

Letter to the Editor

Wasp venom immunotherapy expands a subpopulation of CD4⁺CD25⁺ forkhead box protein 3–positive regulatory T cells expressing the T-cell receptor Vβ2 and Vβ5.1 chains

To the Editor:

There is a broad body of *in vitro* and *in vivo* evidence that the induction of CD4⁺CD25⁺ forkhead box protein 3 (Foxp3)⁺ regulatory T (Treg) cells in the course of immunotherapy plays a major role in achieving tolerance to causative allergens. However, the precise mechanism of Treg cell-mediated deviation/suppression of immune responses induced by immunotherapy is still unresolved. Through almost all studies, the most striking measurable effect of immunotherapy is an increased production of the immunomodulatory cytokines IL-10 by Treg cells and IFN-γ by conventional T cells.¹ We have recently shown that during wasp (*Vespula* species) venom immunotherapy (VIT), Treg cells are specifically activated during the first month, followed by a decrease to baseline levels after 6 months of VIT.² Because both the development and function of individual Treg cells are largely defined by the expression of specific T-cell receptors (TCRs),³ we investigated the expression of specific TCR Vβ chains in the course of VIT.

Therefore we studied whether the enhanced Treg cell activation was accompanied by allergen-specific expansion of a specific Treg cell subpopulation by analyzing 24 TCR Vβ chains longitudinally (ie, 0 month, 1 month, and 6 months after initiation of VIT) in a homogeneous group of 20 patients with wasp venom allergy. Ten male patients (mean age, 44.4 years; age range, 25–69 years) and 10 female patients (mean age, 44.9 years; age range, 29–67 years) who had experienced anaphylaxis after wasp stings during the 2 years before were eligible for VIT, according to national and international recommendations and guidelines.⁴ Venom-specific sensitization was confirmed based on positive intradermal skin test results (ALK-Abelló, Hamburg, Germany), as well as detection of venom-specific serum IgE (ImmunoCAP; Phadia, Uppsala, Sweden). Patients' clinical characteristics are depicted in Table I.⁵

Wasp VIT was performed as described previously.² The incremental phase of VIT was carried out with an aqueous venom preparation during 3 consecutive days in which escalating venom doses were injected subcutaneously into the upper arms. Doses were increased from 0.1 to 100 μg, reaching a total amount of 201.1 μg of venom on day 3. Subsequently, maintenance injections of 100 μg of aluminum hydroxide-adsorbed depot venom preparations were administered after 2 days and 1, 4, and 8 weeks and thereafter for every 6 weeks. After obtaining approval of the local ethics committee and informed consent from the patients, blood samples were drawn directly before the injection of venom. Peripheral venous blood mononuclear cells were immunolabeled with mAbs against CD3, CD4, CD25, Foxp3, CD45RO, and Vβ chains (Vβ kit; Beckman Coulter, Krefeld, Germany) and analyzed by using flow cytometry.

Analyzing all CD3⁺CD4⁺ T cells, the Vβ expression pattern did not change significantly before and 1 month after starting VIT (see Fig E1 in this article's Online Repository at www.jacionline.org). Because of the rather low frequencies of Treg cells in peripheral venous blood (1% to 2% of the peripheral

TABLE I. Characteristics of patients

No. of subjects	20
Age (y), mean ± SD	44.7 ± 13.0
Sex (M/F)	10/10
Anaphylaxis (grade, according to Ring and Messmer ⁵)	
I	0
II	18
III	2
IV	0
Threshold of positive IDT (μg/mL [no. of subjects])	
<i>Vespula</i> species	0.01 (7)
	0.1 (9)
	1.0 (4)
<i>Apis mellifera</i>	0.1 (1)
	1 (1)
Total IgE (kU/L [<100 kU/L]), mean ± SEM	93.2 ± 15.3
Specific IgE (kU/L [<0.35 kU/L]), mean ± SEM	
<i>Vespula</i> species	9.7 ± 4.1
<i>Apis mellifera</i>	0.3 ± 0.1
Atopy	2/20

Values in brackets are reference values. Results of bee venom (*Apis mellifera*) testing of all patients with wasp venom allergy are also indicated.
F, Female; IDT, intradermal test; M, male.

