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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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The honey bee venom allergen Api m 10 displays one major IgE epitope, Api m $10_{160\text{-}174}$

To the Editor,

Yellow jacket and honey bee stings are among the most common elicitors of severe anaphylactic reactions in Europe.[1] The only causal therapy to prevent future anaphylactic reactions is allergen immunotherapy (AIT). AIT is well established for patients allergic to honey bee (HBV) and yellow jacket venom (YJV).[2] However, the efficacy of AIT with HBV is lower than with YJV.[3] So far, the reason for this difference is not clear. Recently, we have identified Api m 10 as a major allergen in HBV[4] and described that a dominant Api m 10 sensitization is associated with an increased risk for treatment failure of AIT in HBV-allergic patients.[5] In addition, we and others reported that Api m 10 is absent or underrepresented in several therapeutic HBV preparations.[5-7]

To characterize the IgE response to Api m 10 in detail, we now analyzed linear IgE epitopes recognized by patients allergic to HBV. Synthetic 15-mer peptides with 12 amino acids overlay were generated (>95% purity, CASLO ApS) spanning the whole amino acid sequence of Api m 10 (isoform variant 1; 204 amino acids) and coupled to macroarrays (Macro Array Diagnostics). Recombinant (r) HBV allergens (rApi m 1, 2, 3, and 10) served as positive controls (for more details see this article's online supporting information). IgE binding was evaluated in sera obtained from HBV-allergic patients prior to initiation of AIT that displayed specific (s) IgE to Api m 10 (>0.35kU_A/L) as determined by ImmunoCAP (ThermoFisher Scientific) (Api m 10-sIgE median [range]: 3.79 [0.59-66.6] kU_A/L; n = 40) and (for more details see online supporting information) in sera from healthy controls without a history of HBV allergy and without Api m 10 sensitization (n = 10). All patients' sera displayed positive IgE reactivity for rApi m 10 in the macroarray analysis (5.37 [0.17-24] kU_A/L) that showed a reasonable correlation with the values obtained by ImmunoCAP (r = .7041, P < .0001). Individual sera displayed IgE reactivity to up to 29 Api m 10 peptides, and collectively 43 peptides of the 64 tested were recognized by IgE of HBV-allergic patients. Three Api m 10 regions were identified that were recognized by more than 40% of tested sera. Higher numbers of recognized peptides correlated with higher Api m 10-sIgE concentrations (r = .6500, P < .0001; data not shown). Healthy control sera did not show IgE binding to any of the Api m 10 peptides or to any of the HBV allergens tested (data not shown).

Interestingly, one peptide, P54 (Api m $10_{160\text{-}174}$; amino acid sequence: ADSDVTTLPTLIGKN), was recognized by 100% of the Api m 10-positive sera and hence represents the dominant linear IgE epitope of Api m 10 (Figure 1A). IgE reactivity to P54 was higher than to any other Api m 10 peptide (Figure 1A) and represented on average 67% of the total Api m 10 peptide-sIgE for the tested patient cohort (data not shown). The correlation of Api m 10-sIgE and P54-sIgE (as

