On the role of antibody affinity in the IgE mediated allergic response

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Abstract

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Abstract

Type I hypersensitivity, also known as classical allergy, is mediated via allergen-specific IgE antibodies bound to type I FcR (FcεRI) on the surface of mast cells and basophils upon cross-linking by allergens. This IgE-mediated cellular activation may be blocked by allergen-specific IgG through multiple mechanisms, including direct neutralization of the allergen or engagement of the inhibitory receptor FcγRIIb which blocks IgE signal transduction. In addition, co-engagement of FcεRI and FcγRIIb by IgE-IgG-allergen immune-complexes causes down-regulation of receptor bound IgE, resulting in desensitization of the cells. Both, activation of FcεRI by allergen-specific IgE and engagement of FcγRIIb by allergen-specific IgG are driven by allergen-binding. Here we delineate the distinct roles of antibody affinity versus avidity in driving these processes and discuss the role of IgG subclasses in inhibiting basophil and mast cell activation.

KEYWORDS

Affinity, allergy, antibody, avidity, IgE.

Future Research Perspectives

Specific immunotherapy should aim at maximizing allergen-specific IgG responses. IgG subclass responses after AIT are not fully understood to date and require ongoing clinical research, further development of platforms e.g. Immunocap® for IgG1 could provide high throughput value. Further study on the characterization of FcγRIIb on human mast cells may further establish evidence of the translational potential of vaccines containing a single ... a major challenge in vaccinology today and is relevant for the development of next generation allergy immunotherapies.

Major milestone discoveries

High avidity interactions, and consequently, low affinity IgE antibodies can trigger cellular activation. Antibody affinity dictates the mechanism of mast cell inhibition, and low affinity IgG antibodies triggering the inhibitory FcγRIIb pathway can show a broader cross-reactivity pattern than previously thought. IgG subclasses plays only a minor role ... and increase induction and maintenance of specific antibodies; the basis of a novel vaccine against peanut allergy.

Glossary

Allergen-specific immunotherapy (AIT): also known as desensitization or hyposensitization; the most common form of specific immunotherapy; involves a course of injections that build up tolerance to particular allergens through small, controlled doses. Allergens: proteins that trigger the immune system of an allergic person to produce unwanted symptoms. Allergy: a chronic condition involving an exaggerated reaction to an otherwise harmless substance, called an allergen. B cells: differentiate into clones of antibody-producing plasma cells; have a central role in allergen tolerance through the production of IgG-blocking antibodies. FcRI: a high-affinity IgE receptor expressed on the cell surface of mast cells and basophils; triggers the IgE-mediated allergic cascade. FcγRIIb: a low-affinity IgG receptor. IgG antibodies can inhibit IgE-mediated mast cell activation through direct engagement of FcγRIIb. Germinal center: a specialized microstructure that forms in secondary lymphoid tissues, producing long-lived antibody-secreting plasma cells and memory B cells, which can provide protection against reinfection; IgE: primary antibody mediator in the allergic response. IgG: most common antibody in the body; used to treat a range of diseases. Mast cells: similar to basophils, mast cells become activated by antigen crosslinking of FcRI receptor-bound IgE to undergo rapid degranulation and release of the inflammatory substance histamine, causing an allergic reaction. Pathogen-associated structural pattern (PASP): a pathogen-associated molecular pattern (PAMP). Cells of the innate immune system utilize pattern recognition receptors to identify viral pathogens by engaging PAMPs e.g. viral structures, Toll-like receptor ligands such as RNA. Toll-like receptors: The Toll-like receptors are Pathogen recognition receptors that have a unique and essential function in animal immunity.

