Determination of immunogenic epitopes in major house dust mite allergen, Der p 2, via nanoallergens

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Abstract

Background: Despite the high prevalence of allergic asthma, currently avoidance of the responsible allergens, which is nearly impossible for allergens such as house dust mite (HDM), remains among the most effective treatments. Consequently, determination of the immunogenic epitopes of allergens will aid in developing a better understanding of the condition for diagnostic and therapeutic purposes. Current methods of epitope identification, however, only evaluate IgE-epitope binding interactions, which is not directly related to epitope immunogenicity.

Objective: To determine and rank the immunogenicity of the epitopes of major HDM allergen, Der p 2.

Methods: We performed degranulation assays with RBL-SX38 cells primed using patient plasma and challenged with nanoallergens which multivalently displayed epitopes to study the relative immunogenicity of various epitopes of Der p 2. Nanoallergens were used to evaluate epitopes individually or in combinations.

Results: When evaluated using three patient samples, three epitopes in two distal regions of Der p 2 were identified as highly immunogenic when presented in combination, while no individual epitope triggered significant degranulation. One of the epitopes (69-DPNACHYMKCPLVKGQQY-86) was identified to be cooperatively immunogenic when combined with other epitopes.

Conclusion: Our study highlights the importance of conformational epitopes in HDM-related allergies. This study also provides further evidence of the versatility of nanoallergens and their value for functional characterization of allergy epitopes, by ranking the Der p 2 epitopes according

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allergen (globular protein), affinity between epitope and IgE is significantly weaker due largely to the increase in conformational entropy (i.e., increased structural conformations) of the amino acid sequence presented without the surrounding protein framework.[24,25] This is particularly important to consider when comparing sequential epitopes (i.e., continuous sequence of amino acids in the protein) versus conformational epitopes (i.e., amino acids in proximity only when the protein is properly folded), as conformational epitopes cannot be mimicked outside of the natural allergen structure without a scaffold for proper structural support. To overcome the weaker monovalent affinity of the short peptide epitopes, here we optimized the valency (i.e., number of epitope copies presented on a nanoallergen) of our nanoallergens by varying the epitope loading and particle size, to increase their avidity (multivalent affinity) for IgE-presenting cells. Increasing the epitope loading could also allow for both F_{AB} arms on a single IgE molecule to interact bivalently with two separate copies of an epitope on the surface of a single nanoallergen, which increases avidity for each IgE-nanoallergen interaction, and in turn increases overall avidity of the nanoallergen, making it more potent. Furthermore, we envision larger-sized particles accommodating a greater number of IgE binding to a single nanoallergen, which results in larger receptor clusters that typically increase the cellular response (i.e., degranulation). Ultimately, synthesis of more potent nanoallergens could allow for the detection of lower affinity immunogenic epitopes that were overlooked in traditional binding assays.

For this study, we focused on a single major allergen from the *Dermatophagoides pteronyssinus* species: Der p 2, to which 70-80% of HDM patients are reported to be sensitized.[26] While HDMs include several species, the most prevalent in the US are *D. pteronyssinus* (Der p) and *D. farinae* (Der f), between which there is typically 80-90% sequence homology (e.g. 88% homology between Der p 2 and Der f 2, group 2 allergens).[27-30] Literature reports on IgE-binding Der p 2 epitopes vary significantly and disagree on whether sequential epitopes of Der p 2 exist.[31-40] Here, we further explore the importance of sequential versus conformational epitopes in HDM allergies.

Literature-reported Der p 2 epitope sequences were determined via studies which tested for epitope binding, typically relying on affinity of the monovalent (one-to-one) epitope-IgE interaction. Such assays fall short of reflecting the complexities of multivalent allergen-IgE-cell interactions *in vivo* and do not directly correlate to an immunogenic response. Importantly, this strategy may result in low-affinity epitopes being overlooked. Bias towards high-affinity epitopes becomes problematic when analyzing patient samples, where the presence of multivalent interactions (i.e., multiple epitopes interacting with multiple IgE) yields a complex relationship between relative abundance and affinity of each IgE, and the immunogenicity of the IgE. In such cases, lower affinity epitope-IgE pairs can play a crucial role, as previously reported.[41,42] Specifically, due to the heterogeneity of IgE on the cell surface, high affinity epitopes alone may not be enough to trigger degranulation, whereas inhibition of lower affinity epitopes may be enough to subdue it.[41] Due to these shortcomings, there is currently no consensus on the major HDM epitopes nor any information on their relative immunogenicity.[26,31-40] The nanoallergen platform is able to evaluate patient samples in an *ex vivo* setting and incorporate multivalent interactions to more accurately simulate the immunogenic activities triggered by the patient IgE. The immunogenic activity can then be quantified through an RBL cell degranulation assay. In

earlier publications, we reported that the nanoallergen platform overcame the limitations of the traditional methods used to identify IgE-binding epitopes,[21,23,43] and here we used a similar approach to identify the relative immunogenicities of Der p 2 epitopes. Furthermore, we have previously demonstrated identification of immunogenic epitopes provides relevant information for the development of new treatments, such as inhibitors.[44,45]

To determine the immunogenic epitopes of Der p 2, in this study, degranulation assays were performed with plasma from patients sensitized to HDM, using nanoallergens loaded multivalently with copies of one epitope at a time (Figure 1B). Assays with nanoallergens presenting a combination of epitopes were also performed to evaluate the possibility of cooperativity when simultaneously presenting multiple epitopes. By testing the ability of the reported epitopes to induce a cellular response, rather than relying on binding affinity alone, a more robust method for identification of the immunogenic epitopes of major HDM allergen Der p 2 is presented. Ultimately, identification of immunogenic portions of the allergen is an important tool for improving the treatment and diagnostic options for HDM allergies and asthma.

