .529$). After VIT, an inverse correlation was found between the Ves v 5-sIgG$_4$ antibody concentrations and the percentage of allergen-IgE complexes bound to CD23 (2-tailed Spearman rank correlation coefficient: $r_s = -0.833$ vs -0.714; $P = .01$ and .047, respectively; Figure 1, *E*).

In conclusion, this study showed that systemic reactions did not occur more often to VIT initiation at the maintenance dose compared with VIT initiation by updosing. However, large local reactions were seen more frequently for several weeks. The difference in systemic reaction rate to venom injected by a living YJ and high-dose purified YJ-venom extract may be explained by a

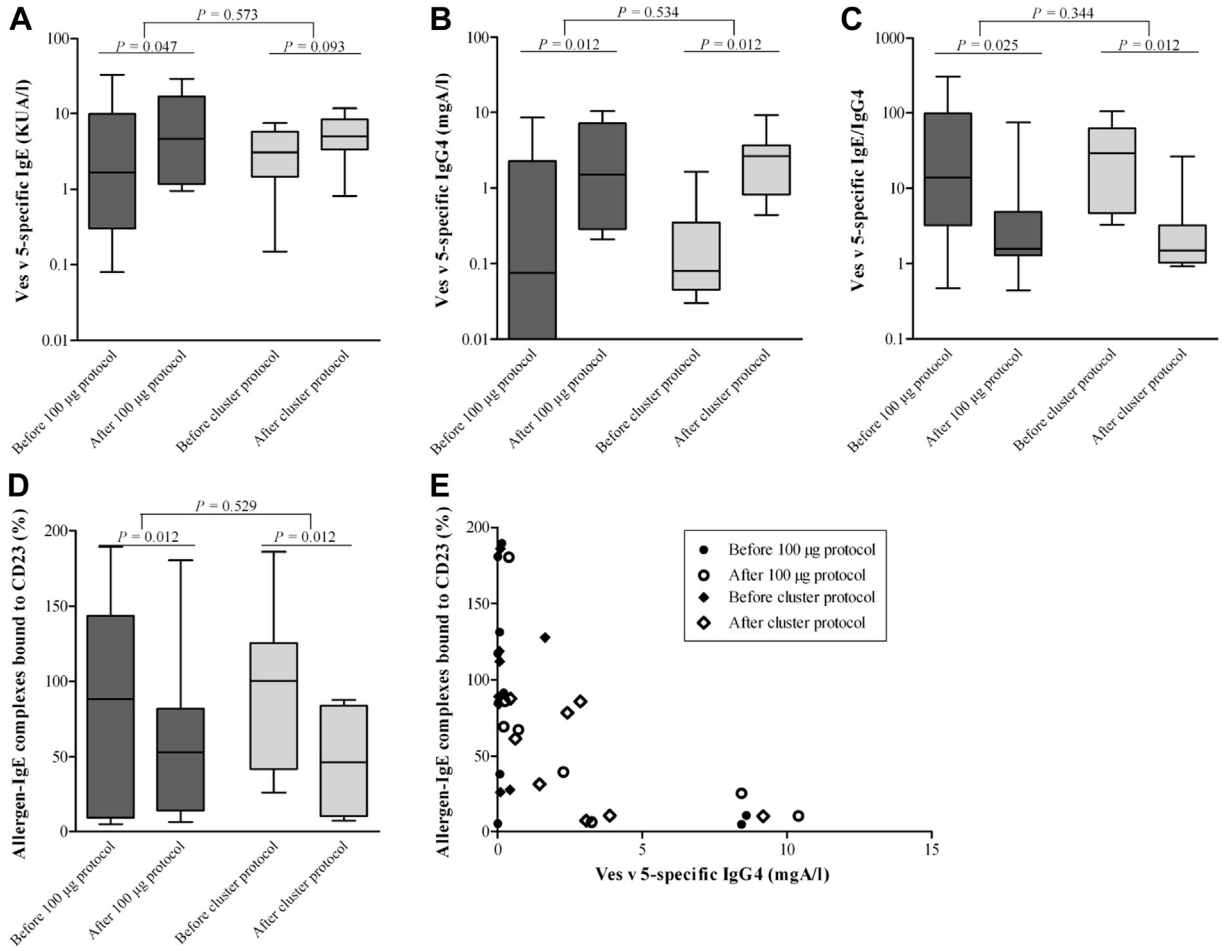

FIGURE 1. **A** and **B,** Ves v 5-sIgE and -sIgG$_4$ antibody concentrations. **C,** Ves v 5-sIgE/IgG$_4$ ratio. **D,** ELIFAB. Data are related to binding with indicator serum alone (100%). **E,** Correlation between Ves v 5-sIgG$_4$ antibody concentrations and percentage of allergen-IgE complexes bound to sCD23. Comparisons of paired samples and unpaired samples were made using Wilcoxon signed-rank test and Mann-Whitney *U* test, respectively. *ELIFAB*, Enzyme-linked immunosorbent-facilitated antigen-binding.

difference in venom composition, which may theoretically be due to underrepresentation of minor allergens or problems in protein recovery and stability as a result of the extraction process.[6] Alternatively, the route of injection (subcutaneously vs intradermal) or the concentration of the venom might cause a potential difference in the reaction rate. Additional studies in a larger number of patients and broader patient category are warranted to investigate whether VIT initiation at the maintenance dose might be a safe and effective treatment regimen in the future. Caution is warranted in the selection of eligible patients. Because we applied very strict exclusion criteria, with an emphasis on mastocytosis, our findings cannot be extrapolated to all YJ venom—allergic patients.