1 Introduction

The discovery of IgE-mediated allergic disease as an indication of hay-fever, or allergic rhinitis, was first described in 1819 by John Bostock, a medical doctor who himself suffered from hay fever. He called the disease catarrhus aestivus, summer catarrh, as it was invariably absent in winter time. Catarrhus aestivus
was a rare disease in the early nineteenth century, and after reporting his own case, it took him more than nine years to identify another 28 cases for publishing a second article\(^2\). At the beginning of the 20th century, allergens were considered being toxins\(^3\). Only with the discovery of IgE antibodies\(^4,5\), it became evident that allergen-specific antibodies are the reason for the aberrant response of the body against pollen.

In clinical practice, there are several possibilities to avoid the activity of IgE and many pharmaceutical interventions aim to prevent IgE production and/or make the immune system more tolerant to allergens by inducing a shift in Th cell responses from Th2 to Th1 or regulatory T cells\(^6,7\); more recently, the concept of mAbs targeting and blocking IgE directly by monoclonal anti-IgE antibodies has been introduced\(^8\). Alternatively, it may be possible to block the action of IgE indirectly by induction of allergen-specific IgG through passive\(^9\) or active vaccination\(^10,11\). This approach is supported by the clinical observation that successful specific immunotherapy may correlate with an increase of the IgG/IgE ratio, and more recently, that polyclonal and monoclonal allergen specific IgG antibodies were able to curb allergic immune responses both in mice and humans as a form of passive vaccination\(^12-14\). Hence, it seems reasonable to conclude that the overarching goal of immunotherapy should be the induction of allergen-specific IgG antibodies.

This raises the question of antibody specificity and quality. Which are the key parameters driving or inhibiting allergic responses? As outlined below, the answer is complex and different for IgE mediated activation by allergen versus IgG mediated neutralization of allergen versus Fc\(\gamma\)RIIb-mediated inhibition of allergic responses (Figure 1). The same is true for antibody specificity, as rules for IgE-mediated activation versus IgG-mediated blockade or Fc\(\gamma\)RIIb-mediated inhibition are fundamentally different. Perhaps unexpectedly, IgG subclasses play only a minor role in inhibiting the allergic response.

### 2 Low affinity IgE antibodies suffice to drive the allergic responses: avidity provides the key

Antibody affinity, also known as binding affinity and defined by its equilibrium constant, is the strength of the interaction between the antigen-binding site on an antibody and a specific epitope on an antigen (monovalent binding) and can be defined by its equilibrium constant (Figure 2A). Antibody avidity represents the overall strength of the antibody-antigen interactions and is influenced by several factors in particular multivalent binding (Figure 2A). In essence, the greater an immunoglobulin’s valency (number of antigen binding sites), the greater its potential avidity as it can bind multiple epitopes on a single antigen – provided the antigen is multivalent\(^15\).

Most classical physico-chemical analysis is performed in solution, in part to avoid the effects of multivalent binding. Nevertheless, standard antibody binding assays, such as ELISA, surface plasmon resonance, and Blolayer Interferometry are performed with antibodies in solution; their ligands are, however, typically bound to two-dimensional surfaces. This opens the possibility for antibodies to bind their ligands with 2 arms, a problem that is typically avoided by complicated coating techniques, including coating at low density to avoid avidity effects (caused by multivalent binding), as affinity (referring to monovalent binding) is much better defined by classical binding models than avidity. However, a notion often overseen is that most pathogens i) are recognized on 2 dimensional surfaces and ii) are highly polyvalent and therefore prone to bind antibodies in a multivalent fashion. Indeed, natural IgM antibodies bind viral particles with great efficiency due to decavalent binding which makes many antibodies stick like glue even if the binding affinity of an individual variable region is low and barely measurable\(^16\). Hence, avidity may be more important in a real-life setting. In the case of IgE bound to Fc\(\varepsilon\)RI on the surface of effector cells, nature adds one level of complexity: lateral diffusion. While in ELISA plates, ligands are fixed on plastic, IgE molecules bound to Fc\(\varepsilon\)RI can rapidly diffuse along the cell surface membrane, a process that strongly facilitates multivalent, high-avidity binding of IgE antibodies to allergens (Figure 2B). In this context, it is interesting to note that the restriction of the movement of receptor-bound IgE within the 2 dimensions of the cell membrane results in high local concentrations of the IgE molecules as well as allergens bound to IgE. Indeed, 10’000 molecules bound to the cell surface of regular sized cells may exhibit a local concentration of >10\(^{-6}\) M within the membrane\(^17\). In addition, membrane bound molecules diffuse on the cell surface with a high velocity, allowing them to circle the cell once every second\(^18\). Hence all these properties foster multivalent binding of single allergens to membrane bound IgE recognizing different epitopes on the allergen. Indeed, we could
show that IgE antibodies exhibiting an affinity as low as $10^{-6}$M for the allergen Fel d 1, were nevertheless able to bind multivalently to FcεRI bound IgE on mast cells and cause degranulation of the cells[19]. This was not possible if the membrane was brought below the Krafft point, the temperature at which cholesterol in membranes freezes, not allowing for lateral diffusion of molecules (Figure 2C). Thus, lateral diffusion of IgE allows for multivalent binding of allergen, causing high avidity interactions, and consequently, low affinity IgE antibodies can trigger cellular activation. This effect may be particularly pronounced for dimeric allergens. Indeed, a case in point is the cat allergen Fel d 1, which is naturally dimeric and causes particularly strong allergies. These data may also explain the often unexpectedly high cross-reactivity between structurally unrelated allergens such as birch allergens and latex.

