Differential Immuno-kinetic Assays of Allergen-Specific Binding for Peanut Allergy Serum Analysis

Rouslan V. Olkhov[1], Edward R. Kaminski[2] and Andrew M. Shaw[1,*]

[1] *College of Life and Environmental Sciences, University of Exeter, Exeter, EX4 4QD, UK*

[2]*Department of Immunology erriford Hospital, Derriford Rd, Plymouth, PL6 8DH, UK*

Corresponding author:

Tel: 01392 723495

Fax: 01392 723434

Email: Andrew.m.shaw@exeter.ac.uk

Abstract

A label-free nanoparticle array platform has been used to detect total peanut allergen-specific binding from whole serum of patients suffering from peanut allergy. The serum from 10 patients was screened against a four-allergen panel of cat and dog dander, dust mite and peanut allergen protein Ara h1. The IgE and IgG contributions to the total specific-binding protein load to Ara h1 was identified using two secondary IgG- and IgE- specific antibodies and were found to contribute less than 50% of the total specific protein load. The total mass of IgE, IgE and the unresolved specific-binding protein, ΔsBP, for Ara h1 provides a new serum profile for high Rast grade patients, 5 and 6 with the IgG/IgE ratio of 4±2 and ΔsBP/IgE ratio of 17 ± 11, neither of which are protective for the small-patient cohort.

Keywords: Ara h1, array, biosensor, IgE, IgG, nanoparticle, peanut allergy

Introduction

Food allergy continues to rise in the US and Europe with peanut allergy suffers producing the largest group of allergy-related fatalities (1-4). In the US as many as 2% of children are peanut allergic, often having multiple allergies, leading to a disproportionately large number of deaths (5) and prompting some scientists to consider an approaching peanut allergy epidemic (6). The exact cause of developing a peanut allergy is unknown (7) and more interestingly 80% of children with peanut allergy grow out of it in early adult life: the mechanism of evolving tolerance is poorly understood (8).

The conventional diagnosis of peanut allergy starts with report of an episode in a clinical history, which may be followed by a skin prick test to measure the weal response and then a test for peanut-specific IgE levels in the serum. A number of peanut allergens have been identified and classified as Ara h 1-11 (9) although the standard ImmunoCap assay platform uses an extract called f13, which is likely a mixture of Ara h 1-3, in its peanut-specific IgE assay. Despite the extensive research into the identification of allergens, the precise epitope presentation in a particular detection platform may lead to significant variations in absolute IgE concentration measurements across platforms, although the results appear to correlate (10). The surface area of the ImmuoCap and the allergen presentation have been optimized to produce the largest consistent IgE absolute concentration determination.

The serum IgE diagnosis of peanut allergy is not precise with conflicts between positive results from skin prick tests and negative serum IgE levels. In one study, only 22% of eight-year-old children showing either a positive result to skin-prick test or serum IgE but did not show a positive response to oral peanut challenge (11). Neither the skin prick test nor the serum IgE levels are an accurate positive predictor of peanut allergy as determined by the oral peanut challenge. Currently, serum concentrations of 15 kUA/L (36 μg /L, Rast grade 3) specific IgE binding to peanut f13 extract and a wheal of 8mm form the skin prick test predicts with greater than 95% accuracy true peanut allergy (12). However, many peanut allergy sufferers have much lower levels with varying degrees of sensitivities, severity and tolerance. Reduction of the f13 extract to a component diagnosis suggests that the Ara h2 is the most efficient with a increased positive predictive value of 95 % as a serum test alone (13) but does not show region specificity (14). A full component analysis Ara h1 – 11 reveals cross-reactivity between food allergies and regional variations (15).

In addition, to IgE allergen specificity, large serum concentrations of IgG for food allergy epitopes (16), specifically the IgG4 isotype, are found in the serum of patients with IgG to food, especially egg white, orange, wheat and rice correlating with IgE to cat, dog, mite and milk (17). Food allergy has been further linked to birch pollen and IgG4 (18). From these observations there would appear

to be a spectrum of epitopes in the food protein digestion products for which there are significant IgG and IgE concentrations in the serum.

Previously, we have measured the effects of competitive binding on nanoparticle biophotonic surfaces between non-specific and specific binding proteins in whole serum (19, 20) leading to interferences in the quantitative response of the assay. This interference effect is not unique to label-free technologies and determining the relative contributions of non-specific and specific binding when screening a complex fluid such as blood is important for any assay. Most platforms use a label to detect only the target, a presumptive test. However, in blood there may be a number of proteins and antibodies that compete specifically with the target protein on the assay which, would be detected by label-free technologies, providing an advantage in screening whole blood for patient diagnosis. In part, competitive specific binding addresses the question of the correlation between measurements on different platforms giving different reported levels of IgE levels (10). Most sensor platforms measure an endpoint with the surface concentration of IgE detected after a long incubation time with the sensor surface. A subsequent sensing event, such as ELISA tagged antibody, then visualizes and quantifies the IgE concentrations. Sensor surfaces with different sensor areas, however, may become quickly filled with specific-binding proteins (sBP) preventing allergy-relevant IgE binging in some patients where sBP dominates the phenotype.