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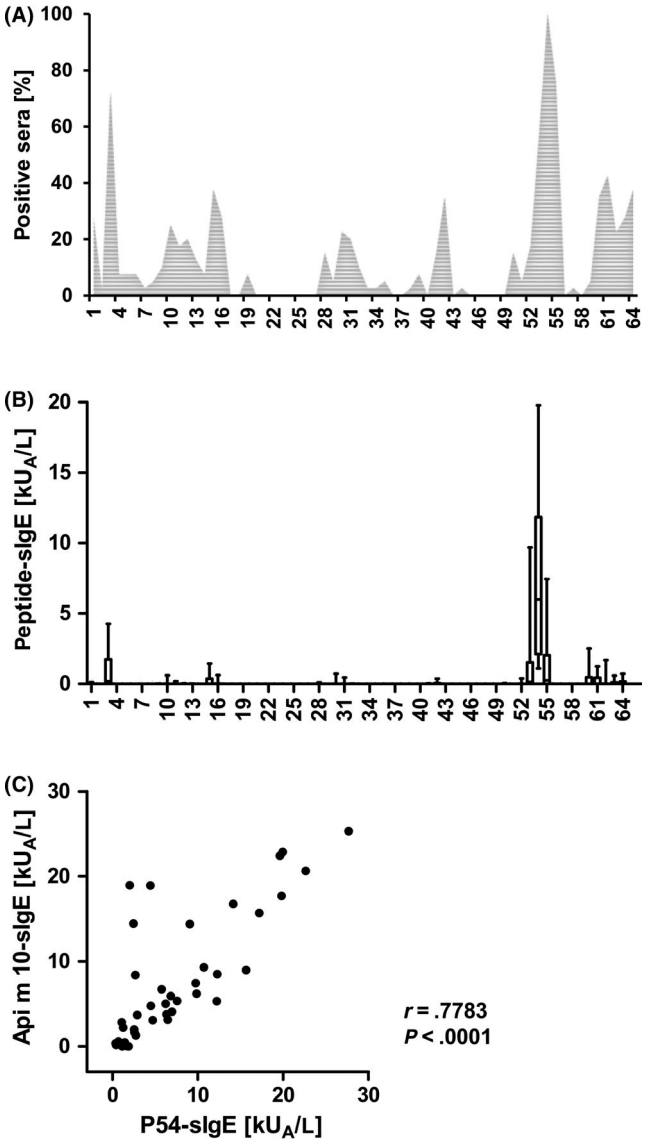

FIGURE 1 IgE reactivity to Api m 10 peptides. A, Frequency of sIgE reactivity to overlapping peptides spanning the sequence of Api m 10 in Api m 10 sensitized patients. B, Concentrations of IgE to Api m 10 peptides. C, Spearman´s correlation of sIgE to P54 and rApi m 10-sIgE

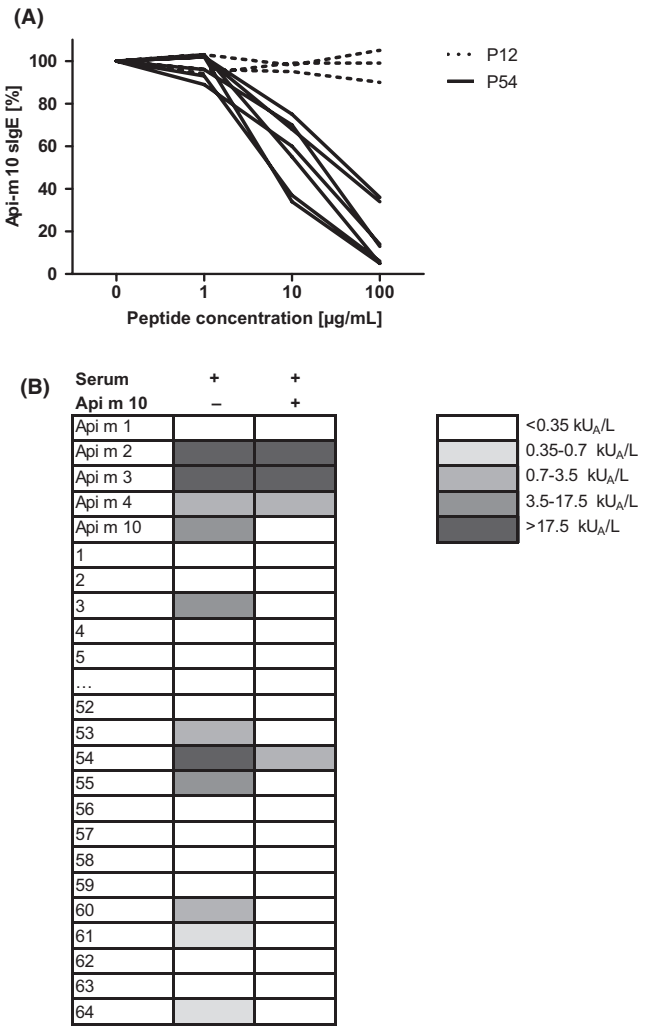

FIGURE 2 Inhibition of IgE binding to rApi m 10 by P54. A, Sera of Api m 10 sensitized patients (n = 7) were preincubated with P54 (or P12) followed by measurement of IgE reactivity to rApi m 10. B, Inhibition of IgE reactivity to Api m 10 peptides by preincubation of one patient serum with rApi m 10

determined by macroarray) showed a positive association (Figure 1C; Spearman's rank correlation coefficient; r = .7783, P < .0001).

