SUPPLEMENTARY MATERIAL

Material and methods

Sequence discovery of fully human Api m 10 specific monoclonal IgE antibody

PBMCs were isolated from beekeepers' blood and frozen. PBMCs were stained with IgD FITC (Biolegend, IA6-2), IgM FITC (Biolegend, MHM-88), CD19 BV650 (Biolegend, SJ25C1), CD3 BV480 (Biolegend, UCHT1), CD14 BV480 (Biolegend, M5E2), live/dead™ fixable aqua dead cell kit (Thermo Scientific), CD38 BV421 (Biolegend, HIT2), and CD27 APC-H7 (Biolegend, M-T271) and IgD/IgM negative Api m 10 specific memory B cells were single-cell sorted into 96-well plates. Sorting and subsequent scRNAseq were performed according to the SMART-Seq® Single Cell Kit User Manual (version 050521). Sequencing was performed on a Illumina NovaSeq 6000, whereafter the sequencing results were analyzed using the RNA-sequence aligner STAR (v. 2.7.10a). B cell receptor sequences were reconstructed using a modified version of the BraCeR pipeline (docker pull nielsphk/bracer:1.3).

Expression of fully human Api m 10 specific monoclonal IgE antibody

The Api m 10 specific IgE mAb heavy- and light chain expression plasmids were custom made at Genscript (Piscataway, NJ). The IgE mAb was expressed using a 1:1 ratio of heavy:light chain encoding plasmid following the "Expi293™ Expression System User Guide" (ThermoFisher, publication number: MAN0019402, revision number: B.0) in 125 mL flasks. Total IgE in the supernatant was determined using the ImmunoCAP system.

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Alanine scan of patient sera IgE reactivity

To evaluate the binding of IgE from patient sera to alanine modified P54, biotinylated peptides were bound to a 384 well NeutrAvidin coated plate (ThermoFisher Scientific, Waltham, MA) with a concentration of 200 ng/ well over night at 4°C. As a positive control the unmodified P54 was used. After blocking with 1% bovine serum albumin (BSA, Applichem, Darmstadt, Germany) in PBS with 0,05% Tween 20 (Carl Roth, Karlsruhe, Germany) for 2 hours at room temperature (RT) diluted serum samples (1:5 in blocking buffer) were added and incubated for 2 hours at RT. For blank reduction, coated wells with blocking buffer only were used for each peptide. The secondary antibody (goat anti human IgE, HRP modified, ThermoFishes Scientific Waltham, MA) was diluted in blocking buffer an added to the plate after washing three times with Dulbecco's PBS (DPBS) containing 0,05 % Tween 20 and incubated for 2 hours at RT. Following another washing step (5 times with DPBS – 0,05 % Tween 20) TMB Substrate (BioLegend, SanDiego, CA) was added and incubated at RT in the dark for 20 minutes. The substrate reaction was stopped with 2 M H_2SO_4 (Carl Roth, Karlsruhe, Germany) and the optical density (OD) at 450 nm was analyzed immediately. After performing blank reduction for each peptide the OD of the unmodified P54 was set to 100 % and the percentages of the ODs from alanine modified P54 were calculated accordingly for all patient sera. Asterisks represent significant difference (95% confidence interval) from P54 IgE binding (* = $P<0.05$, **= $P<0.01$, ***= $P<0.001$, ****= $P<0.0001$).

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IgE mAb 1E10 peptide reactivity screen

An Api m 10 peptide library spanning the entire sequence of Api m 10 variant 1 was generated and provided by CASLO ApS, Lyngby, Denmark, as recently described [6]. A Nunc MaxiSorp™ flat-bottom 96 well plate was coated overnight with 50 µL of 2.5 µg/mL rApi m 10 diluted in PBS. After overnight incubation with the coating reagents at 4 °C, the wells were washed twice with ELISA wash buffer and blocked with 200 µL 2% BSA for 2 hours at 4 °C. Following blocking the wells were washed three times with ELISA wash buffer and incubated for one hour at room temperature with 50 µL of the various peptides at 1 µg/mL pre-incubated 1 hour with anti Api m 10 IgE mAb expression supernatant, 1E10 IgE, at a 1:100 dilution. After incubation with the rIgE the wells were washed four times with ELISA wash buffer and incubated with 100 µL of 1.3 µg/mL rabbit anti-human IgE-conjugated HRP for 1 hour at room temperature. The wells were washed four times in ELISA wash buffer, where after 100 µL TMB (Kem-En-Tec, cat no: 4380A) were added and the plates were shaken for 10 minutes with no light exposure. After the 10 minutes the reaction was stopped with 100 µL 0.5 M sulphuric acid (VWR Chemicals, cat no: 30144.294). The plates were read at 450 nm on an EL808 Ultra Microplate Reader (BioTek Instruments).