The immuno-kinetic response on the label-free biosensor platform is surface-area limited, which means the two target antibodies IgG and IgE compete for the available specific binding sites on the sensor surface coated with the target antigen. This is analgous to the rapid covering of the allergenic surface in the membranes of the upper GI track by IgG which would prevent IgE binding and mitigate the IgE-triggered allergic response. By contrast, the surface area of the ImmunoCap sensors is very large allowing all of the IgE to bind to the surface irrespective of the high IgG concentration resulting in a stable IgE determination and subsequent RAST classification; a serum positive.

In this paper, we investigate the total specific binding proteins, sBP, from patient serum samples to a particular allergen; a broad measure of the patient's allergen phenotype. We investigated the total protein load binding, sBP to the peanut allergen Ara h1 epitopes presented on a gold nanoparticle plasmon sensor surface. The components of sBP are partially resolved into IgG and IgE based on secondary antibody detection of the already-bound proteins.

Materials and Methods

Array Reader

The biophotonic nanoparticle array sensor technology is based on the change in scatted light intensity from nanoparticle-functionalised array elements and has been described in detail elsewhere (20, 21). Briefly, nanoparticles are printed into an 96-element array format with seed nanoparticles of 4 nm in diameter and are then grown 120 nm particles to optimise the scattering sensitivity. Each element of the array is then printed with the target proteins such as the Ara h 1 peanut allergen together with a set of control spots to corrected for temperature changes and local changes in refractive index. The array is introduced into the array reader where it is illuminated with 635 nm radiation from an LED in a near-field configuration. Light scattered by the nanoparticles is collected by a video camera and averaged to produce a real-time kinetic trace as the patient's serum passes over the array. The array element sensitivity variability is within 15% determined by observing the signal change between PBS and 2% glycerol in PBS solutions, with a known RI of 2.7×10^{-3}. The data are presented in response units (RU) with 1 RU conventionally equivalent to a 10^{-6} refractive index change in the bulk analyte (22). Calibration of the kinetic change produces the concentration of the target protein – an immuno-kinetic assay. The detection limit may be enhanced by a secondary detection antibody, which can further identify the IgG or IgE composition of biosensor surface.

Chemicals

Bovine serum albumin, BSA (98%), human fibrinogen and FBR (60%) were obtained from Sigma-Aldrich. Commercially available purified allergens Fel d1 (cat), Can f1 (dog), Der p1 (dust mite), and Ara h1 (peanut), as well as rabbit polyclonal antibodies to Ara h1 were obtained from Indoor Biotechnologies UK; goat polyclonal antibodies to rabbit IgG were supplied by AbD Serotec UK; goat polyclonal antibodies to human IgE and IgG F_c were obtained from R&D systems. Dithiobis-succinimidyl propionate (DTSP, 97%) was from Fluka. The standard buffer used in preparation and dilution of the samples was phosphate buffed saline (PBS) with an additional 0.005% w/w Tween 20 surfactant, supplied by Sigma-Aldrich. A 100 mM aqueous phosphoric acid solution was used as regeneration buffer.

Sensor arrays

The current study employed 12×8 rectangular sensor arrays with round spots of ca. 200 μm diameter and array pitch of 300 μm, Fig. 1a. Bio-functionalized sensors were prepared by inkjet printing allergens along with BSA and FBR proteins onto DTSP-activated gold (20, 23) arrays spots according to the array key shown in Fig. 1a. The printing process delivers a small volume (0.3 nL) of the corresponding allergen or protein solution to a single array element, subsequently the array

is stored in a 80- humidity chamber for 3-4 hours to complete the immobilisation step. The slides are then washed in PBS and stored at 5°C until used with an active lifetime of up to three months.

Clinical samples

Serum samples were obtained from 10 patients who had been tested positive for peanut allergy by specific IgE in the immunology diagnostic laboratory at Derriford Hospital, Plymouth, using the Phadia ImmunoCap system. Results were reported in both kUA/l and as a RAST grade 0 - 6. The study was approved by the National Research Ethics Service Committee South West – Cornwall & Plymouth. The patient samples were anonymised and consent was not required.

