

SUPPLEMENTARY MATERIAL

Supplementary data Figure 1: Alanine scan library of peptide 54.

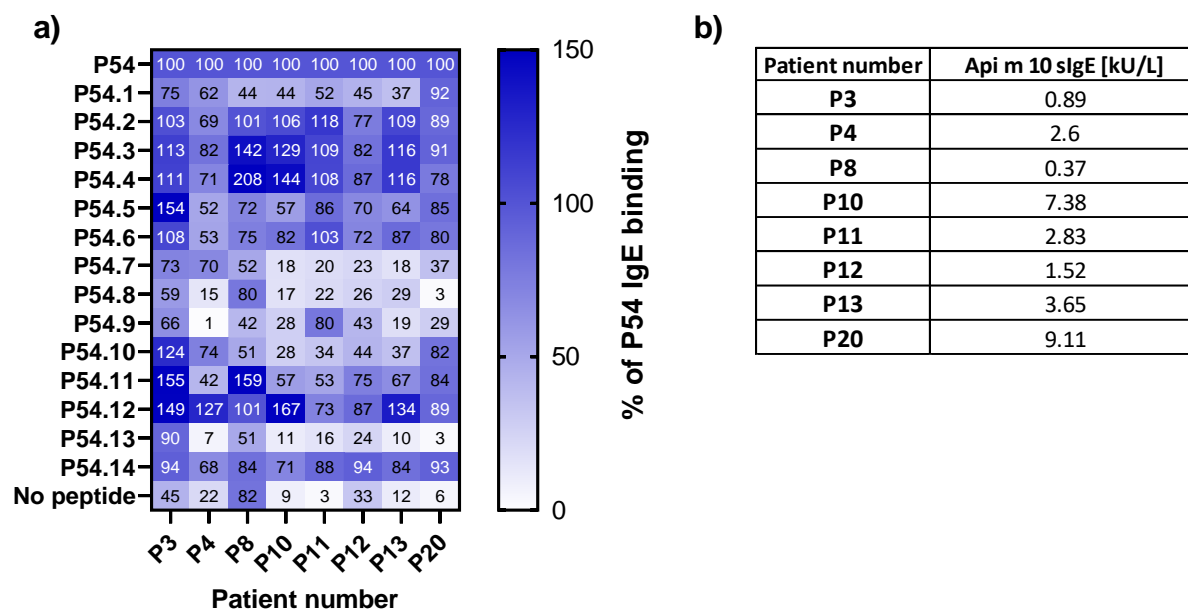
Alanine scan peptides derived from peptide 54 with indicated alanine mutations.

Peptide No	Sequence
P54	ADSDVTTLPTLIGKN
1	AADSDVTTLPTLIGKN
2	ADADVTTLPTLIGKN
3	ADSAVTTLPTLIGKN
4	ADSDAVTTLPTLIGKN
5	ADSDVAITLPTLIGKN
6	ADSDVTALPTLIGKN
7	ADSDVTTAPTLIGKN
8	ADSDVTTLATLIGKN
9	ADSDVTTLPALIGKN
10	ADSDVTTLPTAIGKN
11	ADSDVTTLPTLAGKN
12	ADSDVTTLPTLIAKN
13	ADSDVTTLPTLIGAN
14	ADSDVTTLPTLIGKA

Supplementary data Figure 2: Individual patient immunoreactivity towards Api m 10 peptides and Api m 10.

A: Reactivity of individuals patient IgE towards alanine scan peptides normalized to P54 IgE binding.

B: Individual patients' specific IgE to Api m 10 was determined in the ImmunoCAP system.