METHODS

Materials

All solvents and peptide synthesis reagents were purchased from Millipore-Sigma (St. Louis, MO): dimethylformamide (DMF), piperidine, dichloromethane (DCM), N,N-diisopropylethylamine (DIEA), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU), hydrazine, Oxyma, N,N′-Diisopropylcarbodiimide (DIC), diethyl ether, trifluoroacetic acid (TFA), triisopropylsilane (TIS), isopropanol (IPA), acetonitrile (ACN), chloroform, methanol, cholesterol, bovine serum albumin (BSA), cytochalasin D, poly-N-acetylglucosamine (PNAG), phosphate buffered saline (PBS), palmitic acid, citric acid, sodium citrate, glycine, magnesium chloride hexahydrate, NovaPEG Rink Amide LL resin, and all Novabiochem fmoc-protected amino acids. Fmoc-N-amido-dPEG8-acid and Fmoc-N-amido-dPEG2-acid were purchased from Quanta BioDesign (Plain City, OH). Sodium hydroxide, sodium chloride, and potassium chloride were purchased from VWR (Radnor, PA). 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol)-2000 (DSPE-mPEG2000) ammonium salt, membranes, and all mini-extruder components were purchased from Avanti Polar Lipids (Alabaster, AL). Minimum essential media (MEM), fetal bovine serum (FBS), L-glutamine, penicillin/streptomyocin, G418, calcium chloride, glucose monohydrate, and goat anti-human IgE were purchased from Thermo Fisher Scientific (Waltham, MA). Trypsin was purchased from Corning (Corning, NY). Patient plasma was purchased from PlasmaLab International (Seattle, WA). Isotype monoclonal human IgE were kindly provided by Dr. Scott Smith (Vanderbilt University).

Statistical analysis

All error bars represent standard deviations of triplicates in a single experiment, unless otherwise noted. Degranulation assay data are representative of several experiments. Means,

standard deviation, and p-values were calculated using GraphPad Prism software (San Diego, CA). P-values were calculated with 2-way ANOVA.

Protein analysis

Analysis and study of protein crystal structures for the structural and spatial identification of potential epitopes were performed using UCSF-Chimera, and Der p 2 crystal structure was obtained from the protein data bank (www.rscb.org).

Peptide synthesis

Peptides were synthesized either using a Liberty Blue microwave-assisted peptide synthesizer or manually on a wrist-action shaker following conventional Fmoc chemistry on a NovaPEG Rink Amide LL resin. Fmoc deprotection reactions were performed using 20% piperidine in DMF and ivdde deprotections with 2% hydrazine in DMF. Fmoc-protected amino acids were activated using either DIC/Oxyma on the synthesizer or HBTU/DIEA during manual synthesis. Palmitic acid conjugations were performed manually using HBTU/DIEA for activation. Peptides were cleaved using cocktails of TFA/H2O/TIS (95/2.5/2.5%) or, if the epitopes contained cysteine residues, TFA/H2O/EDT/TIS (94/2.5/2.5/1%). Post cleaving, TFA cocktails were removed via a Buchi rotavapor.

Peptide cyclization was performed after initial purification of the epitope-lipid conjugates. Epitope-lipid conjugate powder was dissolved in 10 mL DMF and 100 μL DIEA (per 10 mg of product), then the solution was allowed to react overnight at RT with gentle stirring. After reacting, the solvent was evaporated using a rotavapor, and the cyclized epitope-lipid conjugates were re-purified, as described below.

Peptide purification and characterization

Peptides were purified using RP-HPLC on an Agilent 1200 Series. Peptide-lipid conjugates were purified with a Zorbax semi-prep C3 column and IPA/ACN/H2O gradient. All products were purified to >90% purity. Following purification, product identity was confirmed by Q-TOF mass spectrometry.

Cell culture

RBL-SX38 cells were cultured in Minimum Essential Media with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin/streptomyocin, and 1.2 mg/mL of G418, a selective agent for the human FcεRI-expressing RBL cells, as previously described.[21] Cells were split every two to three days. For the purposes of this experiment no cells were kept in culture longer than 4 weeks. Cells were kindly provided by Dr Jean-Pierre Kinet.

Nanoallergen preparation and characterization

Nanoallergens were prepared by the dry film hydration method, as described previously.[46] In vials, the purified epitope-lipid conjugates, at desired molar stoichiometric ratios, were combined with bulk lipid and polyethylene glycol (PEG2000) conjugated lipids, all dissolved in volatile solvent. The PEG2000-lipids were added to limit aggregation of nanoallergens and nonspecific interactions between nanoallergens and cells. For the nanoallergens that displayed multiple copies of one or more epitopes

simultaneously, a lipid mixture was used following the formula 95-(X+Y+Z):5:X:Y:Z Bulk-Lipid:PEG2000:EpitopeX:EpitopeY:EpitopeZ, where X, Y, and Z were varied from 0-8 mol% (Figure 2B). An additional 5 mol% of cholesterol was included to further stabilize the liposomes. Volatile solvents were evaporated with a gentle flow of nitrogen, and then vials were kept in a desiccator overnight to form a thin dry lipid film. Films were rehydrated in PBS above T_G (55°C) and extruded through polycarbonate membrane filters, with pores of the desired size (i.e. 100 or 200 nm). Nanoallergen size was characterized with dynamic light scattering (DLS) analysis (Figure 2C & D), using 658 nm light observed at a fixed angle of 90°, on a Brookhaven NanoBrook Omni. DLS analyses were performed at the Center for Environmental Science and Technology at the University of Notre Dame.

Liposome extruder purification (LEP)

To determine epitope incorporation, nanoallergens were first purified via liposome extruder purification (LEP), as described previously.[46] Briefly, nanoallergens solutions were extruded through 30 nm polycarbonate membranes at room temperature, to remove any unincorporated lipids. Then, pre-LEP (before purification) and post-LEP (purified) samples of each nanoallergen were evaluated via RP-HPLC, and area under the curve (AUC) from the chromatogram of each was determined. Pre-LEP AUC was 100% incorporation; therefore, percent incorporation was calculated as: [post-LEP AUC]/[pre-LEP AUC] x 100%.