To verify the significance of P54 in HBV allergy, ImmunoCAP inhibition experiments were performed. Preincubation of sIgE Api m 10-positive sera (n = 7) with increasing concentrations of P54 (1-100 µg/mL) resulted in a dose-dependent inhibition of IgE reactivity to rApi m 10 as determined by ImmunoCAP (Figure 2A), while no inhibition was observed with the control peptide P12 (n = 3). Vice versa, preincubation of serum with rApi m 10 inhibited binding of IgE to the Api m 10 peptides on the macroarray (Figure 2B), while binding of sIgE to other HBV allergens on the macroarray (such as rApi m 2, 3, or 4) was not inhibited (for more details see online supporting information).

Api m 10 is a major allergen in HBV and a marker allergen to discriminate HBV from YJV sensitization.[8] In addition, a dominant Api m 10 sensitization has been associated with an increased risk for AIT treatment failure in HBV allergy.[5] Although Api m 10 is a major allergen in HBV allergy, the quantity of Api m 10 in the venom is of low abundance (approx. 1%).[9] In addition, Api m 10 is easily degraded in reconstituted venom preparation[6] and has been reported to be underrepresented or absent in therapeutic venom preparations,[5-7] suggesting a potential link with treatment failure in dominantly Api m 10 sensitized HBV-allergic patients. However, the lack of results from prospective clinical studies makes it difficult to exclude other predominant sensitizations as risk factors for treatment failure and to conclude that the therapeutic failure of HBV VIT is really due to the lack of Api m 10 in therapeutic preparations. Indeed, currently we do not even know whether and how much Api m 10 is required for effective HBV AIT in Api m 10

sensitized patients. Provided that a link between lack of Api m 10 and lower therapeutic efficacy can be confirmed in prospective studies, strategies to overcome this lack may be of particular interest. In theory, this lack could be compensated by spiking therapeutic HBV preparations with rApi m 10. However, due to the intrinsic instability of Api m 10, alternative strategies may be needed. In this context, the identification of a major Api m 10 IgE epitope, such as P54, that is recognized by all tested sera may become highly relevant. A short immunodominant peptide is easier to produce and thus may have relevant advantages over the use of full-length rApi m 10. Mapping of corresponding T-cell epitopes of Api m 10 is currently underway to address the relevance of P54 for the induction of protective T-cell responses. Finally, due to its dominant IgE reactivity, P54 may substitute rApi m 10 in diagnostic approaches, for example, in a peptide-based sIgE assay. Larger patient samples are needed to address the feasibility and assay performance of this approach.

In summary, we identified a major IgE epitope of the HBV allergen Api m 10 that is recognized by all sera from HBV-allergic patients sensitized to Api m 10. This may be of use for diagnostic purposes and potentially also for augmentation of therapeutic HBV preparations that lack Api m 10 immunoreactivity.

KEYWORDS

allergy treatment, basic immunology, IgE, immunotherapy and tolerance induction, personalized medicine, venom and insect allergy

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CONFLICTS OF INTEREST

MMR, CM, AJ, MM, ES, SM and AR declare that they have no potential conflict of interest. WP has given lectures for ALK-Abelló and Thermo Fisher Scientific, served on advisory boards for ALK-Abelló and received research funding from ALK-Abelló. TJ has given lectures for ALK-Abelló, Allergy therapeutics/ Bencard, Novartis, and Thermo Fisher Scientific, served on advisory boards for ALK-Abelló, Allergopharma, Allergy therapeutics/ Bencard, and Novartis and received research funding by ALK-Abelló, Novartis, and Thermo Fisher Scientific.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Anxiety and depression may associate with poorer control and quality of life in adults with asthma