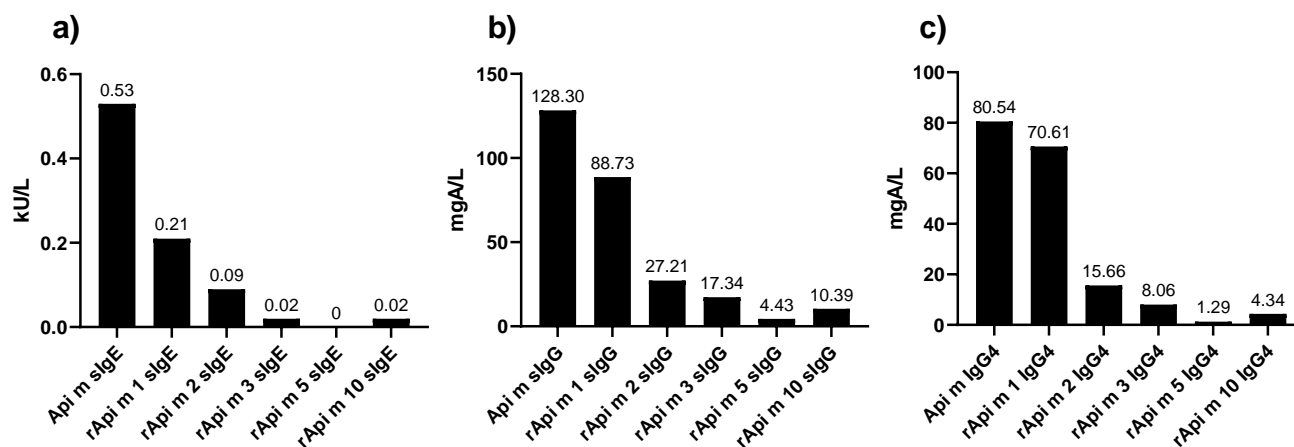


Supplementary data Figure 3: Beekeeper serological data

A: Beekeeper sIgE serological data determined using the ImmunoCAP system

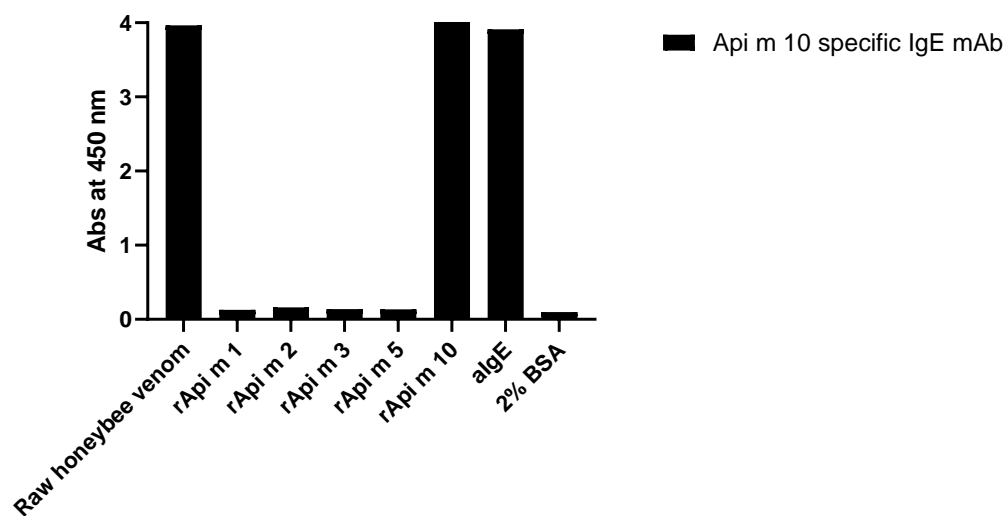
B: Beekeeper sIgG serological data determined using the ImmunoCAP system