Degranulation assays

RBL degranulation assays are performed as previously described.[21,43] Briefly, RBL cells are plated at 50,000 cells/mL in a 96-well culture plate (100 μL per well) and allowed to attach overnight until cells are confluent on plate. The media is then removed, and cells are incubated overnight in 10% (v/v) human plasma in full growth media. The next morning, the cells are washed twice with sterile PBS and media is replaced to remove impurities and stabilize the cells before the assay. After a 3-hour incubation, cells are washed twice with Tyrodes buffer, and allergens are introduced to elicit degranulation responses. Positive and negative control wells are included on each plate. Negative control wells contain cells only. Either TritonX or anti-IgE were added to positive control wells. After incubating for 1.5 h, the PNAG substrate is introduced to initiate β-hexosaminidase enzymatic cleavage reactions. The reaction is stopped with glycine buffer (pH 10.7) after 1 hour at 37°C. Enzymatic activity is monitored and analyzed with a SpectraMax M2e plate reader (λ = 405 nm). All degranulation assays are run in triplicate. Degranulation is shown as a percent of the positive control.

A control group was used to confirm the epitopes caused degranulation due to interaction with specific IgE-FcERI complexes. For the "No IgE" group, no IgE or plasma was used to prime the RBL-SX38 cells, instead only fresh media was added during incubation. For the "Isotype Control" group, the RBL-SX38 cells were primed with monoclonal human IgE specific to peanuts. We confirmed that RBL-SX38 cells primed with this antibody do not react to the dust mite allergen (Figure 3B & C). Isotype control was used for all assays, unless otherwise noted.

RESULTS

Design, synthesis, and characterization of nanoallergens

The nanoallergen platform provides a means to evaluate epitope immunogenicity with precise control over epitope loading and particle size with low variability between batches (Figure 2).[21] The precision in the stoichiometry of loaded epitope per nanoallergen is achieved by assembling the liposome from pure building blocks, including the epitope-presenting peptide-lipid conjugates (Figure 2A & B, eFigure 1A). The epitope-lipid conjugates were produced with solid phase peptide synthesis, using Fmoc chemistry as previously described.[43,45,47] The conjugates were purified via reverse phase HPLC (RP-HPLC) and characterized with Q-TOF mass spectrometry (eFigure 1B, eTable 1). A typical concern, when displaying peptides or small molecules on a surface, is that the peptide may hinder itself by becoming buried in the hydrophobic region of the lipid bilayer. To overcome this concern and achieve maximum efficiency in displaying the epitopes on the nanoallergen surface, a linker composed of ethylene glycol (EG) and three lysines was used (eFigure 1A), which enhances the presentation of the epitope to the IgE by improving partitioning of the epitope into the aqueous phase, as previously reported.[43]

The nanoallergens were prepared from pure components (Figure 2B) via the dry film hydration method, as previously described.[21,22,46] Nanoallergen size was characterized with dynamic light scattering (DLS) analysis (Figure 2C & D). For the 200 nm nanoallergens, effective particle diameter ranged 150-175 nm with low polydispersity (<0.17); the 100 nm effective particle diameters ranged 95-120 nm with polydispersity <0.15. Percent epitope loading of >80% incorporation was confirmed using RP-HPLC analysis on nanoallergen samples purified via liposome extruder purification (LEP), as described previously (eFigure 1C & D).[46]

Cross-analysis of literature-reports to identify allergen epitopes for further immunogenicity evaluation

For Der p 2, literature studies report both continuous (sequential amino acids) and conformational (nonsequential) IgE-binding epitopes. Overlapping portions of both sequential and conformational epitopes reported in multiple literature sources were identified for immunogenic evaluation in this study. The evaluated epitopes are highlighted in the crystal structure of Der p 2 (Figure 3A) and listed in Table 1. Several epitopes were designed and synthesized in multiple configurations (i.e., linear or cyclic; full or truncated), to evaluate whether differences in 3D conformational structure of said epitopes, when presented outside of the protein scaffold, had any meaningful causative effect on their ability to bind sIgE and induce degranulation.

The relative immunogenicity of each of the eleven synthesized epitopes was determined using the nanoallergens to display the epitopes and perform degranulation assays. Nanoallergens were loaded with a precisely controlled mole percent (typically 1-8%) of each epitope-lipid conjugate, either presenting copies of a single epitope or a combination of multiple epitopes. Degranulation assays with patient plasma-primed RBL-SX38 cells were performed to compare the intensity of degranulation induced by the nanoallergens.

Verification of patient sample Der p 2 sensitivity

Three samples of plasma were obtained from patients reported to be allergic to dust mites. Since the epitopes of Der p 2 were under closer analysis, it was imperative to verify that the patient samples would respond to dust mite proteins as well as to Der p 2, specifically. Degranulation assays were performed on cells primed with each of the three patient samples and challenged with varying concentrations of either dust mite extract (Figure 3B) or purified Der p 2 (Figure 3C). All three samples demonstrated degranulation to the extract, but only patients 1 and 3 proved to have IgE specific to Der p 2. Therefore, all future assays reported here were performed with patients 1 and 3 only.

Single-epitope immunogenicity analysis with nanoallergen assay

To determine the immunogenicity of the epitopes identified from literature reports, each epitope was loaded at a previously optimized[43] valency of 2 mol% on 100 nm nanoallergens, such that nanoallergens presented multivalent copies of a certain epitope. Multivalent presentation of a single epitope or a combination of epitopes on the same platform is necessary for IgE-crosslinking and to trigger degranulation. Patient plasma-primed RBL-SX38 cells were challenged with each of the single-epitope presenting nanoallergens (Figure 3D & E; TritonX positive control, no IgE negative control). Even at the maximum nanoallergen concentrations (1 nM) tested, no significant triggering of degranulation was observed from any of the nanoallergens presenting single epitopes. The nanoallergen concentration was limited to a maximum of 1 nM due to concerns relating to specificity, where controls indicated that some nanoallergens, at concentrations above 1nM, nonspecifically interacted with the cells and yielded potentially unreliable degranulation measurements (data not shown).