CD3⁺CD4⁺ T-cell population), only Vβ chains expressed with higher frequencies (Vβ2, Vβ3, Vβ5.1, Vβ8, Vβ13.1, and Vβ17 in our population; see Fig E1), could be reasonably analyzed in activated/memory Treg cells (CD4⁺CD25⁺Foxp3⁺CD45RO⁺), resting/naive Treg cells (CD4⁺CD25⁺Foxp3⁺CD45RO[−]), memory T cells (CD4⁺CD25[−]Foxp3[−]CD45RO⁺), and naive T cells (CD4⁺CD25[−]Foxp3[−]CD45RO[−]) by using a flow cytometric gating approach, as shown in Fig 1, A.⁶ Whereas the frequencies of Vβ3⁺, Vβ8⁺, Vβ13.1⁺, and Vβ17⁺ T cells did not change throughout the observation period in either T-cell subset (see Fig E2 in this article's Online Repository at www.jacionline.org), the relative numbers of Vβ2⁺ and Vβ5.1⁺ cells among activated/memory Treg cells significantly increased after 1 month of VIT (mean ± SEM Vβ2 expression: 10.5% ± 0.58% compared with 1 month: 11.3% ± 0.5%, $P = .048$; mean ± SEM Vβ5.1 expression: 6.2% ± 0.39% compared with 1 month: 6.9% ± 0.5%, $P = .031$; Fig 1, B). This is also highlighted by the individual changes in frequency distribution (see Fig E3 in this article's Online Repository at www.jacionline.org). Simultaneous measurements demonstrated that the expression of these TCR Vβ chains remained unchanged in conventional memory T cells (Fig 1, B, and see Fig E3). Interestingly, 6 months after initiating VIT, the frequency of Vβ2⁺ and Vβ5.1⁺ expressing memory Treg cells returned to baseline levels. Additional analysis of CD127 demonstrated that the identified Treg cells were not recently activated conventional T cells because they were CD127[−] or CD127^{low} (data not shown).⁷ In contrast, neither resting/naive Treg cells nor naive conventional T cells showed a specific change in TCR Vβ2 or Vβ5.1 chain use (data not shown). Because this study focused on high-expression Vβ chains, it cannot be ruled out that other initially low-frequency Vβ populations might expand in the course of VIT.

This transient increase in the frequencies of Vβ2⁺ and Vβ5.1⁺ TCR-expressing cells after 1 month of VIT was only