3 High affinity antibodies are required for allergen-neutralization

The most straightforward way for IgG antibodies to inhibit activation of basophils and mast cells by allergens is direct neutralization of the allergen in competition with allergen-specific IgE[10,20]. This mechanism, now, corresponds to the above discussed and by physico-chemists’ preferred interaction of antibodies with ligands; it happens in solution, or at least within tissue fluids and antibodies typically bind with one arm only. Hence, because this epitope-specific interaction is a competitive one, it is largely promoted by high affinity and monovalency, in contrast to avidity which only plays a minor role in direct neutralization. Indeed, low affinity IgG antibodies failed to neutralize allergens and did not block basophil/mast cell activation both in vitro as well as in vivo[21]. As a further restriction, neutralizing antibodies must recognize the same allergen as the IgE and preferably even the same epitope. This contrasts with interaction mediated via FcγRIIb, as described below. Indeed, low affinity IgG antibodies fail to neutralize the allergen but may efficiently block cellular activation by engaging FcγRIIb.

4 Λογο αφφινιτψ αντιβοδιες συφφιςε το ενγαγε ΦςγΡΙΙβ: αβρογατινγ ΙγΕ σιγναλλινγ φορ σινγελε αλλεργενε

Using Fel d 1 as a model antigen, we have shown that low affinity IgG antibodies fail to neutralize the allergen but nevertheless can efficiently block mast cell/basophil activation[21,22]. This unexpected finding was entirely FcγRIIb dependent. Furthermore, the low affinity antibodies must have a different epitope specificity than the IgE, as only in this case, low affinity IgG could block mast cell activation by interaction with FcεRI-bound IgE and engagement of the inhibitory FcγRIIb. Again, it is likely that lateral diffusion and avidity stabilization is important in the process, as low affinity IgG may need to rapidly engage with and be stabilized by FcγRIIb for effective inhibition of cellular activation. Thus, in this way, also low affinity IgG antibodies can “poison” IgE-signalling by engaging FcγRIIb. There is an interesting analogy from enzyme kinetics for the two types of inhibition. Allergen neutralization corresponds to competitive inhibition, requiring high affinity ligands for effective competition. In contrast, engagement of FcγRIIb corresponds to con-competitive inhibition, rather independent of high affinity[23].