Cooperative epitope immunogenicity analysis with nanoallergen degranulation assay

Since nanoallergens presenting single epitopes did not demonstrate any detectable immunogenicity, cooperativity between epitopes was investigated next by presenting the multiple epitopes simultaneously, in combinations, on the same nanoallergen. Using epitopes which are spatially distant on the natural allergen (Figure 4A & 4B), two nanoallergen batches presenting two separate combinations of epitopes were prepared. Nanoallergens presenting combination-1, epitopes 2, 5, 7, and 9, showed no statistically significant degranulation compared to the isotype control (Figure 4C; no IgE negative control). Nanoallergens presenting combination-2, epitopes 1c, 3, 4, and 8, induced degranulation at nanoallergen concentrations of 10-1000 pM for both patient samples (Figure 4D; no IgE negative control). These results indicated that two or more of the epitopes in combination-2 were immunogenic and that the combination of epitopes was having a cooperative effect.

To improve the signal-to-noise ratio of the assay, combination-2 nanoallergens were used to optimize nanoallergen diameter and percent epitope loading for maximum degranulation sensitivity with minimum nonspecificity (eFigure 2). For subsequent experiments described below, 200nm nanoallergens and 4% total loading were used.

Nanoallergens (200 nm, 4% total peptide) loaded with pairs of epitopes from combination-2, (i.e. epitopes 1c&3, 1c&4, 1c&8, 3&4, 3&8, 4&8), were prepared and used in degranulation assays. Analysis of the results with the epitope pair nanoallergens indicated that epitope 4 had the strongest observable immunogenicity among combination-2 epitopes, as it produced degranulation when paired with any of the other three epitopes (Figure 5B, D, F). Epitope 1c was the least immunogenic of the four, inducing no significant degranulation when paired with either epitope 3 or 8 (Figure 5A & 5C). Degranulation induced by epitope pair 3&8 indicated these epitopes have slightly higher immunogenicity than epitope 1c, as the epitope pair 3&8 induced 2-fold higher degranulation on average than either 1c&3 or 1c&8 (Figure 5E & 5G).

For patient 1, at 1 nM nanoallergen concentration, epitope pairs 1c&4, 3&4, and 4&8 all achieved 36-44% degranulation in comparison to only 20% achieved with epitope pair 3&8 (Figure 5G). While the degranulation response was 2-to-3-fold lower for patient 3 than for patient 1, patient 3 showed similar trends in immunogenicity.

Epitopes 1s, 2, 5, 6, 6c, and 9 also triggered degranulation, to varying degrees, when presented in combination with epitope 4. These results can be seen in Figure 6.

DISCUSSION

Here, we described how nanoallergens were used to identify the relative immunogenicity of allergen epitopes of dust mite protein Der p 2. The results of this study, upon evaluation of linear and cyclic versions, demonstrated that sequential epitopes of Der p 2 were not highly immunogenic on their own. Instead, presenting combinations of Der p 2 epitopes was necessary to produce significant cellular response, suggesting that immunodominant Der p 2 epitopes had to be conformational. The well-formulated nanoallergens made it possible to identify cooperative immunogenicity among epitopes 1c, 3, 4, and 8 and to rank the relative levels of immunogenicity between these epitopes. In the end, epitope 4 proved to be the most immunogenic, followed by epitopes 3 and 8, and with epitope 1c the least immunogenic. The remainder of the literature-reported epitopes did not display any significant immunogenicity in our analysis. It is noteworthy that the epitopes identified throughout this report align with results from a recently published study that determined two regions on Der p 2 to which certain human monoclonal IgE bind.[48] Specifically, one of these regions supports our identification of epitopes 3 and 4 as immunogenic epitopes, while the other region partially overlaps with epitope 8. While powerful, previous studies required complicated NMR-based evaluations, which necessitate large financial input, time-consuming processes, and difficult to obtain resources, such as large amounts of purified human monoclonal IgE. Our methodology, on the other hand, achieved similar results with the heterogeneous pool of IgE in patient samples, at much lower cost and without the need for synthesis of human monoclonal IgE.

Given the proximity of epitopes 3 and 4 on the natural allergen (Figure 4B), they are unlikely to be simultaneously binding to two separate sIgE, as steric hindrances would prevent two IgE from binding to two very closely positioned sites. We hypothesize that the mechanism by which these epitopes induce degranulation, when presented on a

nanoallergen, is related to one or more of the following binding scenarios: i) portions of Epitopes 3 and 4 may simultaneously bind to the antigen binding site of a single IgE clone, analogous to the interaction of an IgE with a conformational epitope (Figure 7A), ii) alternatively, it is possible epitopes 3 and 4 are recognized individually by the same IgE clone, albeit with different affinity for each epitope (Figure 7B), or iii) two distinct IgE clones may exist, one that binds epitope 3 and one that binds epitope 4. (Figure 7C). If the first case was true, it is possible that epitope 3 provided structural support to keep epitope 4 in its suitable conformation for binding, or vice versa. Conversely, epitopes 1c and 8 are located more spatially distant from epitope 4 on the natural allergen, therefore we would expect epitopes 1c, 8 and 4 to be binding to different monoclonal sIgE (Figure 7C).

In the case of epitope pairs 1c&4 and 4&8, we believe that epitope 4, possibly a higher affinity epitope, may be acting as an anchor (Figure 7C). Association rate constants for biomolecular interactions are generally independent of overall binding affinity (i.e., the on-rate constant (k_{on}) should be similar for most epitopes). Instead, variations in dissociation rate constants (k_{off}), as well as relative abundance of each sIgE in the patient sample, are expected to correlate to the residence time of the nanoallergen staying bound to the cell-surface IgE. Epitope 4 could have served as an anchor by providing sufficient residence time for cellular receptor-IgE complexes to rearrange and bind to the other epitopes on a nanoallergen, thereby promoting crosslinking and initiating degranulation. We predict that the pairing of epitopes 3&8 acts in a similar manner, as these epitopes are also positioned on opposite ends of the natural allergen.

