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Artificial Intelligence-Assisted Hypoallergenic Sui p 2 Design as a Potential Prophylactic Vaccine for House Dust Mites Allergies

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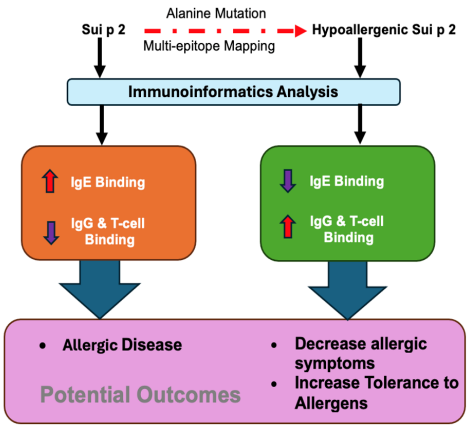
Graphical Abstract



Abstract

House dust mite (HDM) group 2 allergens are clinically significant IgE-binding proteins that trigger allergic diseases such as asthma, rhinitis, and dermatitis. The HDM group 2 allergen homolog Sui p 2 could be a major allergen in the HDM species *Suidasia pontifica*. The formulation of hypoallergenic Sui p 2 is crucial in advancing a prophylactic vaccine targeting HDM allergies. This study utilized artificial intelligence-based software in mapping the linear and discontinued B-cell and T-cell epitopes of the native Sui p 2 followed by alanine mutagenesis for the hypoallergenic construct. Protein prediction servers were used to predict the proteins' allergenicity, toxicity, homology, and physicochemical properties. Twenty-nine (29) amino acid (AA) residues were changed into alanine to create the hypoallergenic design, resulting in 9.8% IgE binding reduction, lesser AA IgE binding residues, less toxic, 7.5-fold higher IgG binding fragments, and tripled MHC II T-cell epitopes compared to its native form. The hypoallergenic design was highly conserved regarding its sequence and structure homology. This study demonstrated the potential of alanine mutagenesis in developing a more safe and efficacious allergen-specific vaccine design.

Keywords: Sui p 2; prophylaxis; artificial intelligence; vaccine; epitopes, house dust mites

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Introduction

Allergic asthma, allergic rhinitis, and atopic dermatitis are some of the most common manifestations of IgE-mediated hypersensitivity reactions due to exposure to allergens, activating a Th2-biased immune response among atopic individuals. Th2 cells produce pro-inflammatory cytokines, which activate plasma B cells to produce allergen-specific IgE (sIgE). The elevated amount of sIgE, caused by recurrent allergen exposure, binds to the high-affinity receptors located on the membrane of mast cells, basophils, and eosinophils, which triggers the release of inflammatory mediators that result in vasodilation, mucus production, and inflammation [1] . Allergic diseases affect the quality of life of a significant proportion of the world's population, estimated between 10-40% in various geographical regions [2], [3]. In the Philippines, atopy patients are highly sensitized to house dust mites (HDM) [4]. Worldwide, HDM sensitization peaks at an alarming 15.9%- 81.7% [5], [6], [7], [8], [9], making HDM a major aeroallergen source that triggers allergic reactions.

The HDM species *Suidasia pontifica* (Sp) has been reported to trigger 39-74% IgE sensitization among the allergic population [10], [11]. Furthermore, Sp was found to cross-react with other HDM species, indicating its role in the parallel sensitization of allergic diseases [12]. Recently, a group 2 allergen homolog, Sui p 2, was sequenced and characterized through in silico analysis [13]. Given the well-documented record of HDM Group 2 allergens, exhibiting high IgE-binding activity [14], in addition to the fact that no study has developed a hypoallergenic prophylactic vaccine targeting Sui p 2, the current study is of utmost importance.

Rational hypoallergenic vaccine [15], [16] design has advanced with molecular biology techniques and in-silico analysis. Veering away from conventional hypoallergenic vaccine design through the addition of IgE antagonist compounds or chemical binding-altering agents that inhibit type 1 hypersensitive reactions [17], allergen in silico epitope mapping is now widely available through the advent of artificial intelligence-integrated bioinformatics tools [18]. Direct point mutation on the immunodominant epitopes poses a promise in developing hypoallergenic constructs [19], [20]. Alanine scanning works on the same principle, to which site-direct alanine mutations enable the probing of the critical amino acid for the structure and function of a protein [21]. This technique is well applied in IgE and IgG epitope mapping for allergens, as alanine reduces steric hindrances important for extrapolating antibody binding capability [22], [23], [24].

Considering the promise of genetically engineered hypoallergenic prophylaxis and alanine scanning technology, this study utilized an A.I.-based rational vaccine design for Sui p 2 allergies. This seeks to address the high demand for allergy vaccines, which pose a potential intervention for the allergy burden of the 21st century.