5 ΦςγΡΙΙβ σηοως ινηιβιτορψ αςτιvιτψ αςροσς επιτοπες ανδ αλλεργενς ιν αλλεργεν μιξιτες: αβρογατινγ ΙγΕ σιγναλλινγ φορ μυλτιπλε αλλεργενε

A key difference between allergen neutralization and engagement of FcγRIIb is the breadth of activities, not only in terms of affinity but also in terms of specificity. As discussed above, neutralization is only possible for a given epitope on a given allergen. Unexpectedly, engagement of FcγRIIb not only blocks activation of IgE antibodies with a different epitope than the IgG exhibits but is also effective for entirely different allergens as long as the allergen recognized by the IgG antibody is present in the mixture. Specifically, peanut-allergic local and systemic responses against whole peanut allergen extract could be blocked by immunization against a single allergen, specifically Ara h1 or Ara h2[13,24]. Even though mice were allergic to multiple peanut allergens, immunization against a single allergen was sufficient to block allergic responses. Furthermore, polyclonal antibodies against Ara h 1 or Ara h 2, as well as a mAb against Ara h 2 were able to abrogate allergic responses induced by the peanut extract[25], confirming earlier data for cat allergy, an allergy which is, however, mostly driven by the single allergen Fel d 1[26]. This protective effect of Ara h 1 and Ara h 2 antibodies was again strictly dependent on the presence of functional FcγRIIb. In absence of
the inhibitory receptor, no reduction in allergic symptoms could be observed. Hence, IgG complexes with a single allergen are able to abrogate IgE-signalling induced by complex allergen mixtures.

6 Role of the IgG subclass

Clinical efficacy of allergen-specific immunotherapy (AIT) or vaccination best correlates with increased levels of IgG4 in humans. Hence, it is generally assumed that IgG4 is the key IgG subclass for clinical outcomes. However, as pointed out previously\textsuperscript{27}, the correlation between clinical efficacy and IgG4 may simply be due to the fact that the way we are currently performing immunotherapy is preferentially inducing IgG4 rather than other subclasses; mostly due to the absence of strong toll-like receptor or other innate ligands in the formulations. It should be noted that a correlation between clinical outcome and allergen-specific IgG4 has been reported in some but not all studies. Hence, IgG4 is not a validated biomarker\textsuperscript{28}. Negative results may in part be due to suboptimal designs of conventional immunotherapy. Recently, however, it was demonstrated that IgG1 and IgG4 appeared in mucosal fluids after AIT with genetically modified allergens\textsuperscript{29}. IgG subclass responses after to AIT are not fully understood to date and are therefore an active area of ongoing clinical research.

We compared the ability of 3 different mAbs against Fel d 1 expressed in a IgG1 or IgG4 format (exhibiting identical epitope specificities despite being expressed as IgG1 or IgG4) for their ability to block primary human basophil activation via allergen-neutralization or engagement of FcγRIIb\textsuperscript{27}. Indeed, both antibody subclasses had the same ability to block basophil activation in a quantitative manner. These observations were confirmed when the affinity of the antibodies for recombinant FcγRIIb was assessed by BIolayer Interferometry showing similar affinities for IgG1 and IgG4. Hence, it seems that different IgG subclasses – at least IgG1 and IgG4 – are capable of similar allergen neutralization and FcγRIIb engagement. It is now planned to further assess the diversity and functionality of antibody profiles in response to a vaccine against peanut allergy which is in clinical development (PROTECT trial; ClinicalTrials.gov Identifier: NCT05476497).

7 Current trends in AIT

Current trends in AIT seek novel routes of allergen administration as e.g. the use of allergens or allergoids formulated with adjuvant systems or native allergens displayed on virus-like particles (VLPs). Many of these novel approaches favour induction of IgG1 rather than IgG4\textsuperscript{14}. It could therefore be considered that allergoids either share some structural features with viral antigens as eg forming aggregates that present the epitopes in a more rigid and repetitive structures compared to native allergens. Ordered and repetitive surfaces are unique features of viral and bacterial surfaces and, hence, considered to be a pathogen-associated structural pattern (PASP)\textsuperscript{30}.

