

Letter to the Editor

Tolerant beekeepers display venom-specific functional IgG₄ antibodies in the absence of specific IgE

To the Editor:

Beekeepers are exposed to multiple stings in season although they are immune to the effects apart from local swellings at the site of the sting. This is in contrast to venom-allergic individuals who are in general stung only occasionally yet remain at risk of IgE-mediated anaphylaxis. To assess the mechanism of tolerance in beekeepers, we measured their serum allergen-specific IgE and IgG₄ levels. We measured the ability of their serum to form bee venom IgE-allergen complexes (the IgE-facilitated-allergen binding [FAB] assay). Results were compared to those in bee venom-allergic subjects (positive control) and normal healthy individuals (negative control). We also measured the ability of serum from tolerant beekeepers to inhibit allergen-IgE binding in the IgE-FAB assay. To determine whether any observed differences in beekeepers are sustained in the absence of exposure, we compared immunoreactive and functional immunoglobulin levels in beekeepers inside/outside the beekeeping season.

Approval was obtained from the local ethics committee, and the study was performed with the written informed consent of the participants. Sera from 10 nonallergic (tolerant) beekeepers (mean age, 48.2 years; men/women, 10/0) were analyzed in July, corresponding to the peak beekeeping season, and compared with sera from 10 bee venom-allergic subjects (mean age, 40.5 years; men/women, 4/6) and from 10 nonallergic healthy controls (mean age, 40.0 years; men/women, 4/6). Sera from beekeepers were additionally analyzed outside the season in January. Phospholipase A2-specific IgG₄ and IgE levels were measured by using ImmunoCAP. IgE functional activity of participants' sera was assessed by the IgE-FAB assay as described previously.¹ Briefly, sera were incubated at 37°C with 3 µg/mL of *Apis mellifera* extract. Allergen-IgE complexes were incubated for 1 hour at 4°C to bind CD23 on the surface of B cells. Complexes bound to B cells were detected by flow cytometry by using a polyclonal human anti-IgE phycoerythrin-labeled antibody. In addition, serum inhibitory activity for IgE-FAB was measured in beekeepers' serum obtained inside and outside the beekeeping season by incubating with an indicator serum (bee venom-allergic serum with elevated specific IgE level of >20 kU/mL) at 37°C with 3 µg/mL of *A. mellifera* extract. To explore the likely IgG association of this serum inhibitory activity, pooled serum from 3 bee-tolerant individuals was fractionated by affinity chromatography by using a Protein G column (Protein G minispin column, Generon, Berkshire, United Kingdom) and the inhibitory activity of the pooled serum was compared with that of IgG-enriched and IgG-depleted fractions, as previously described.²

In tolerant beekeepers compared with bee venom-allergic individuals, there were marked elevations in allergen-specific IgG₄ levels during the summer (median [interquartile range (IQR)], 76.00 [42.75-250.5]) (Fig 1, A) whereas allergen-specific IgE and IgE FAB were virtually absent (median [IQR], 0.14 [0.06-0.23]; 0.02 [0.0-0.80]) (Fig 1, B and C). In contrast, in venom-allergic subjects, there were markedly lower but detectable levels of specific IgG₄ (median [IQR], 2.20 [0.91-10.55]) (Fig 1, A) whereas specific IgE and IgE FAB levels were elevated (median

[IQR], 4.84 [1.89-10.64]; 4.20 [3.59-5.74]) (Fig 1, B and C). In normal controls, there was complete absence of specific IgG₄ (median [IQR], 0.05 [0.04-0.08]), IgE (median [IQR], 0.03 [0.02-0.08]), and IgE-FAB (median [IQR], 0.00 [0.0-0.27]) (Fig 1, A-C).

To determine whether seasonal repeated field stings in tolerant beekeepers resulted in "protective" IgG-associated serum inhibitory activity (as observed during bee venom immunotherapy [VIT]), we measured the ability of their serum to inhibit IgE-FAB by using an indicator system as described previously¹ (Fig 2). Both IgG₄ and serum inhibitory activity for IgE-FAB was markedly elevated in season (median [IQR], 76.00 [42.75-250.5]; 75.63 [61.93-88.02]) (Fig 2, A and C). Although there was a small but significant decrease in both IgG₄ and serum inhibitory activity out of season (median [IQR], 47.50 [10-188]; 71.04 [43.34-77.10]) (Fig 2, A and C), levels remained substantially elevated, whereas serum IgE was virtually undetectable both inside and outside of season (Fig 2, B). Separation of pooled sera into IgG-containing and IgG-depleted fractions confirmed that greater than 90% of the serum inhibitory activity for bee venom allergen-IgE binding to B cells resided within the IgG fraction (data not shown).