Results

The multi-allergen array performance, Fig. 1, was tested for each of the dog, cat, dustmite and peanut allergen proteins in a series of experiments with purified samples of their respective anti-allergen antibodies. Purified allergen-specific human immunoglobulins are not readily available commercially for Ara h1 and the sensor response was characterised using standard solutions of rabbit anti Ara h 1 polyclonal IgG antibody, a-Ara-IgG. Solutions of known concentrations a-Ara-IgG diluted with PBS were injected over the sensor slides for approximately 16 minutes, Fig. 2a. IgG concentrations greater than 3 nM show a characteristic kinetic binding response with an association rate consistent with an antibody-antigen interaction. A switch to the running PBS buffer leads to removal of the adsorbed antibody with a slow dissociation rate. The ratio of the dissociation rate to the association defines the affinity of the antibody, typically K_D = 10 nM (24). At lower antibody concentrations, below the detection limit for the primary binding event, a detection antibody (goat anti rabbit IgG) was introduced to bind to the a-Ara-IgG to enhance the signal and identify the component binding to the surface. After the primary-secondary detection sequence was completed, the sensor was regenerated with 100 mM phosphoric acid solution, shown in Fig. 2a as step (d).

The observed detection limits for the primary detection event is 300 pM (45 ng ml^{-1}) and secondary detection step is 4 pM (6 ng ml^{-1}) ((based on 2 SD noise); a 7-fold enhancement factor. The regeneration step allows many measurements to be made using the same array. The calibration curves are presented Fig. 2b and relate explicitly for the concentration determination of pure antibodies in PBS dilutions and must be corrected for the effects of the patient sera. The presence of other serum proteins in high concentrations extensively affects concentration determination and we have derived previously the method to determine quantitatively accurate results (19). There are no purified human allergen-specific IgE/IgG for the control assays and an approximate quantitative estimate only is possible.

The patient sample serum is diluted to 12% in PBS from a patient with known peanut allergy RAST grade 5 as determined by the f13 Immunocap assay. The binding specificity was tested on the array for Ara h1 is specific to this assay and there is no correlation with binding to epitopes presented by Fel d 1, Can f 1 and Der p 1, Fig. 3a. The experimental sequence consists of a primary sample injection step, characterised by clearly visible bulk refractive index change associated with the bulk refractive index of the serum, followed by a slower surface binding in all channels dominated by the response in the Ara h1 assay. Two protein assays for FBR and BSA were used as internal control standards for temperature drift, LED intensity changes and as a non-specific binding reference. After the first buffer wash, an identification assay was performed with secondary detection IgG antibodies for the 6 human immunoglobin types: E, G, A, M, and D for which only a positive response was observed for IgG. Finally, the sensor surface was regenerated with the phosphoric acid buffer. Further sample injection profiles and kinetic responses may be seen in the supplementary material, Fig. S1 and Fig. S2.

The patient serum set clearly shows specific binding to the Ara h 1 allergen with no significant binding to the positive controls. However, there is significant variation in both the total sBP and the detected IgE and IgG with varying composition, indicating different immuno-recognition profiles. Fig. 3b shows the data recorded for a RAST grade 5 sample: a strong primary response in specific binding to the peanut allergen channel is followed by slow wash-off when the flow was switched from sample to running buffer. Then two detection antibodies were employed to identify the surface bound proteins, human anti-IgE and anti-IgG were injected at ca. 25 and 45 minutes, respectively. The order of injections of the two detection antibodies is important: when anti-IgE was injected first, the response is significantly stronger than when it was injected secondly, after anti-IgG. The protocol was refined to ensure the IgE detection step is performed first with IgG second: the surface concentration estimations were consistent with this detection sequence.

Determination of the surface concentration of IgG, IgE and sBP requires a calibration step. On the assumption that the detection polyclonal antibody has the same epitope affinity as the anti-rabbit detection antibody, the calibration curve Fig. 2b can be used to derive component contributions to the total specific binding mass. The mass balance estimation for the data presented in Fig. 3b is : total specific binding protein load, sBP, is equivalent to a solution phase concentration of 3.5nM, of which IgE = 0.13nM, IgG = 0.36nM. Only 14% of the total surface concentration may be identified, total sBP, indicating that 86% of the total epitope-specific binding is unidentified, ΔsBP = 86%. Another example of sBP analysis is presented in the supplementary material, Fig. S3. For this RAST grade 6 patient; here the identification steps show that only 20% of the total protein load was identified as IgE or IgG with IgG in a ninefold excess.

The new epitope profile parameters for a patient serum are now sBP, IgE, IgG and ΔsBP and these were assessed for the contributions to Ara h 1 for all 10 patient samples, Fig. 4a. The left-hand axis shows the observed response, while the right-hand axis is marked with an effective total sBP concentration based on assumption that the analyte species has a molecular mass similar to the immunoglobulin proteins, 150kDa. The patient cohort consisted of mainly RAST grade 5 and 6 samples all of whom had demonstrated a clinical allergic response to peanut. Correcting for the amplification factor of the detection/identification antibody, Fig. 2b, the identified combined IgE+IgG load is significantly lower that the observed total response in sBP, with a mean (IgE+IgG)/sBP = 0.18 ± 0.10 value (mean ± SD) for the cohort.