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METHODS

Exclusion criteria

Patients were prohibited from participation if they had severe cardiopulmonary disease, insufficiently controlled asthma, immune deficiencies, autoimmune diseases, malignancy, or severe kidney failure, used β-blockers or immunosuppressive drugs, and were pregnant.[E1] In addition, patients were not included if they were younger than 18 years, had double sensitization to YJ venom and bee venom, experienced a grade IV sting reaction with loss of consciousness or incontinence, or suffered from mastocytosis. Patients were screened for mastocytosis by baseline serum tryptase, urine methylhistamine and methylimidazole acetic acid, and skin inspection for urticaria pigmentosa.

Hymenoptera venom-specific serum antibodies and mast cell parameters

Concentrations of sIgE and $sIgG_4$ antibodies against YJ venom and Ves v 5, and bee venom-sIgE, were measured by the ImmunoCAP system (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden). Serum tryptase levels were determined with the B_{12} assay using ImmunoCAP Tryptase reagents and the Phadia 250 analysis device (Thermo Fisher Scientific). Levels of methylhistamine and methylimidazole acetic acid were determined by an isotope-dilution mass fragmentographic method as described previously.[E2]

Short protocol cell-free enzyme-linked immunosorbent-facilitated antigen-binding assay

In brief, 20 μL of IgG-depleted indicator serum exhibiting high major allergen Ves v 5-sIgE levels (>100 kUA/L) was mixed with either 20 μL of patient serum or 20 μL of medium in the presence of 5 μL recombinant Ves v 5. Because YJ venom extract consists of a complex mixture of different allergens, the major allergen Ves v 5 was used as a surrogate to allow defined assay conditions. The indicator serum was previously shown to exhibit an optimal antibody/allergen ratio for complex formation at 3 μg/mL Ves v 5.[E3] The solution was preincubated at 37°C for 1 hour to allow for formation of allergen-IgE complexes. After preincubation, allergen-IgE complexes were transferred to soluble CD23-coated plates (R&D Systems, Wiesbaden-Nordenstadt, Germany) and incubated for 1 hour at room temperature. Next, free sIgE was washed away and allergen-IgE complexes bound to immobilized soluble CD23 (Sigma-Aldrich, Schnellorf, Germany) were detected by adding biotin-conjugated antihuman IgE antibody (BD Biosciences, Heidelberg, Germany), streptavidin-peroxidase, and 3,3',5,5'-tetramethylbenzidine (both Sigma-Aldrich). All samples were measured in duplicate.

TABLE E1. Clinical and laboratory patient characteristics

Characteristic	100-μg protocol (n = 10)	Cluster protocol (n = 8)	Comparators (n = 20)
Age (y)	43.1 ± 12.4	52.9 ± 5.5	51.25 ± 12.2
Sex: male, n (%)	6 (60.0)	6 (75.0)	10 (50.0)
Field sting reaction (grade), n (%)			
I	1 (10.0)	1 (12.5)	3 (15.0)
II	2 (20.0)	2 (25.0)	3 (15.0)
III	3 (30.0)	1 (12.5)	7 (35.0)
IV	4 (40.0)	4 (50.0)	7 (35.0)
Interval field sting - start VIT or sting challenge (mo)	9.7 (8.4-20.6)	9.6 (7.7-10.4)	11.2 (8.5-25.0)
YJ venom-sIgE (kU/L)	3.51 (1.47-7.22)	7.96 (3.28-16.62)	5.15 (3.06-8.35)
Tryptase (μg/L)	5.0 (3.9-6.7)	5.5 (3.7-6.0)	4.3 (3.5-5.1)
MH (μmol/mol creatinine)	93 (62-125)	113 (73-114)	83 (60-108)
MIMA (mmol/mol creatinine)	1.1 (0.9-1.4)	1.0 (0.9-1.3)	1.4 (1.2-1.6)*

MH, Methylhistamine; *MIMA*, methylimidazole acetic acid.
Mean ± SD or median (interquartile ranges). Group differences were tested using the independent samples *t* test, Mann-Whitney *U* test, or Kruskal Wallis test.
*$P < .05$, patients on cluster protocol vs sting challenge comparator cohort.

TABLE E2. Overview of clinical and laboratory characteristics of reactors to sting challenges

		Patient 1	Patient 2	Patient 3	Patient 4
Symptoms		Within 2 min: Feeling of impending doom, anxiety and confusion; metallic taste in mouth; tachycardia; swollen lips and eyelids; obstructed nose and red sclera eyes; flushing of head, chest, and arms; nausea, abdominal cramping, and urge to defecate Intervention with epinephrine after 3 min Pronounced shaking afterwards	After 20 min: Headache and heavy feeling; flushing of face, neck, chest, and upper part of back; urticaria neck, back, and abdomen	After 35 min: Increase in blood pressure; tachycardia; headache; swelling of the neck; change in voice; urticaria on neck, chest, and back	After 11 min: Flushing of face, chest, abdomen, and arm; metallic taste in mouth; feeling of deafness; swollen throat with tingling sensation; edema eyes; itchiness all over body
Tryptase (μg/L)	Before sting	4.2	4.6	6.0	4.5
	1 h after sting	18.4	6.2	6.8	7.8
	2 h after sting	11.2	5.4	6.7	4.1
MH (μmol/mol creatinine)	Before sting	68	96	214	113
	1 h after sting	191	179	274	681
MIMA (mmol/mol creatinine)	Before sting	1	1.3	1.9	1.4
	1 h after sting	4.7	1.4	2.1	9.9

MH, Methylhistamine; *MIMA*, methylimidazole acetic acid.

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