Peptide P53-57 dose-response inhibition of 1E10 IgE mAb binding to rApi m 10

Coating of rApi m 10 were performed as described above. IgE mAb 1E10 expression supernatant was preincubated in 1:100 dilution with P53-57 at 0,5 ng/mL to 10000 ng/mL in a dilution series for one hour before being added to the rApi m 10 coated plates. After one hour incubation detection of bound IgE was performed as described above.

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Alanine scan library inhibition of IgE mAb 1E10 binding

The Api m 10 P54 peptide alanine scan library, with every non-alanine residue in the P54 Api m 10 peptide mutated to alanine in 14 different peptides was generated and provided by CASLO ApS, Lyngby, Denmark. A Nunc MaxiSorp™ flat-bottom 96 well plate was coated overnight with 50 µL of 2.5 ug/mL rApi m 10, 2% BSA, or 10 µg/mL rabbit anti human IgE diluted in PBS. After overnight incubation at 4 °C with the coating reagents the wells were washed twice with ELISA wash buffer and blocked with 200 µL 2% BSA for 2 hours at 4 °C. Following blocking the wells were washed three times with ELISA wash buffer and incubated for one hour at room temperature with 50 µL of the various peptides at 1 µg/mL pre-incubated 1 hour with anti Api m 10 IgE mAb, 1E10 IgE, at a 1:100 dilution. IgE detection and plate reading were performed as described above.

IgE mAb 1E10 Api m specificity determination

A Nunc MaxiSorp™ flat-bottom 96 well plate (Thermofisher, cat no: 44240421) was coated overnight with 50 µL freeze-dried raw honeybee venom or Api m SCIT drug substance, rApi m 1, rApi m 2, rApi m 3, rApi m 5, rApi m 10, 10 µg/mL rabbit anti human IgE, and 2% BSA. The concentration of raw honeybee venom and Api m SCIT drug substance was 50 µg/mL and the concentration of single allergens was 2.5 µg/mL. 2% BSA and 10 µg/mL rabbit anti human IgE were used as a negative and positive control, respectively. All the reagents were diluted in DPBS. After overnight incubation with the coating reagents at 4°C, the wells were washed twice with ELISA wash buffer and blocked with 200 µL 2% BSA for 2 hours at 4°C. The wells were washed three times with ELISA wash buffer and incubated with 50

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µL of the various rIgE's supernatants diluted 1:10 in 2% BSA for one hour at room temperature. After incubation with the rIgE the wells were washed four times with ELISA wash buffer and incubated with 100 µL of 1.3 µg/mL rabbit anti-human IgE-conjugated HRP (DAKO, cat no: P0295) for 1 hour at room temperature. The wells were washed four times in ELISA wash buffer, where after 100 µL TMB (Kem-En-Tec, cat no: 4380A) were added and the plates were shaken for 10 minutes in the dark. After 10 min the reaction was stopped with 100 µL 0.5 M sulphuric acid (VWR Chemicals, cat no: 30144.294). The plates were read at 450 nm on an EL808 Ultra Microplate Reader (BioTek Instruments).

Affinity determination

All experiments were conducted on the Octet RED96e (Sartorius) at 30°C, 1000 RPM agitation, and 2 Hz frequency (high sensitivity setting). Data were processed with Data Analysis HT 11.1 software (Sartorius). High Precision Streptavidin (SAX) Biosensors (FortéBio) were conditioned for a minimum of 10 minutes in kinetic buffer (DPBS, 0.1% BSA, 0.02% Tween-20, pH 7.4) before starting the experiment. Enzymatically biotinylated sdab026 (patent WO 2012'/175740 A1) with an AviTag™ at the C terminal end, was loaded onto the SAX biosensors at a concentration of 0.375 µg/mL for 180 seconds with a resulting signal of approximately 0.7 nm. Sensors were then dipped into kinetic buffer until a stable baseline was achieved. Then the sensors were dipped into human recombinant IgE mAb or buffer as a control at concentration of 9 µg/mL for 300 seconds. Sensors were then dipped into the allergen of interest in a two-fold dilution series, or buffer as a negative control, for 300 seconds. Sensors were then dipped into kinetic buffer for dissociation for 1800 seconds.

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