Discussion

We have performed label-free measurements of the total allergen-specific binding to Ara h1 peanut allergen from a small patient cohort (*n*=10), Fig. 4a. In each case, the measurements were compared with the RAST grade determined by the conventional immunology laboratory methods using the Phadia Immuocap specific-IgE test. There is a positive correlation between the ImmunoCap and the sensor array Ara h1 total sBP measured values with a correlation coefficient R^2 = 0.64, Fig. 4b. The f13 epitope presentation is not resolved into the specific Ara h components and the specific binding to Ara h1 appears to represent only a small fraction f13 binding, with additional binding likely to be attributed to Ara h1 and h3 in the whole peanut extract. Allergen surface preparation is important in all instrumental platforms, including the tethering chemistries, to ensure there is a sufficiently large surface area of the allergen and that the target eptope is presented correctly. In our experiments, the active epitope presentation has been preserved as judged by the specific activity of antibodies binding to the surface, (20, 21, 24, 25).

The ratio of the sensor surface area to the sample size is important: a large surface area will allow all serum components sBP, IgG, IgE and ΔsBP to bind to the sensor surface with a sufficiently larger number of binding sites to accommodate all proteins. Subsequent detection of IgE with an IgE-specific antibody will then give a consistent result for the determination of IgE concentration in the patient serum: this is most likely the case for the ImmunoCap platform. However, if the surface area binding site capacity is low, the ΔsBP and IgG binding will compete kinetically with IgE for the available sites on the sensor surface, Fig. 5, limiting IgE deposition and producing a reduced IgE detected level. The array spot surface area is significantly smaller than the ImmunoCap platform and, in part, this is compensated for in the choice of a small volume of patient serum, 250 µL, so as not to saturate the sensor surface. In this way, we measure the effect of kinetic of competitive binding of IgE, IgG and ΔsBP based on their relative concentrations. Epitope

presentation and the competitive binding effect will produce different IgE determinations on different platforms (10) if the sensor surface.

The kinetic, however, measurement could be closer to the clinical response where a potential allergen protein is covered rapidly by whatever sBP component is present in the serum or in the membrane mucosa with only IgE triggering the allergic response. A high concentration of competitive component in the patient could provide a potential mechanism for a serum positive, IgE patient to not show a clinical response. Desensitisation studies for bee venom have shown patients with an increasing exposure to venom have an increasing IgG titre and a consequent decrease in IgE titre (26). Further studies however have suggested that simple measurement of serum IgG to bee and wasp venom is not sufficient to predict severity of response to a challenge (27). Antibody subtype namely IgG4 has also been proposed in the role of blocking antibody (28) where the IgG4/IgE ratio for non-allergic bee keepers has been reported as in excess of one thousand.

In contrast to the IgE- and IgG-specific sensors, the scattering nanoparticle platform can measure the total mass of protein binding to a particular allergen and measure the relative binding affinities/avidities for the association with the allergen on the surface. The measurement is than a sensitivity profile of all of the available epitopes to sBP within the patient's serum phenotype. Subsequent identification and quantification of IgE and IgG (all subtypes) on the surface allows the contributions from each of these immunoglobulin to be determined with ratios varying up to 4±2, significantly smaller than the protection ratio seen with the non-allergic bee keepers. Two factors are however important for protection, the ratio of IgG to IgE and also the absolute concentration of IgG: to be protective it must effectively block the available allergen surface area. A protective level was not present in the serum of any of the patients tested as all showed clinical symptoms of allergy.

Importantly, and novel to this investigation, using the nanoparticle array sensor surface as an epiotope-specific mass sensor has been able to quantify all proteins with specific binding (K_D ~ 10 nM<) to the target allergen Ara h1. The determined concentrations of IgE and IgG only account for 50% of the total specific binding to the Ara h1 epitope in all patients. Again, the concentration of ΔsBP is not protective for the patients at the current levels, but is a new specific binding protein for Ara h1 present in the blood of peanut suffers, Fig. 4a. The cumulative effect of the IgG and ΔsBP are could therefore be protective as neither are readily displaced from the Ara h1 epitope and would block it for IgE binding.

Conclusions

The immuno-kinetic assay approach was used to study patient serum samples on a scattering nanoparticle array-format multi-allergen biosensor, focusing on peanut allergy detection. The sensors are demonstrably sensitive and selective towards allergen-specific binding producing a new epitope profile of the allergy sufferers' serum. For peanut sufferers specifically, the serum epitope-binding profile for Ara h1 was determined, resolving total mass binding to the epitope into IgG, IgE and an unknown ΔsBP. The ratio IgG and ΔsBP to IgE and their absolute concentration could provide protection for patients, blocking the IgE response kinetically. The ratio IgG/IgE ratio in our small patient cohort is 4±2 and does not offer protection for the high RAST grade patients. The biosensor platform has potential to identify the presence of new allergen-specific markers against specific allergens to understand the clinical presentation of allergy and serum composition and to provide a companion diagnostic for new desensitisation immune-therapies.