As reported in previous publications, identification of immunodominant epitopes has led to the development of inhibitors, specific for the immunogenic sIgE, which successfully blocked or reduced the intensity of allergic responses to peanuts and drug allergens.[44,45,49] Moreover, identification of immunogenic sequential epitopes (which may be a portion of, or mimic, conformational epitopes) provides a more cost-effective and technically feasible roadmap to the development of allergen-specific inhibitors, compared to conformational epitopes. Thus, we predict that understanding and ranking HDM epitope immunogenicity will aid in the development of a potential preventative treatment for HDM-related asthma. The extension of such technology to the clinic could translate to personalized medicine, as diagnostic identification of immunogenic epitopes in patients can allow for use of personalized therapeutics for inhibition of specific IgE relevant to the patient's allergy. Using a platform such as nanoallergens could determine which epitopes a patient is allergic to, via an ex vivo diagnostic similar to those described in this study or as a skin prick test. Then epitope-specific therapeutics would allow for more accurate and specific treatment of allergic condition. For example, identification of immunodominant epitopes can allow for the development of IgE-specific inhibitors, which could treat HDM allergies and asthma by selectively blocking IgE from binding to HDM allergens. Another potential approach would be developing sIgG against the immunogenic HDM epitopes, which could be used as a monoclonal antibody therapy to capture allergens before allergen-IgE-FcεRI binding can occur.[50] IgG that is randomly generated from the parent allergen will not be guaranteed to block or outcompete IgE binding. Therefore, if a patient is administered only the IgG specific to the relevant epitopes, instead of a mixture of IgG of varying efficacy, the cost of the IgG therapy should decrease.

In conclusion, this report described a novel method to investigate HDM allergic asthma at the molecular level, and a potential new approach to inhibitor development for this condition. Inarguably, the more alternative approaches clinicians have in their arsenal with which to treat asthma and allergies, the better their chances are to effectively treat the condition and improve patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

FUNDING SOURCE:

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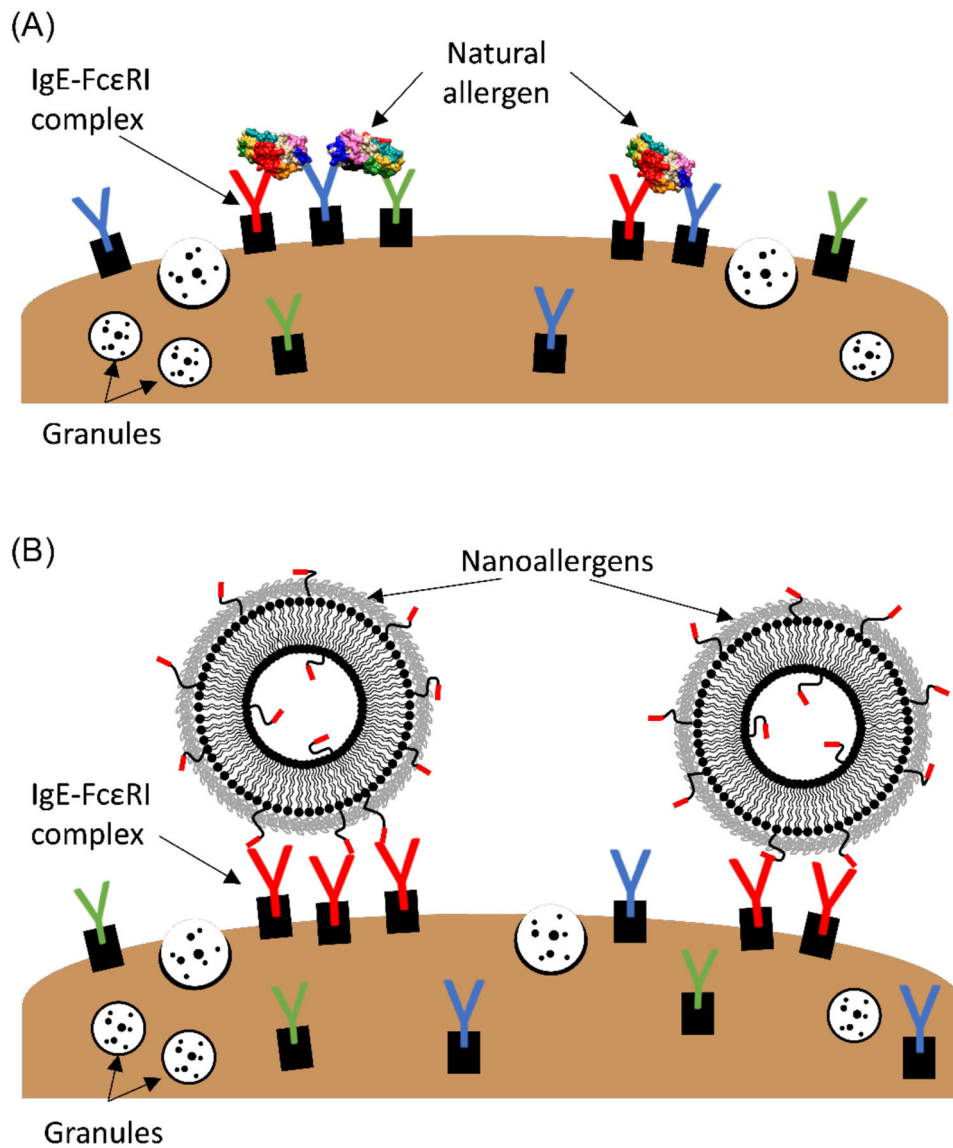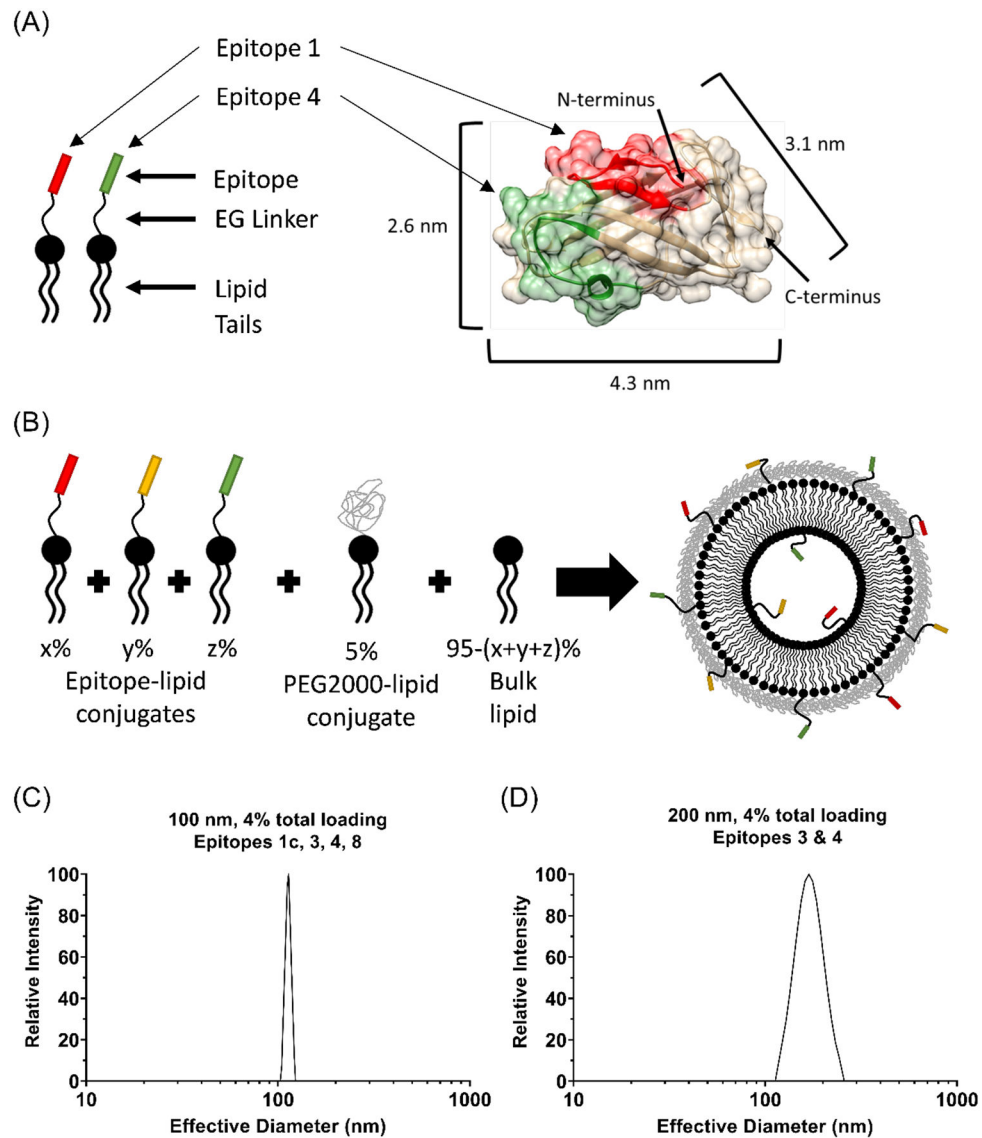
Figure 1.
Cartoon representation of the multivalent binding and crosslinking of specific IgE-FcεRI complexes on RBL cell surface by natural dust mite allergen Der p 2 (A) and nanoallergens presenting immunogenic epitopes (B), inducing intracellular signaling cascades resulting in degranulation. Drawings are not to scale.