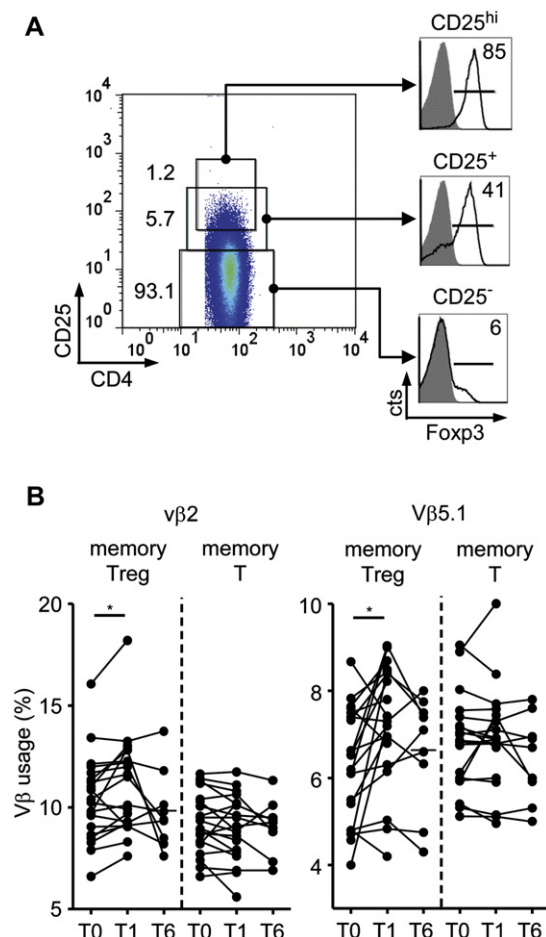


FIG 1. TCR V β use in memory Treg and memory T cells. **A**, Gating approach for CD4⁺CD25⁺Foxp3⁺ T cells. Percentages of CD25⁺ and Foxp3⁺ T cells are indicated, respectively. cts, Counts. **B**, Significant increase in V β 2- and V β 5.1-expressing memory Treg cells after 1 month (T1) of VIT. N = 20 (T0 and T1) and n = 9 (T6). *P < .05 (repeated-measures ANOVA, followed by the Tukey-Kramer test).

observed among Treg cells with an activated/memory phenotype, which are usually highly suppressive *in vitro*. As shown recently, they might also account for the VIT-induced inhibition of wasp venom-driven T-cell proliferation *in vitro* occurring in parallel.^{2,6} The transient and relatively short-lived increase in specific V β chain use most likely reflects the highly dynamic and adaptive phenotype of Treg cells in general and of activated/memory Treg cells in particular.⁶ In contrast to memory T cells, Treg cells are characterized by a rapid turnover rate, as reflected by a short half-life time of 11 days and a high proliferation rate.^{2,8}

It is tempting to speculate that these oligoclonally expanded Treg cells with restricted V β use might be allergen-specific Treg cells, which are able to selectively suppress the wasp venom-induced allergic immune response. This hypothesis is supported by the finding that in a natural model of immune regulation, multiple bee stings in healthy nonallergic beekeepers

induced clonal switching from dominant venom antigen-specific T_H1 and T_H2 cells toward dominant IL-10-secreting Treg cells persisting 3 months after cessation of allergen exposure.⁹

Numerous studies provide steadily increasing evidence for a functional role of Treg cells during immunotherapy of allergic patients by actively restoring allergen tolerance.¹ However, to our knowledge, this is the first report on the effect of VIT on the TCR repertoire of Treg cells. We could demonstrate a transient increase in the frequencies of V β 2 and V β 5.1 TCR chain-expressing cells among Treg cells in the course of VIT, which might indicate the oligoclonal activation and expansion of allergen-specific Treg cells. These findings provide new insights into the role of allergen-specific Treg cells during immunotherapy and might pave the way for a further refinement of allergen immunotherapy through even more selective targeting of Treg cells expressing the V β 2 and V β 5.1 TCRs.

We thank Claudia R  th for technical assistance and Petra Pfeuffer and Petra Raith for performing blood sampling and intradermal skin testing.

Andreas Kerstan, MD^a

Niklas Beyersdorf, MD^b

Johanna Stoevesandt, MD^a

Axel Trautmann, MD^a

From ^athe Department of Dermatology, Venereology, and Allergology, University Hospital of W  rzburg, W  rzburg, Germany, and ^bthe Institute for Virology and Immunobiology, University of W  rzburg, W  rzburg, Germany. E-mail: kerstan_a@klinik.uni-wuerzburg.de.

Supported by the Deutsche Forschungsgemeinschaft (TR460/3-1 and -2 to A.K. and A.T.) and the IZKF W  rzburg (Z-2/18 to A.K.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

- Ozdemir C, Kucuksezer UC, Akdis M, Akdis CA. Mechanisms of immunotherapy to wasp and bee venom. *Clin Exp Allergy* 2011;41:1226-34.
- Kerstan A, Albert C, Klein D, Br  cker EB, Trautmann A. Wasp venom immunotherapy induces activation and homing of CD4(+)CD25(+) forkhead box protein 3-positive regulatory T cells controlling T(H)1 responses. *J Allergy Clin Immunol* 2011;127:495-501.
- Pacholczyk R, Kern J. The T-cell receptor repertoire of regulatory T cells. *Immunology* 2008;125:450-8.
- Golden DB, Moffitt J, Nicklas RA, Freeman T, Graft DF, Reisman RE, et al. Stinging insect hypersensitivity: a practice parameter update 2011. *J Allergy Clin Immunol* 2011;127:852-4.
- Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1977;1:466-9.
- Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human CD4⁺ T cells expressing the FoxP3 transcription factor. *Immunity* 2009;30:899-911.
- Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med* 2006;203:1693-700.
- Vukmanovic-Stejic M, Zhang Y, Cook JE, Fletcher JM, McQuaid A, Masters JE, et al. Human CD4⁺ CD25hi Foxp3⁺ regulatory T cells are derived by rapid turnover of memory populations *in vivo*. *J Clin Invest* 2006;116:2423-33.
- Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med* 2008;205:2887-98.