Acknowledgement

The research was funded by the BBSRC and the EPSRC.

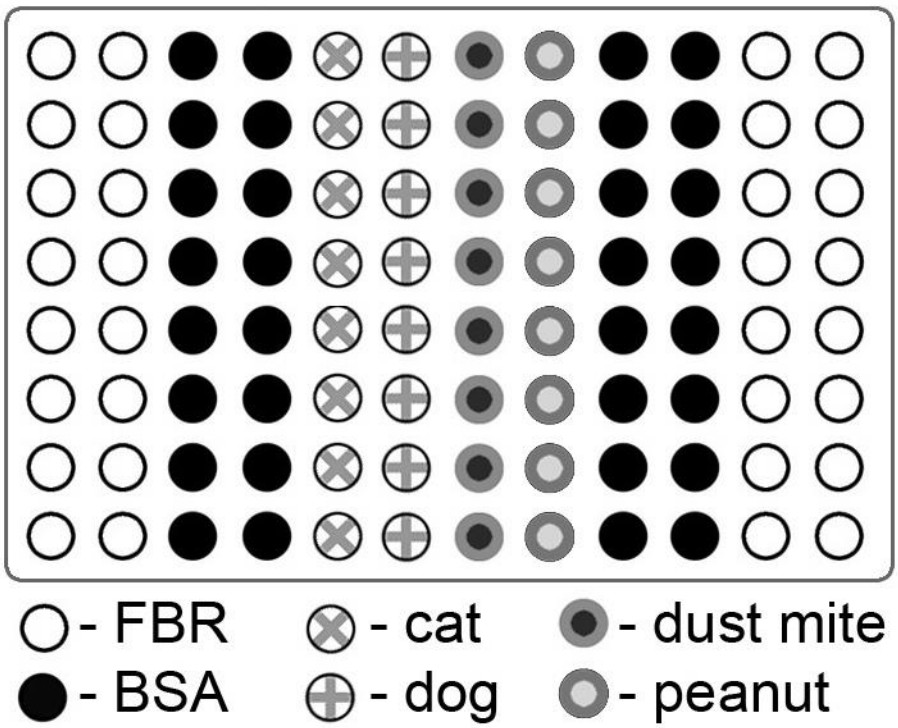

Fig. 1 Experimental platform. **a**: sensor array bio-functionalization printing mask.

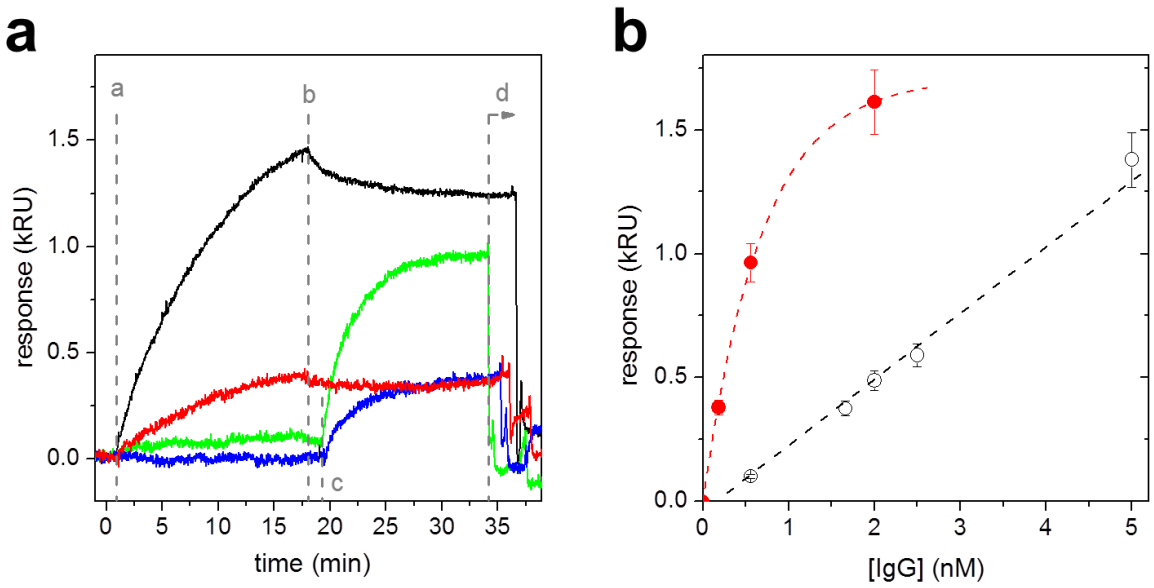

Fig. 2 Peanut Ara h1 Calibration. **a**: kinetic traces with different rabbit a-Ara-IgG antibody concentrations in analyte: 5, 1.7, 0.6, 0.2 nM in the order of stronger to weaker responses. Injection sequence: a – sample, b – running buffer, c – detection goat anti rabbit IgG (only 0.6 and 0.2 nM measurements), d – regeneration. **b**: standard curves derived from the primary responses, open circles, and from enhanced responses after the detection antibody was used, filled circles. The a-Ara-IgG detection limit in the primary step is 300 pM and after the detection antibody was applied – 40 pM