Such considerations may offer insights into the surprisingly large treatment effect size in a recent exploratory field trial using a mixed Grass-SCIT-based allergoid combined with a novel adjuvant system (MCT\textregistered; MPL\textregistered) in the late stages of clinical development\textsuperscript{31}. Approximately a 40% reduction of the Combined Symptom and Medication Score were achieved during the peak Grass Pollen Season (GPS) compared to placebo using a new regimen of only six pre-seasonal monthly injections\textsuperscript{31}. In this study, grass-specific serum of IgG4 (n=37) and total IgG (n=10) was statistically significantly increased (LS mean ± SE: +3.34 mg/L ±0.946, p = .0006 and 79.94 mg/L ±111.10 (p = .0004)) at the start of the grass pollen season and remained elevated at the end of the grass pollen season.

It is worth noting that detection of specific IgG1 antibodies is not possible with the high throughput ImmunoCap Phadia platform used to support large-scale sampling from clinical trials. However, a grass specific (mix) IgG is commercially available and covers all subclasses including IgG1 and IgG4. Based on the fact that IgG1 makes up 60% of all IgG subclasses while IgG4 only 4%, measurement of grass specific total IgG will likely mainly represent changes in IgG1 levels.

Given recent developments in the field studying the importance of IgG subclasses and the classification of IgG1 as an equally dominant IgE-blocking antibody as IgG4, this will be a further potential biomarker correlate of the pivotal phase III trial, where specific IgG1 data is currently being collected for a sub-
population-group of patients on active treatment. This will add further clinical evidence whether IgG1 and IgG4 subclass induction are equally important and both may constitute a hallmark of successful short-course pre-seasonal AIT; attributed to its adjuvant system (including a toll-like receptor 4 ligand) and nature of ligands (allergoid as opposed to native allergens) in the formulation.

8 TLR7/8 signalling broadens antibody repertoire, affinity, and avidity

Despite the above-described ability of low affinity allergen-specific IgG antibodies to block type I hypersensitivity responses, it remains common thinking that high affinity antibodies would be more effective to also neutralize the allergen. In addition, induction of high affinity antibodies continues to be a general and common goal in vaccinology. For that, induction of germinal centers (GC), also known as secondary follicles, is a key goal, as GCs are the anatomical location of hypermutation and antibody affinity maturation. Induction of GCs is driven by antigen deposition on follicular dendritic cells (FDCs) and effective stimulation of B cells. Repetitive display of antigens in general and allergens specifically has been found to induce strong and rapid B cell responses due to effective B cell receptor cross-linking as well as engagement of the innate immune system[10]. Indeed, natural IgM recognizes repetitive epitopes on viruses and virus-like particles (VLPs), causing activation of the classical pathway of complement (via C1q), leading to the deposition of these particles on FDCs and to GC-reactions[16]. In addition, VLPs may package RNA from E. coli during VLP production, when assembling inside bacteria. This RNA has not only been observed to effectively drive class switching to IgG and IgA but also to increase the affinity of the induced antibodies as well as to facilitate maintenance of a broad immunoglobulin repertoire against both VLPs as well as displayed allergens[32]. In a recent preclinical model of peanut allergy, the presence of RNA in VLPs (Cucumber mosaic virus-like particle; CuMV_TT) displaying the peanut allergen Ara h 2 (CuMV_TT-Ara h 2; VLP Peanut) proved essential for induction of protective IgG responses against peanut allergy; here the role of TLR-signalling and its influence on the vaccine’s efficacy profile was explored in TLR7 knock out (KO) mice as well as VLPs loaded with low amounts of RNA[33]. Unexpectedly the total amount of Ara h 2 specific antibodies was not affected by TLR 7 signalling; in contrast, when mice were immunized with the product containing approximately half the total RNA content, a significant reduction of Ara h 2 specific IgG titers was observed in Wild Type and TLR 7 KO mice. Not only the total amount of IgG antibodies was affected but also IgG subclasses and the number of high avidity IgG antibodies (Figure 3). In essence, Ara h 2 specific total IgG responses were highly dependent on the VLP Peanut carried RNA. The observed difference in low RNA versus lack of TLR 7/8 is explained by the fact that the RNA loaded in VLPs also engages TLR 3, contributing to the overall immunogenicity profile. Indeed, the bacterial RNA contained in VLP Peanut exists in both single- and double-stranded forms, stimulating TLR 7 and TLR 3. A missing TLR 7 signal can therefore partially be compensated by a TLR 3-derived signal as both TLRs are expressed in B cells[34,35].