Materials and methods

Multi-Epitope Mapping for Sui p 2 allergen

B-cell Epitope Mapping. The nucleotide sequence of Sui p 2 was retrieved from GenBank using the accession number KY449406.1 and was translated using Expasy (https://web.expasy.org/translate/). The signaling peptide was deleted. Using the Bepipred 2.0 server (http://tools.iedb.org/bcell/), the B-cell linear epitope, with a threshold value of ≤ 0.5, was predicted. B-cell conformational epitope prediction, on the other hand, was done using DiscoTope 1.1 (http://tools.iedb.org/discotope/) utilizing its default settings. The IgG-producing B-cell epitope was then determined using the IgPred server, with a threshold of 0.9 (http://crdd.osdd.net/raghava/igpred/bkp-biology-direct/prot-experi.html).

T-cell epitope Mapping. Major histocompatibility complex II is essential in eliciting immune response, which is why the T-cell epitope mapping was analyzed. To predict the possible MHC II T-cell epitopes, TepiTool (http://tools.iedb.org/tepitool/) was employed. The input parameters were the top 26 frequent class II human alleles for promiscuous binding under the HLA-DQ, HLA-DR, and HLA-DP to cater to a wide range of alleles and ethnicities.

Homology Analysis

A homology analysis ensured that the hypoallergenic construct would have conserved regions relative to its native form. This is essential to build the immune tolerance towards the allergen of interest. NCBI Protein Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE =BlastSearch&LINK_LOC=blasthome) was used to determine the local alignment of the hypoallergenic sequence. The native and hypoallergenic construct was then subjected to A.I.-based tertiary structure modeling AlphaFold2 (https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/Alpha Fold2.ipynb). The best protein model was quality-checked using the ERRAT and PROCHECK tools in UCLA SAVES (https://saves.mbi.ucla.edu/). The TM-score was calculated using the Yang Zhang Lab computation server (https://zhanglab.comp.nus.edu.sg/forum/viewtopic.php?t=1113). Protein visualization and analysis were done using UCSF Chimera.

Construction of the Hypoallergenic Sui p 2

The hypoallergenic Sui p 2 construction was based on the principle of alanine scanning (22). The hypoallergenic construct must abide by the following set of conditions: (1) lowered allergenicity; (2) reduced B-cell linear epitope fragments (<8 amino acid residue); (3) decreased discontinuous epitopes; (4) increased IgG-producing linear B-cell epitopes; (5) increased T-cell MHC-II epitopes; (6) reduced toxicity; and (7) conserve at least 50% of the identity of the native gene. Additional alanine mutations beyond the mapped epitopes were done to ensure that the certain sequence configuration would suffice the conditions stated previously, namely mutating the cysteine-cysteine residue of the native Sui p 2. The toxicity and allergenicity analysis of the proteins was determined using the machine learning server AlgPred2 server (https://webs.iiitd.edu.in/raghava/algpred2/) (25), while the protein toxicity analysis was determined using ToxinPred

(http://crdd.osdd.net/raghava/toxinpred/) (26). Protein Physicochemical properties were calculated using Expasy ProtParam Tool (https://web.expasy.org/protparam/).

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Native Suip2      GEMKFQDCGHGEVKKLLVSDCSCDYCIIHKGKKLSMEADFVANQDSPTAV   50
Hypo Suip2        GEMKFQDAAAGAVKKLLVSDASGDYAIAHAGAALSMEADFVANQDSPTAV   50

Native Suip2      IKISAKVNGVELQVPGIETNGCHHMKCPLVKGQSYQFKYDMVIPQILPNV  100
Hypo Suip2        IKISAKVNGVEAAVAAIAAAAAHHMAAPAAAAASYQFKYDMVIPAALANV  100

Native Suip2      KADVTASLTGAHGLLACGTVHSEVQT  126
Hypo Suip2        KADVTASLTGAHGLLAAGTVHSEVQT  126
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Figure 1. Pairwise alignment of native and hypoallergenic Sui p 2 sequences. Emphasized are the concordant linear and discontinued B-cell epitopes (red) and the combined MHC II T-cell and IgG-producing B-cell epitopes (boxed with broken lines) in the native sequence. Furthermore, the alanine mutated amino acid residues (cyan) were highlighted in the

The total sequence recovered from the NCBI consisted of 585 base pairs with an open reading frame of 134 amino acids. The first 8 signaling peptide sequences were removed, yielding a total of 126 amino acids, which were used as the final native Sui p 2.