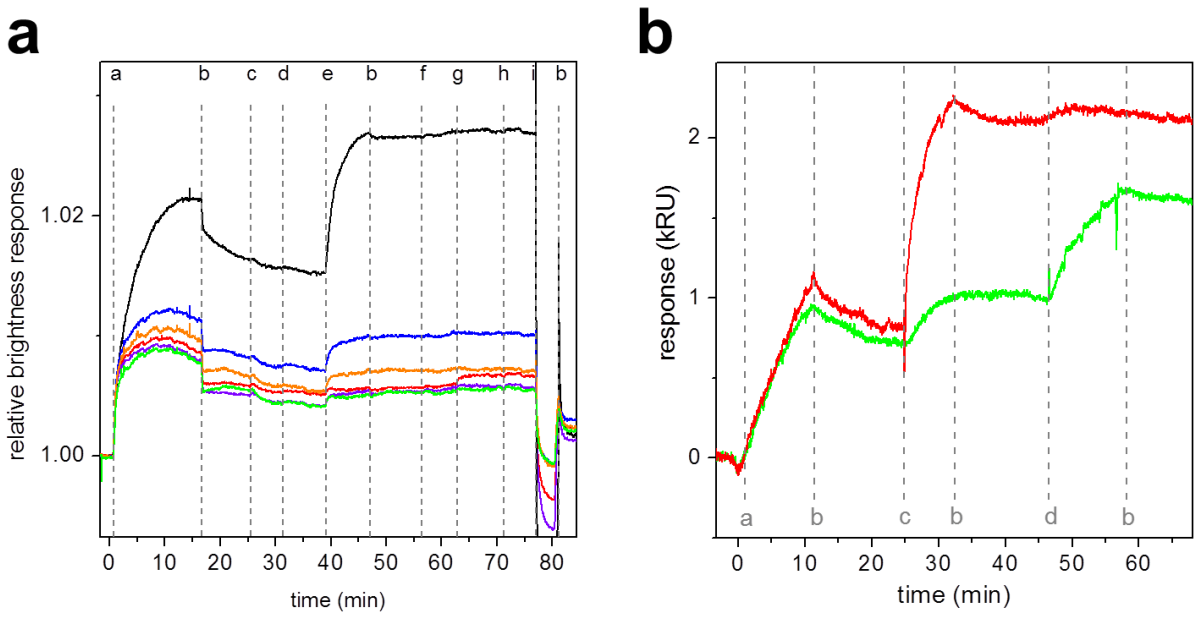

Fig. 3 Example of the raw sensograms obtained in assay of the peanut allergic, RAST grade 5, patient serum. The injection sequence: a – sample consisted of 12% serum diluted in PBS; b – PBS buffer, c – anti-IgE (R&D), d – anti-IgE (Bethyl), e – anti-IgGc, f – anti-IgA, g – anti-IgM, h – anti-IgD, i – regeneration buffer. The order of sensor channels from top to bottom within sample injection: peanut, dust mite, cat, FBR, BSA, dog