To the Editor,

Asthma is a major cause of global health problems and affects more than 300 million individuals worldwide. Furthermore, the global burden of asthma has increased by almost 30% in the past 20 years.[1] Recent studies in different populations have shown that people with asthma have a higher prevalence of anxiety and depression than the general population.[2] A recent national cross-sectional study reported that there were approximately 45 million adults with asthma in China, but co-existing mental health conditions in asthma are often overlooked during the clinical treatment.[3] The impact of psychological disorders in people with asthma, especially those with uncontrolled symptoms, has not been fully elucidated.[4] Therefore, in this study, we aimed to investigate the prevalence of anxiety and depression in people with asthma in China. We also sought to examine associations between psychological conditions and patient-centered outcomes, including disease control and quality of life among adults with asthma.

The study protocol was approved by the Ethics Committee of Ningbo First Hospital, and all the participants provided written informed consent. Details for recruitment and methods are provided in this article's online supporting information (Data S1, Figure S1).

105 male and 151 female participants were recruited in this study, with a mean age of 50.2 ± 15.2 years old, mean BMI of 23.6 ± 4.0, and mean asthma duration of 14.5 ± 17.9 years. Using the Hospital Anxiety and Depression Scale (HADS), 35 (13.7%) patients were found to have symptoms of anxiety and 26 (10.2%) to have symptoms of depression. Characteristics of subjects and significant comparisons are summarized in Table 1.

Lower lung functions were found in people with higher anxiety symptoms from the HADS score (Table 1). In terms of quality of life, the Asthma Quality of Life Questionnaire (AQLQ) scores decreased with increasing HADS-A score in all domains (Table 2). The Asthma Control Questionnaire (ACQ) score was positively associated with the HADS-A score (Table 2). Patients with high levels of anxiety symptoms were more likely to have uncontrolled asthma than patients with low levels of anxiety (85.7% vs 51.1%). Similarly, lower FEV_1, PEF, $MMEF_{75/25,}$ and $MMEF_{75/25}$% were found in patients with higher depression scores. The AQLQ scores were inversely associated with the HADS-D score in domains of the total, activity limitation, asthma symptoms, psychological status, and self-health care (Table 2). The ACQ score was higher in patients with higher HADS-D score (Table 2).

In this study, a high prevalence of anxiety (13.7%) and depression (10.2%) was observed in patients with asthma. Results suggested that higher levels of anxiety symptoms and depression symptoms are associated with lower lung function, poor asthma control, and lower quality of life. Moreover, we further analyzed our data in three different severity levels (well-controlled, partially controlled, and uncontrolled). Symptoms of anxiety and depression were more severe in patients with asthma who had uncontrolled disease, when comparing with those with well or partially controlled disease.

Patients with asthma in other developed countries, such as in Europe and the USA, have a higher prevalence of anxiety and depression than the levels found in our study within a Chinese population. For anxiety, prevalence in other countries is at 11%-37% and prevalence of depression has been evidenced at 18%.[4,5] This difference in prevalence of anxiety and depression may be attributable to variance in socioeconomic status between different

Abbreviations: AQLQ, the Asthma Quality of Life Questionnaire; BMI, body mass index; FEV_1 (%), predicted one-second forced expiratory volume; FEV_1/FVC, forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) ratio; FEV_1/L, one-second forced expiratory volume; FVC (%), predicted forced vital capacity; FVC/L, forced vital capacity; HADS, the Hospital Anxiety and Depression Scale; HADS-A, the anxiety subscale of the Hospital Anxiety and Depression Scale; HADS-D, the depression subscale of the Hospital Anxiety and Depression Scale; $MMEF_{75/25}$ (%), predicted maximal mid-expiratory flow; $MMEF_{75/25}$/L, maximal mid-expiratory flow; PEF (%), predicted peak expiratory flow; PEF/L, peak expiratory flow.