The multi-epitope mapping in the Sui p 2 proteins guided the alanine mutagenesis for the hypoallergenic design. Thirty-five (35) amino acid residues were predicted to be a potential epitope; which was much greater compared with the initial study on Sui p 2, which only reported to have only 16 amino acid residues based on the conserved epitope regions of the Der f 2 and Der p 2 [13] . This increased predicted epitopes can be attributed to a bigger dataset in the prediction servers. About seven amino acids from the previous study [13] were successfully mapped and mutated, resulting in 29 out of 126 amino acids being changed into alanine, constituting about 23.02% sequence mutations relative to the original Sui p 2 gene. These mutations will potentially disrupt the IgE binding and allergenicity while increasing the immunologic profile of the hypoallergenic construct.

B-cell linear epitopes are important in producing sIgE antibodies, which evoke allergic responses[27], [28]. The hypoallergenic Sui p 2 presents a shorter epitope fragment length (1-4 amino acids) than its native counterpart (1-13 amino acids). The shortening of the epitope fragments could be an indicative aspect in the disruption of the B-cell binding of the allergen, this is important in prohibiting the production of sIgE on the allergen. Length is crucial in antibody interaction with proteins. B-cell linear epitopes usually range from 11 to 50 amino acids and are often stable at 38 amino acid residues [29].

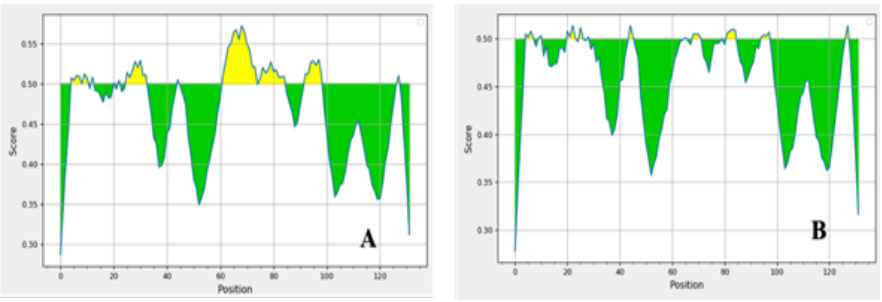

Figure 2. B-cell linear epitope prediction of the amino acid residues of the native Sui p 2 (A) and the hypoallergenic Sui p 2 (B) showing positive epitopes (yellow) and negative epitopes (green) based on scores at a threshold of 0.5.

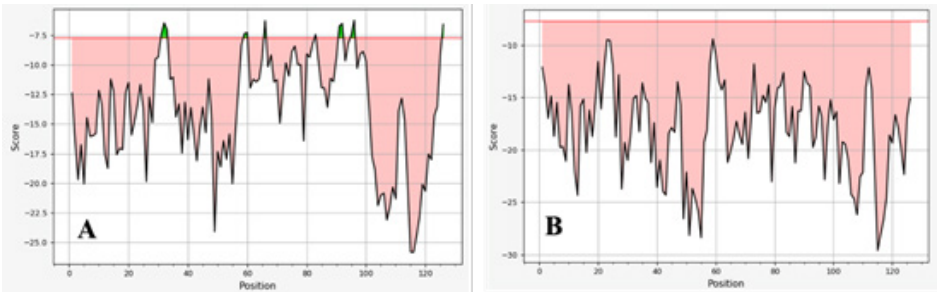

Figure 3. B-cell discontinuous epitope prediction of the amino acid residues of the native Sui p 2 (A) and the hypoallergenic Sui p 2 (B) showing positive epitopes (green) and negative epitopes (red) based on threshold score of -7.7.

Lowering the length of the epitopes results in a drastic change in their binding capacity to the immune cells [30]. The B-cell epitope prediction score of the hypoallergenic construct was lower, having an average score of 0.457, compared to the native Sui p 2, 0.465 (Figure 2).

The lowering of the prediction score can be attributed to the alanine mutations. Alanine only contains a basic and less bulky structure, and changing the sequences with alanine will eliminate the possible atomic interaction that causes antibody binding affinity beyond the beta chain [21], hence the reduction seen in the epitope prediction.

Conformational epitopes play a big role part in eliciting immune response. About 90% of the naturally occurring B-cell epitopes are discontinued [31]. Mutations on these epitopes significantly reduce the allergens' IgE binding capacity while increasing the protective IgG binding sites on the protein derivatives [32]. The hypoallergenic construct was seen to have a complete reduction in the discontinuous epitope of the hypoallergenic design. This indicates that alanine mutagenesis effectively disrupts essential epitopes and possibly hampers their allergenic potentials.