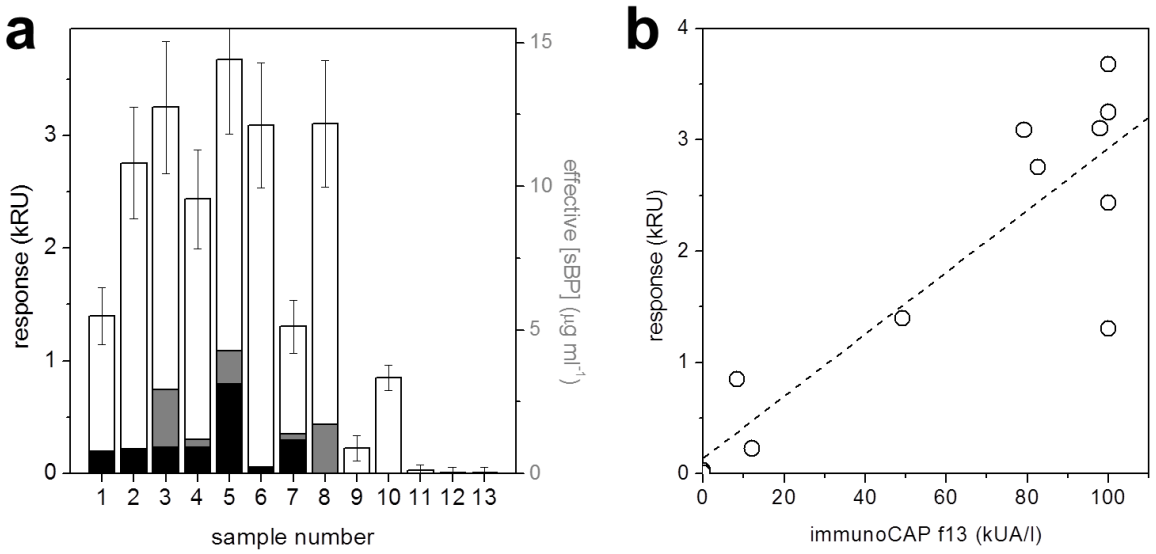

Fig. 4 Summary of clinical samples tests for peanut allergy. **a**: response in peanut allergen channel, approximate scale equivalent to sBP concentration is linked on the right axis. Samples 1-8 are graded as RAST 5 or 6 and are presented with partially resolved IgE (gray) and IgG (black) contributions in the total mass response sBP (white). Samples 9 and 10, RAST grade 3, were not resolved with detection antibodies. Control samples 11 and 12 are sera graded as RAST 3 and 6 towards other, non-peanut allergens (cat dander and dust mite), and sample 13 is a control serum with no allergy description. **b**: correlation between observed peanut allergens specific response and known allergy grades from clinical laboratory, R^2 is 0.64. The ImmunoCap values normally reported as kUA/L are converted into nM using IgE molecular weight 180 kDa and 1UA=2.4ng.

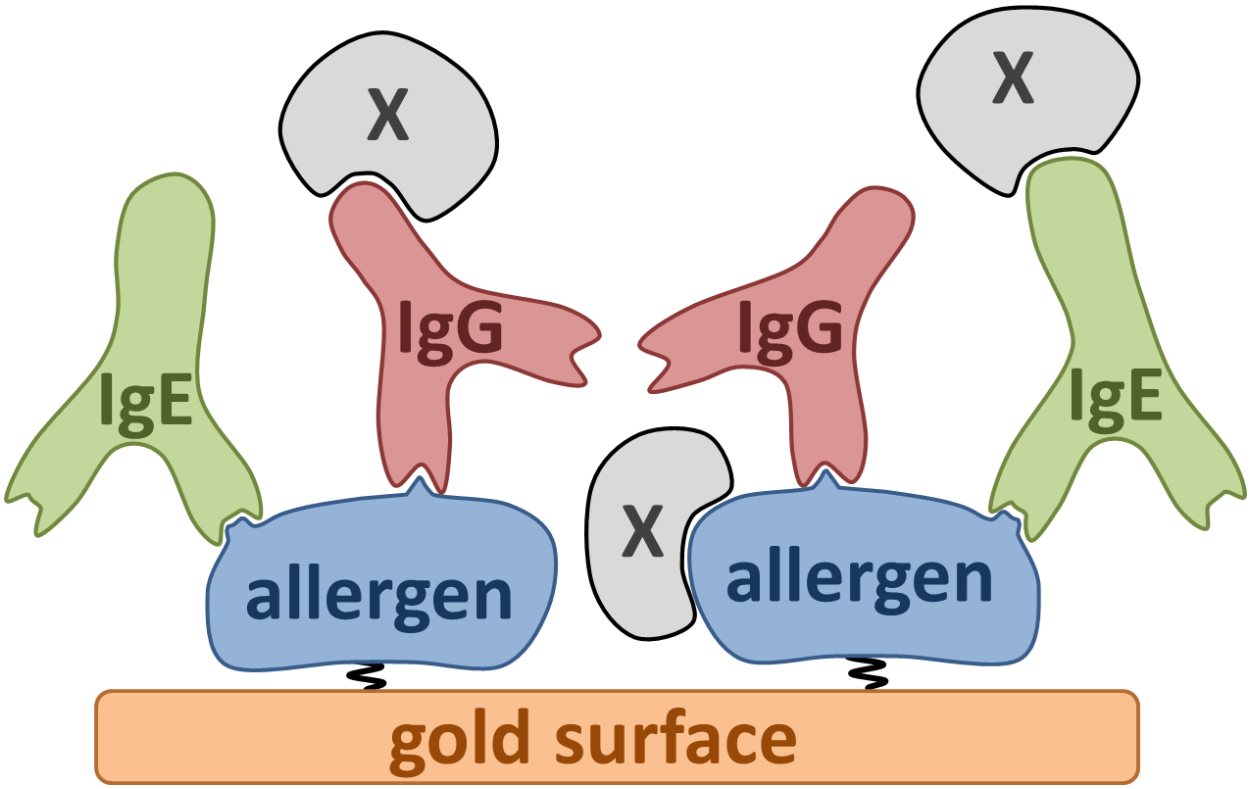

Fig. 5 Schematic binding of analyte species to the surface functionalized with allergen. In addition to specific IgE and IgG there seem to be some extra deposited material X (ΔsBP) either as itself or in the form of complexes X-IgE/IgG