Additionally, there seems to be a different dependency on the VLP-carried RNA between antigen- and carrier-specific immune responses. The observation that VLP-specific antibodies were less dependent on TLR signalling than Ara h 2 specific antibodies may be explained by the fact that the CuMV_TT subunits are more densely packed at a lower distance than the Ara h 2 molecules, overcoming TLR dependence. This indicates that the overall IgG response is dictated by a 2-dimensional integral of TLR stimulation and antigen density. We have previously seen that B cell activation may be driven by integrated overall signals, as TLR-signalling could overcome IL-21-dependence of B cell responses[36]; an observation reminiscent of the observed independent of TLR-signalling.

Hence, repetitive display of allergens on VLPs packaged with RNA appears an attractive way to increase induction and maintenance of high affinity antibodies (Figure 4). In studies on mast cell interaction with VLPs displaying Fel d 1 or peanut allergens, a substantially reduced interaction with IgE bound to mast cells was observed and an even more pronounced failure to significantly activate the FcεRI mediated signalling cascade. Specifically, under conditions where similar or higher amounts of allergen were bound to IgE on mast cells, free allergens induced strong cellular activation while allergens on VLPs failed to do so[13,14]. Together, it was therefore an attractive choice to bring Ara h 2 displayed on VLPs (VLP Peanut) into clinical development[24].
9 Conclusions

The affinity as well as the avidity of IgE-allergen interactions play critical roles for activation of effector cells and allergic reactions. It is well accepted that specific allergen-binding of antibodies is required for cellular activation. However, the low affinity of FcεRI-bound IgE for allergens can be compensated by bivalent, high avidity binding to multiple epitopes on allergens. This appears to be the mechanism responsible for the often not expected allergen cross-reactivity which may be driven by low affinity but high avidity interactions. Similarly, IgG antibodies with low affinity for the allergen were also sufficient to inhibit mast cell activation by engaging the inhibitory FcγRIIb. Furthermore, polyclonal and monoclonal antibodies specific for a single allergen were able to block allergic symptoms mediated by whole allergen extract. This effect was also mediated by FcγRIIb and was rather independent of the affinity of the antibodies for the allergen as low affinity antibodies were also effective. Interestingly, comparison of two different IgG subclasses, IgG1 and IgG4 showed the same capacity to block mast cell activation and to bind to FcγRIIb, suggesting similar clinical efficacy for both antibody subclasses. This is currently being further validated in the clinical setting for short-course allergoid treatment options which utilizes an adjuvant system employing MPL (TLR 4 agonist).

Finally, even if FcγRIIb-mediated inhibition can be triggered by low affinity IgG antibodies, allergen neutralization still requires high affinity antibodies which are generated in the germinal centers. The generation of such antibody responses has been shown to be optimized by repetitive display and TLR signalling in B cells, which can be triggered by immunization with virus-like particles packaged with RNA and displaying antigens in a repetitive fashion.

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Author contributions

M.F. Bachmann, P.S. Krenger, M.O. Mohsen, M.F. Kramer, S. Starchenka, M. Vogel, P. Whitehead, M.D. Heath wrote, revised, and approved this manuscript.

Conflict of Interests

M.F. Bachmann, M.O. Mohsen, M. F. Kramer, S. Starchenka, M.D. Heath are involved in the development of allergen-specific immunotherapies within Allergy Therapeutic LTD and Saiba AG.