Figure 2.
(A) Epitope-lipid conjugate depiction of Der p 2 epitopes. Der p 2 crystal structure (PDB-ID 1ktj) with epitopes color-coded and allergen dimensions depicted. (B) Cartoon depiction of liposome assembly from components at specific stoichiometries. (C), (D) Representative DLS data shown for nominally 100 nm (C) and 200 nm (D) diameter nanoallergens.
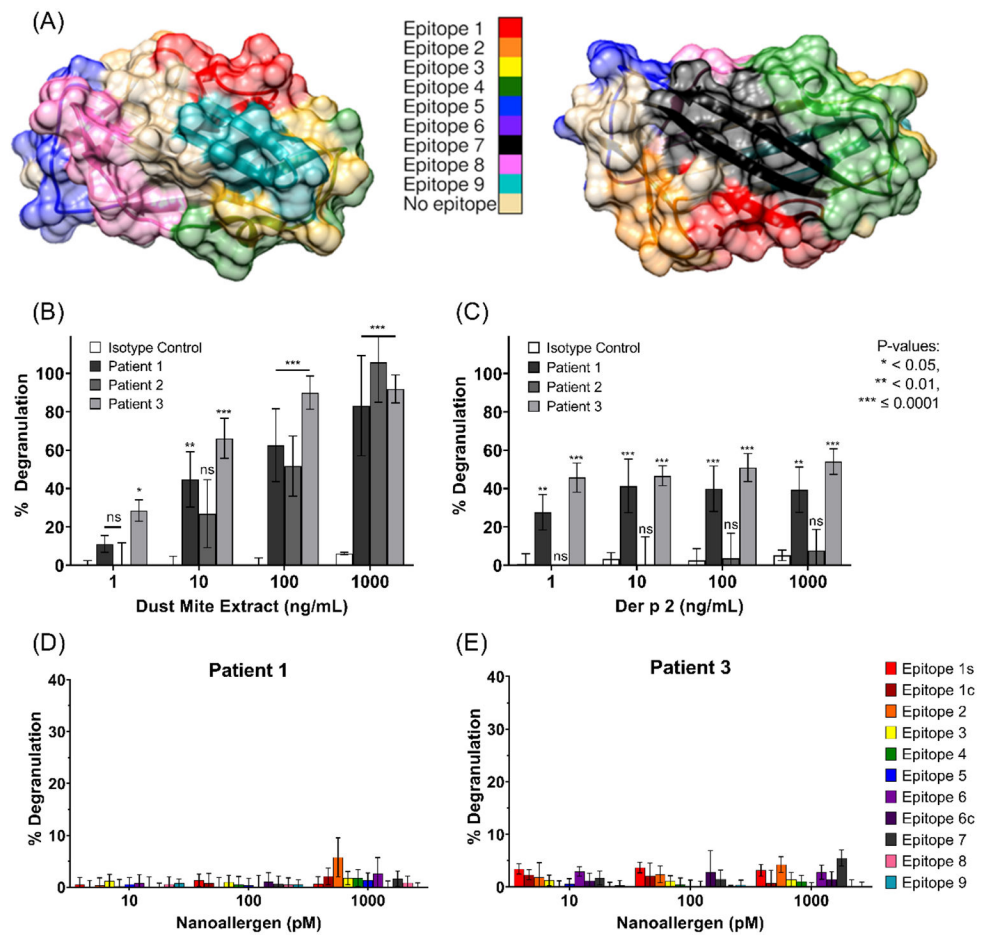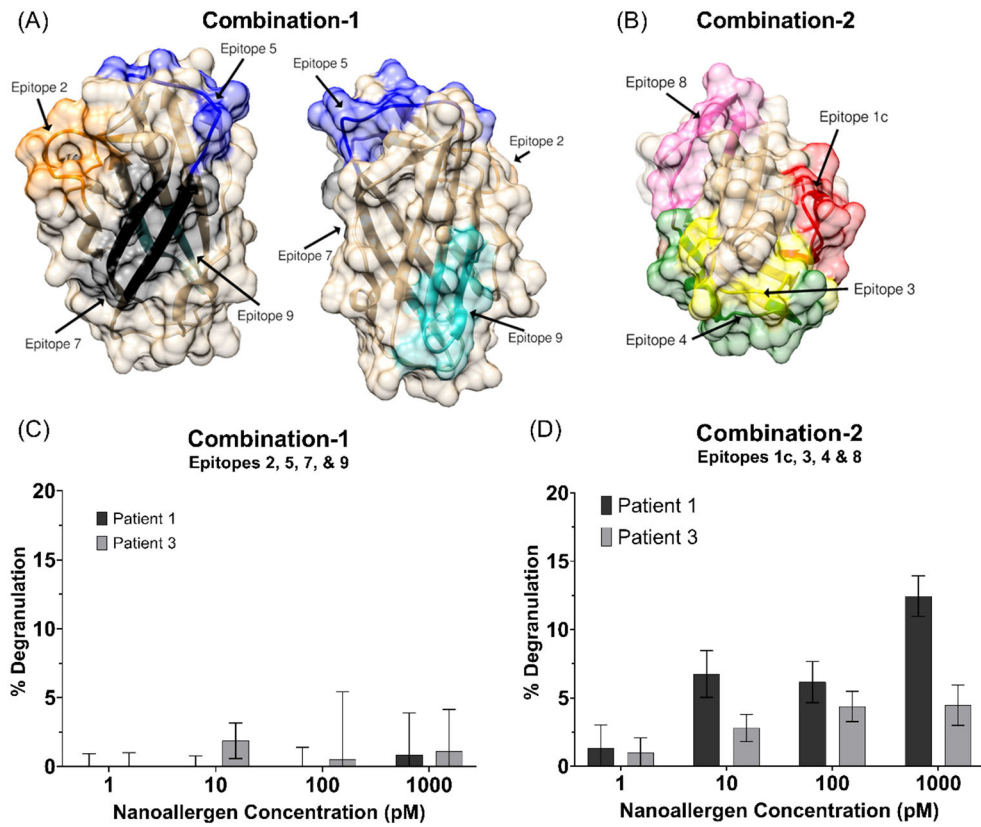
Figure 3.
(A) Der p 2 crystal structure (PDB-ID 1ktj) with epitopes color-coded. Evaluating sensitivity of patient plasma or isotype IgE primed RBL-cells to (B) dust mite extract or (C) Der p 2 via degranulation assays. Cells primed with patient-1 (D) or patient-3 (E) plasma were challenged with nanoallergens presenting copies of a single epitope.