C: Beekeeper sIgG4 serological data determined using the ImmunoCAP system



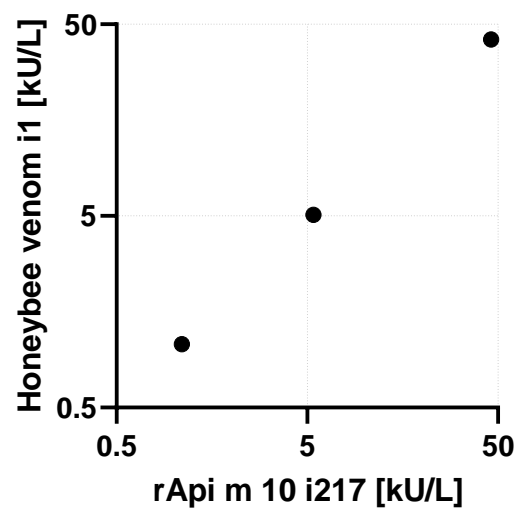
Supplementary data Figure 4: Immunoreactivity analysis of the anti-Api m 10 IgE antibody.

Immunoreactivity and specificity of the Api m 10 specific IgE mAb was assessed by ELISA using cellular supernatant diluted 1:10 and a set of recombinant HBV allergens as compared to venom. Detection of bound IgE was performed using the anti-human IgE alkaline phosphatase conjugate.



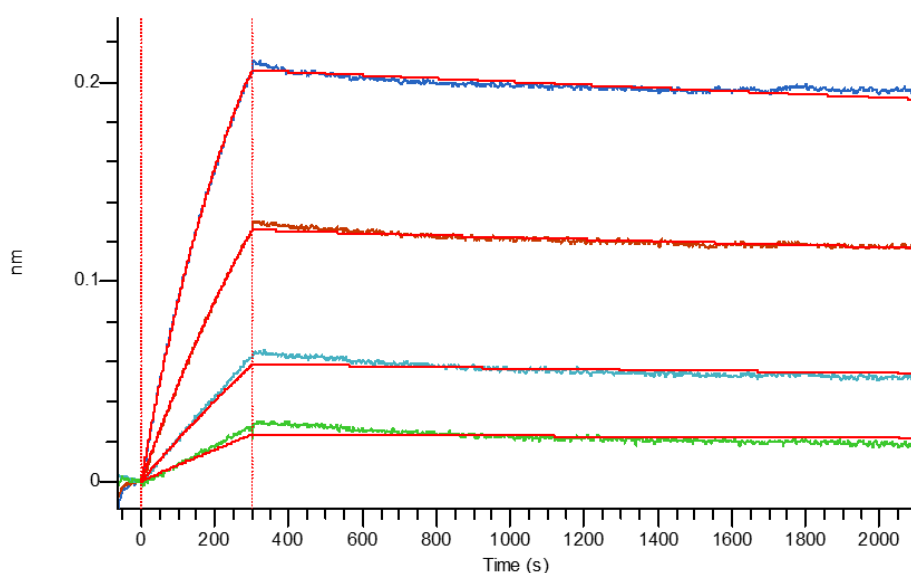
Supplementary data Figure 5: Determination of IgE Immunoreactivity using the immunoCAP.

Specific IgE of the monoclonal IgE antibody 1E10 to Api m 10 as compared to HBV was determined in the ImmunoCAP system.



Supplementary data Figure 6: Affinity determination using bio-layer interferometry (BLI).

Sensorgrams of varying concentrations of rApi m 10 binding to immobilized Api m 10 specific IgE mAb using BLI with 300 and 1800 seconds of association and dissociation, respectively. A 1:1 global fit binding model was fitted on the sensorgrams resulting in the affinity variables in the table.



mAb ID	KD (M)	KD error (M)	ka (1/M*s)	kd (1/s)	χ^2	R²
Api m 10 specific IgE mAb	1.372e ⁻¹⁰	1.18e ⁻¹²	299900	4,12e ⁻⁵	0,0761	0,999

Supplementary data Figure 7: Epitope identification of the mAb specific for Api m 10

Inhibition of the mAb immunoreactivity towards rApi m 10 by the 64 overlapping peptides (left). Non-inhibiting peptides are marked yellow, inhibiting peptides marked blue. Dose-response curves of peptides P53-57 inhibiting the mAb binding to rApi m 10 (right).