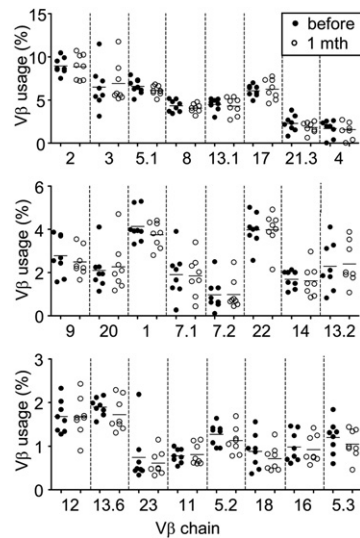


FIG E1. TCR Vβ chain expression of CD3⁺CD4⁺ T cells. TCR Vβ repertoire was analyzed by costaining with 24 different anti-TCR Vβ antibodies before and 1 month after starting VIT. Each *circle* represents 1 patient. *Dashes* indicate means.

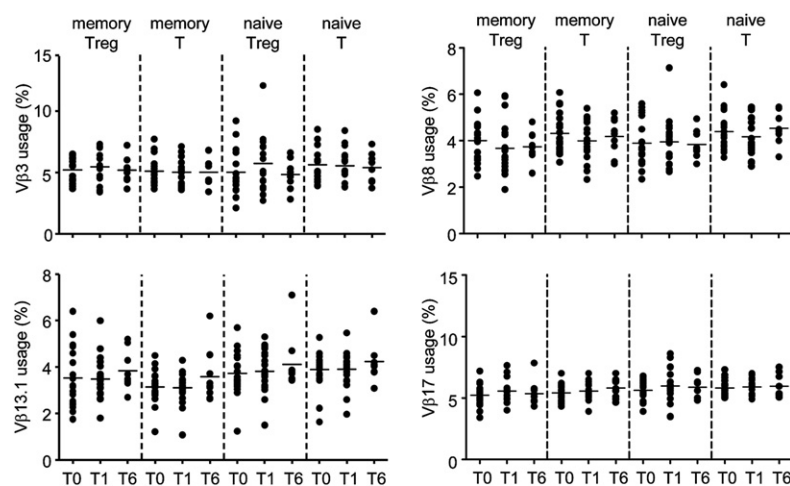


FIG E2. TCR Vβ3, Vβ8, Vβ13.1, and Vβ17 use of T-cell subsets. TCR Vβ chains were determined before (*T0*), 1 month (*T1*), or 6 months (*T6*) after starting VIT. N = 20 (*T0* and *T1*) and n = 9 (*T6*). Each circle represents 1 patient. Dashes indicate means.

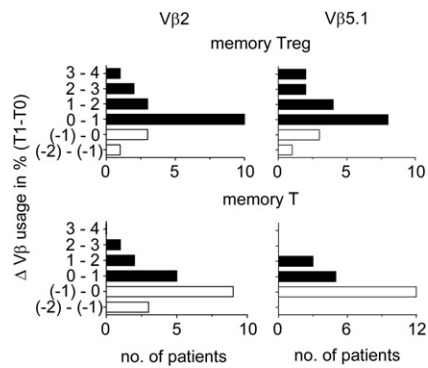


FIG E3. Individual changes in Vβ2 and Vβ5.1 frequency distribution of memory Treg and memory T cells after 1 month of VIT (*T1*) compared with the start of VIT (*T0*). Change (Δ) in expression is depicted in 1% intervals.