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This review discusses the various hypotheses for the mechanism of immunotherapy puts forward the concept that allergens may be viewed as 'protoxins' which need to be activated by IgE antibodies. As such, protoxin-neutralizing IgG antibodies are the key effector molecules of treatment and as such viewing allergens as protoxins may therefore allow outlining a path for the development of more efficient immunotherapies.


In this model of peanut allergy, the induction of IgG antibodies against a single allergen combined with [Alexander's text in Greek]


*Low-affinity IgE antibodies are able to bind allergens bivalently on the surface of mast cells, leading to high-avidity interactions, as such allergen cross-reactivity may be low-affinity but high-avidity binding between IgE antibodies and cross-reactive allergen.*


Antibody affinity dictates the mechanism of mast cell inhibition, and IgG antibodies triggering the
ινηιβιτορψ ΦςγΡΙΙβ πατηωαψ ςαν σηοω α βροαδερ χροο-ρεαςτιvιτψ παττερν τηαν πρειοωολψ τηρουγη.


IgG1 binds with a similar affinity to the FcγRIIb as IgG4 and is comparable at blocking human basophil activation from allergic patients; both by neutralizing the allergen as well as engaging the inhibitory receptor FcγRIIb.


The generation of antibody responses was shown to be optimized by repetitive display and

**FIGURES**
Figure 1. Model illustrating the impact of IgG antibodies on Type I hypersensitivity. Allergens are taken up by antigen presenting cell which present processed antigen to T<sub>H</sub> cells. T<sub>H</sub> cells help B cells to produce IgE which binds to FcεRI on the surface of mast cell and sensitize them. Allergen-specific IgG antibodies neutralize allergens and/or engage the inhibitory receptor FcγRIIb thereby agonist mast cell activation.

Figure 2. Strength of interaction between antibody and antigen. A) Difference between antibody affinity and avidity. B-C) Lateral movement at 37°C facilitates multivalent and high-avidity binding of IgE antibodies to allergens, while temperatures below the Krafft point at (e.g. 4°C) inhibit lateral movement and multivalent binding.

Figure 3. The RNA dependency of VLP Peanut to induce high-avidity antibodies and to drive antibody class switching. A) Illustration of VLP Peanut (PDB file). VLP Peanut consists of CuMV-derived subunits (PDB code 1F15) and CuMV subunits that were modified by the incorporation of Ara h2 (PDB code 3OB4). For the illustration, the Ara h 2 monomer was manually placed near the insertion loop present in the CuMV subunit by using COOT. Based on the obtained modified PDB file and icosahedral symmetry operators the image was created in pymol. The figure was curtesy of Prof Kaspars Tars, Riga B) Cartoon representation of VLP Peanut encapsulated RNA. C) Ara h2 specific total IgG responses are highly dependent on the VLP Peanut-carried RNA. Illustrations depicted in B and C created with Biorender.com.

Figure 4. A cartoon illustrating a VLP nanoparticle-based vaccine against peanut allergy. The nanoparticles are packed with ssRNA, a TLR7/8 ligand, and display Ara h2 antigen. The natural pentameric IgM antibody can bind the VLPs with low affinity/high avidity. Such interaction contributes to the activation of classical complement cascade and binding of the formed immune-complex on FDCs for effective formation of GCs. This vaccine may induce high-affinity antibodies, drive antibody class-switching and establish long-lived memory. Abs; antibodies.
A. **Affinity** vs **Avidity**

B. **Lateral Movement** at **37°C**

C. **Krafft Point** at **4°C**, **No Lateral Movement**

*Figure 2, Bachmann et al.*
Figure C: A bar chart showing the serum IgG levels of Ara h 2 in WT mice, TLR 7 KO mice, CuMV17 control, VLP Peanut low RNA in WT mice, VLP Peanut in TLR 7 KO mice, and VLP Peanut in WT mice.
Figure 4. Bachmann et al.