Our findings in beekeepers have similarities to those observed during bee VIT where significant increases in bee venom-specific IgG₄ serum antibody levels during VIT maintenance³ were observed, likely due to an accompanying increase in IL-10-secreting regulatory T cells.⁴ In our studies of bee VIT in children, we also found parallel significant increases in serum inhibitory activity for IgE-FAB.³ However, high levels of allergen-specific IgG₄ antibodies and serum inhibitory activity for IgE-FAB were not sustained at 2 years following VIT withdrawal. In tolerant beekeepers, venom-specific IgG₄ levels remained significantly elevated between seasons as compared with both normal controls and venom-allergic patients with a small but significant decline during winter in the absence of stings.

In our previous study of bee VIT,³ we observed a significant decline in venom-specific serum IgE antibodies during VIT with a further decline at 2 years following VIT withdrawal. We speculated that long-term clinical protection following bee VIT withdrawal⁵ may result from the prolonged suppression of allergen-specific IgE rather than sustained elevations in blocking IgG₄ antibodies. Similarly, we identified low/absent levels of specific IgE in our beekeepers.

Our current data in tolerant beekeepers suggest that their protection from allergic reactions afforded by repeated stings may be due to *either* high levels of immunoreactive IgG₄ antibodies with blocking activity *and/or* low levels of venom-specific IgE antibodies. With regard to the underlying mechanism of long-term tolerance in beekeepers, this dichotomy could be further addressed in a future study involving long-term withdrawal from beekeeping, although this may be difficult to achieve for cultural reasons.

Our findings in tolerant beekeepers of a decline in immunoreactive IgG₄ antibody levels between seasons, and in our previous study of VIT in children, are in contrast to studies of antibody levels during grass pollen immunotherapy.⁶ Grass pollen-treated individuals maintained persistent IgG-associated serum inhibitory activity for FAB for at least 2 years following the withdrawal of immunotherapy. One explanation for differences in protection afforded by beekeeping or bee VIT compared with grass pollen