Figure 4.
(A) Location of epitopes 2, 5, 7, and 9 (combination-1) or (B) epitopes 1c, 3, 4, and 8 (combination-2) are shown on Der p 2 crystal structure (PDB-ID 1ktj). Degranulation assays with nanoallergens presenting (C) combination-1 or (D) combination-2 epitopes loaded at 4% total on 100 nm liposomes.
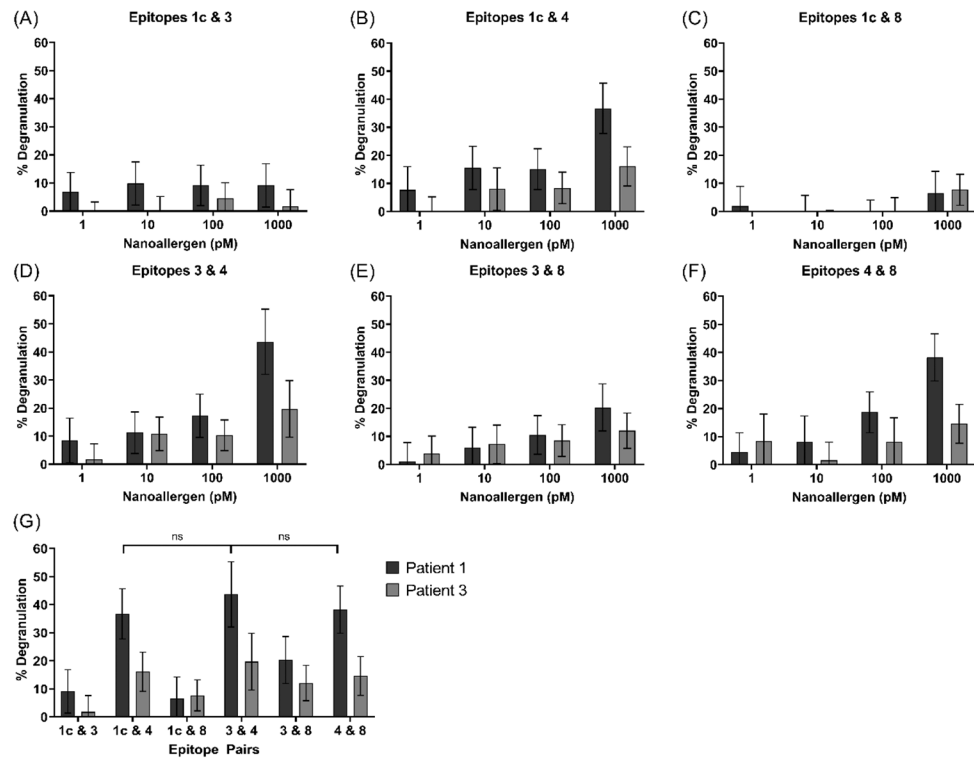
Figure 5.
Degranulation triggered by 200 nm nanoallergens presenting 4% total loading of each epitope pair: (A) 1c & 3, (B) 1c & 4, (C) 1c & 8, (D) 3 & 4, (E) 3 & 8, and (F) 4 & 8. (G) Comparison of degranulation due to pairs of epitopes, as observed at 1000 pM nanoallergen concentration.
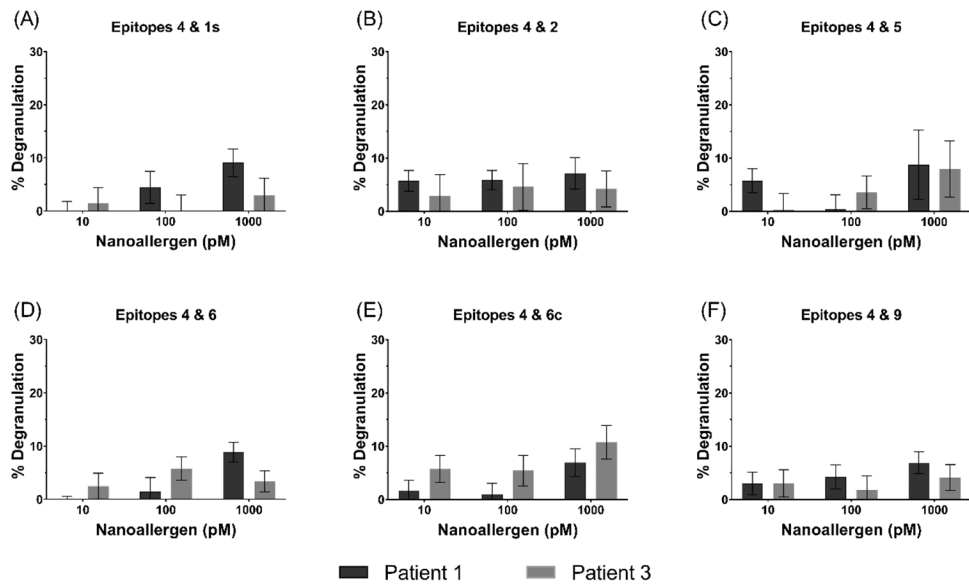
Figure 6.
Cooperativity analysis of epitopes 1s, 2, 5, 6, 6c, or 9 paired with epitope 4.
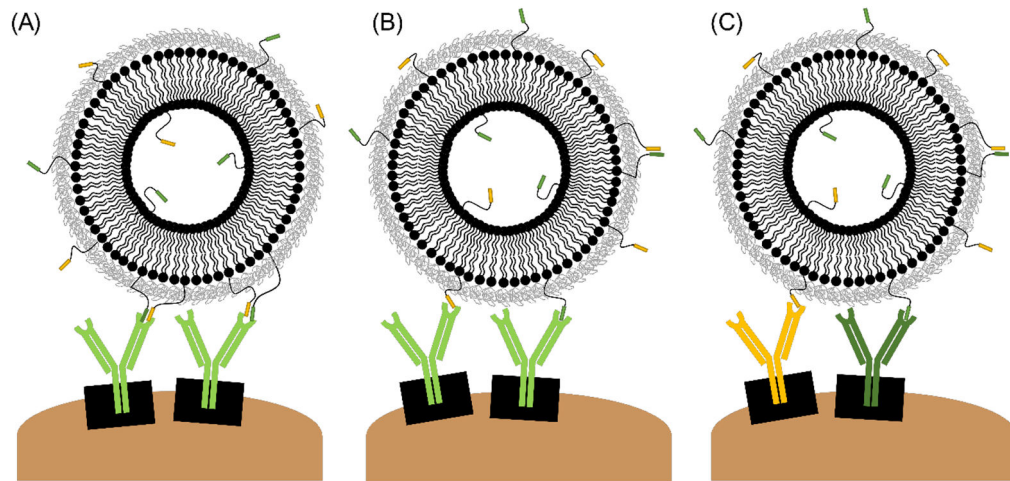
Figure 7.
Hypothesized binding scenarios for cooperative epitopes may occur individually or in combination: (A) Two epitopes bind to the same antigen binding site. (B) Two epitopes bind to separate copies of the same IgE. (C) Epitopes bind to separate IgE specific for different regions of the allergen. Not to scale.

Table 1.

Sequences of the Der p 2 epitopes identified from literature

#	Sequence	Residues	Notes	References
1s	KDSANHE	6-12	Loop without β-sheets	22,23,30
1c	CEVDVKDSANHEIKKVLC	1-18	N-terminus; cyclized	22,23
2	GCHGSEPCI	20-28	cyclized	15,23-25
3	EANQNTKTAK	42-51		15,23,26,27
4c	DPNACHYMKCPLVKGQQY	69-86	α-helix; cyclized	15,22,23,25,27,28
5	VPKIAPKSEN	94-103		23,27,28
6	KASIDGLEVD	55-64		26,27
6c	CSIDGLEC	56-63	cyclized	26,27
7	CPFQLEAVGGGDIKYTWNC	34-40,87-93	2 β-sheets, attached via 3 Gly; cyclized	15,23,26,27
8	ASIDGLEVDVPGID	56-69	extension of Epitope 6	26,27
9	CKVMGDDGVLAC	108-119	cyclized	25,27,29