References

1. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol2010;125(2, Supplement 2):S116-S25.
2. Sicherer SH, Muñoz-Furlong A, Godbold JH, Sampson HA. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. J Allergy Clin Immunol2010;125(6):1322-6.
3. Bock SA, Muñoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. J Allergy Clin Immunol2007;119(4):1016-8.
4. Eigenmann PA, Zamora SA. An internet-based survey on the circumstances of food-induced reactions following the diagnosis of IgE-mediated food allergy. Allergy2002;57(5):449-53.
5. Hourihane JOB. Peanut Allergy. Pediatr Clin North Am2011;58(2):445-58.
6. Sicherer SH, Sampson HA. Peanut allergy: Emerging concepts and approaches for an apparent epidemic. J Allergy Clin Immunol2007;120(3):491-503.
7. Husain Z, Schwartz RA. Peanut allergy: An increasingly common life-threatening disorder. J Am Acad Dermatol2012;66(1):136-43.
8. Nicolaou N, Poorafshar M, Murray C, Simpson A, Winell H, Kerry G, et al. Allergy or tolerance in children sensitized to peanut: Prevalence and differentiation using component-resolved diagnostics. The Journal of allergy and clinical immunology;125(1):191-7.e13.
9. Cabanos C, Urabe H, Tandang-Silvas MR, Utsumi S, Mikami B, Maruyama N. Crystal structure of the major peanut allergen Ara h 1. Mol Immunol2011;49(1–2):115-23.
10. Wang J, Godbold JH, Sampson HA. Correlation of serum allergy (IgE) tests performed by different assay systems. J Allergy Clin Immunol2008;121(5):1219-24.
11. Finkelman FD. Peanut allergy and anaphylaxis. Curr Opin Immunol2010;22(6):783-8.
12. Roberts G, Lack G, the Avon Longitudinal Study of P, Children Study T. Diagnosing peanut allergy with skin prick and specific IgE testing. J Allergy Clin Immunol2005;115(6):1291-6.
13. Dang TD, Tang M, Choo S, Licciardi PV, Koplin JJ, Martin PE, et al. Increasing the accuracy of peanut allergy diagnosis by using Ara h 2. J Allergy Clin Immunol;129(4):1056-63.
14. Nicolaou N, Murray C, Belgrave D, Poorafshar M, Simpson A, Custovic A. Quantification of specific IgE to whole peanut extract and peanut components in prediction of peanut allergy. J Allergy Clin Immunol2011;127(3):684-5.
15. Finkelman FD. Peanut allergy and anaphylaxis. Curr Opin Immunol;22(6):783-8.
16. Vickery BP, Scurlock AM, Jones SM, Burks AW. Mechanisms of immune tolerance relevant to food allergy. J Allergy Clin Immunol;127(3):576-84.
17. Eysink, De J, Bindels, Scharp-Van Der L, De G, Stapel, et al. Relation between IgG antibodies to foods and IgE antibodies to milk, egg, cat, dog and/or mite in a cross-sectional study. Clin Exp Allergy1999;29(5):604-10.
18. Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen–related food allergy: Clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol2011;127(3):616-22.e1.
19. Jansen van Vuuren B, Read T, Olkhov RV, Shaw AM. Human serum albumin interference on plasmon-based immunokinetic assay for antibody screening in model blood sera. Analytical Biochemistry2010;405(1):114-20.
20. Olkhov RV, Fowke JD, Shaw AM. Whole serum BSA antibody screening using a label-free biophotonic nanoparticle array. Analytical Biochemistry 2009;385:234-41.
21. Olkhov RV, Shaw AM. Label-free antibody–antigen binding detection by optical sensor array based on surface-synthesized gold nanoparticles. Biosensors and Bioelectronics 2008;23:1298-302.
22. Biacore User Training Documentation: GE Healthcare.
23. Hermanson GT, Mallia AK, Smith PK. Immobilized affinity ligand techniques: Academic Press; 1992.
24. Olkhov RV, Shaw AM. Quantitative label-free screening for antibodies using scattering biophotonic microarray imaging. Analytical Biochemistry2010;396(1):30-5.
25. Rich RL, Papalia GA, Flynn PJ, Furneisen J, Quinn J, Klein JS, et al. A global benchmark study using affinity-based biosensors. Anal Biochem2009;386(2):194-216.
26. Clayton WF, Reisman RE, Mueller U, Arbesman CE. Modified rapid venom desensitization. Clinical & Experimental Allergy1983;13(2):123-9.
27. Ewan PW, Deighton J, Wilson AB, Lachmann PJ. Venom-specific IgG antibodies in bee and wasp allergy: lack of correlation with protection from stings. Clinical & Experimental Allergy1993;23(8):647-60.

28. Fujita H, Soyka M, Akdis M, Akdis C. Mechanisms of allergen-specific immunotherapy. Clinical and Translational Allergy;2(1):2.