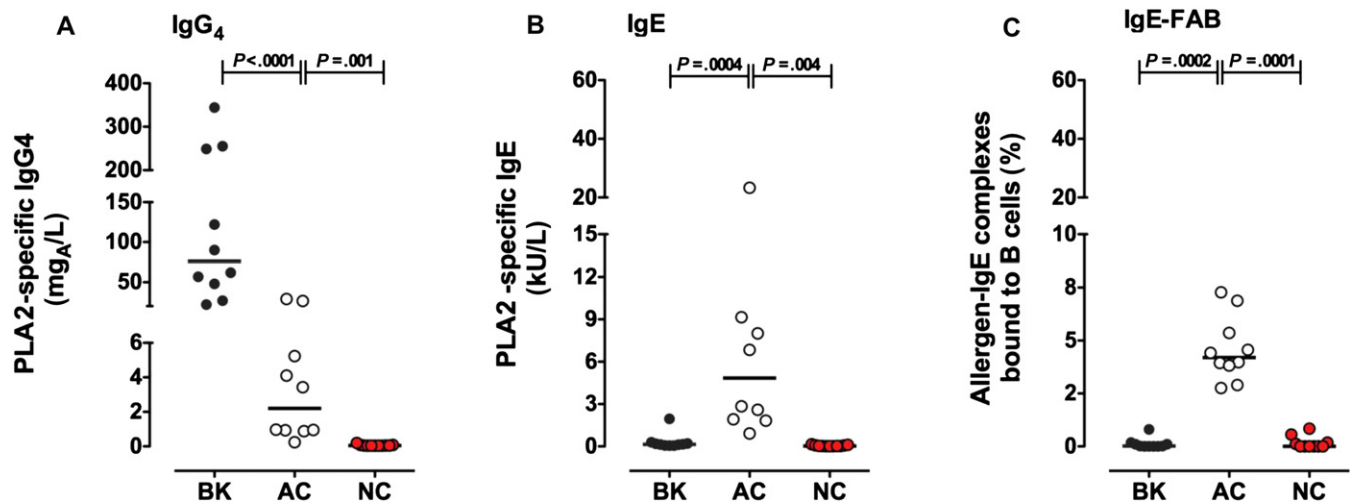


FIG 1. A, Serum PLA2-specific IgG₄ antibodies. B, Serum PLA2-specific IgE antibodies. C, The level of serum IgE-associated binding activity for allergen-IgE complexes to B cells. Data are given for tolerant beekeepers (BK), bee venom-allergic controls (AC), and nonatopic normal healthy controls (NC). Mann-Whitney test ($P < .05$ considered significant). PLA2, Phospholipase A2.

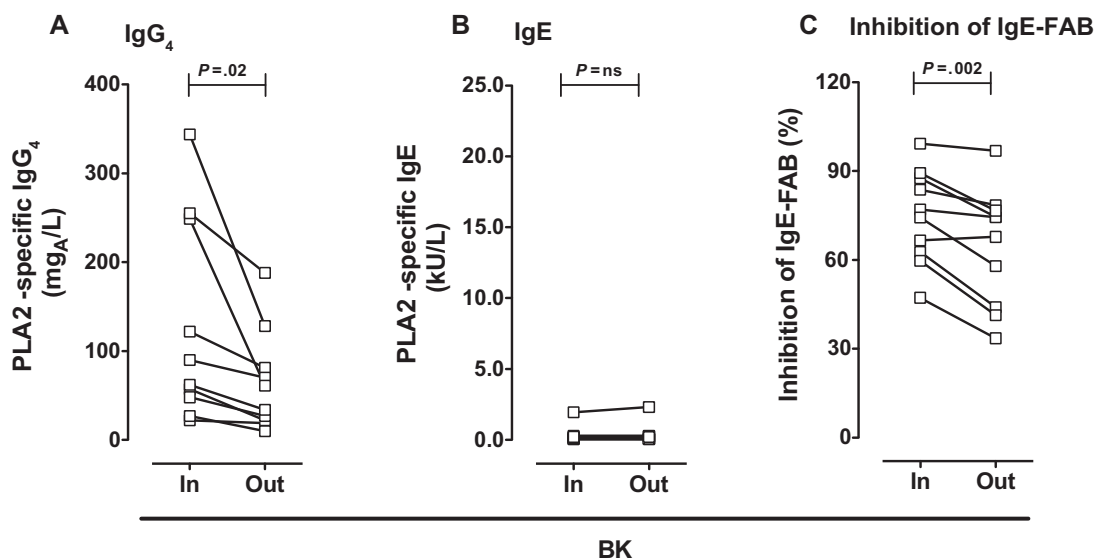


FIG 2. A, Serum PLA2-specific IgG₄ antibodies. B, Serum PLA2-specific IgE antibodies. C, The level of serum IgG-associated inhibitory activity for binding of allergen-IgE complexes to B cells. Data are given for tolerant beekeepers inside (July) and outside (January) the beekeeping season. Wilcoxon matched-pairs signed ranks test ($P < .05$ considered significant). PLA2, Phospholipase A2.

immunotherapy may be the intermittent and systemic exposure to venom that contrasts with the low-dose, particulate and mucosal exposure to grass pollen that is responsible for the disease and alternative mechanisms of tolerance induction.

Eva-Maria Varga, MD^a
 Fahima Kausar, MSc^b
 Werner Aberer, MD^c
 Maximilian Zach, MD^a
 Ernst Eber, MD^a
 Stephen R. Durham, MD^b
 Mohamed H. Shamji, PhD^b

From ^athe Respiratory and Allergic Disease Division, Department of Paediatrics, Medical University Graz, Austria; ^bAllergy and Clinical Immunology, National Heart and

Lung Institute, Imperial College London, United Kingdom; and ^cthe Department of Environmental Dermatology and Allergy, Medical University Graz, Austria.
 E-mail: s.durham@imperial.ac.uk.

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