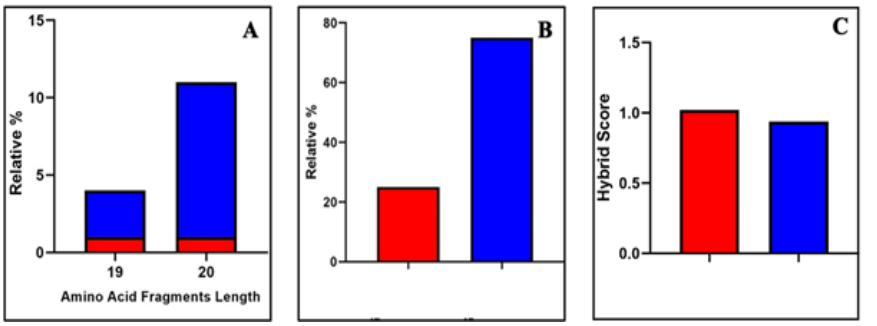

Figure 4. The IgG-producing B cell epitopes, MHC-II T-cell linear epitopes, and Allergenic protein prediction of the native Sui p 2 (red) and the hypoallergenic Sui p 2 (blue). The relative percentage of IgG-producing B-cell linear prediction (A) indicated amino acid fragment lengths at a threshold of 0.9. The relative percentages of MHC-II T-cell linear epitopes (B) were predicted using the 26 most frequent human alleles. The allergenicity prediction score (C) was calculated using the combined SVM and IgE epitope prediction scoring.

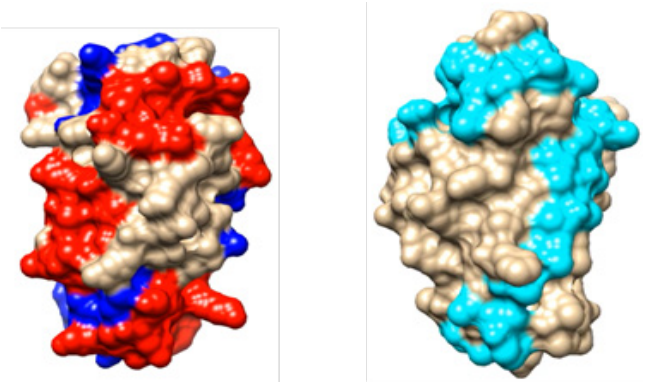

Figure 5. 3-Dimensional modeling of the native Sui p 2 (Left) and hypoallergenic Sui p 2 (Right). In the native Sui p 2 structure, the B-cell epitopes (red), while the T-cell and IgG-producing B-cell epitopes (blue) were identified. The alanine mutated residues on the hypoallergenic Sui p 2 were also highlighted (cyan).

The antibody IgG is established to have a neutralizing effect that plays an integral role in immune protection. IgG could inhibit the IgE binding sites on the allergens, making them incapable of eliciting allergic responses [33]. Increasing the IgG binding sites on the vaccine design is essential to boost the protective immune activity against allergens [34]. That is why the IgG-producing linear B-cell binding sites of the hypoallergenic Sui p 2 were increased by 7.5-fold, or about 650% (Figure 4A), relative to native Sui p 2. Even though most IgG epitopes predicted in the hypoallergenic construct were superimposed from one another, the IgG binding sites are inevitably increased relative to its original sequence. These are a promising result considering that the new construct would have greater recognition sites and potentially even antibody-specific production for the IgG, which will increase the possibility of immune tolerance against Sui p 2.

Furthermore, increased T-cell MHC-II epitopes indicate a good vaccine design, as T cells are essential for building immunologic tolerance towards the target allergens [34]. This was well observed in the relative binding fragments in Figure 4B. The T-cell MHC-II epitopes were tripled on the hypoallergenic construct, and the MHC-II binding alleles also increased, ranging from 17-25. T-regulatory cells could be activated when we increase the T-cell epitopes present, resulting in a cascading reaction, producing a protective IgG4 antibody. IgG4 neutralizes allergens and inhibits degranulation and cytokine production for the manifestation of allergic responses [35] . With this, it can be inferred that the hypoallergenic Sui p 2 design will most likely induce immune tolerance towards the native Sui p 2.

The preceding epitope mapping was further validated through the hybrid score of the allergenic protein prediction. The hybrid score was based on the combined result of the support vector machine learning (SVM) and the IgE epitope prediction of the server [25]. The allergenicity of the hypoallergenic construct was reduced by about 9.8%. Additionally, hypoallergenic Sui p 2 was non-toxic compared to the native Sui p 2, which contains toxic peptides starting from the 19th amino acid residue until the 28th (SDCSGDYCII).

Allergenicity and toxicity profile reduction are important aspects of producing hypoallergenic vaccines; this will ensure that the vaccine formulation is safe and efficacious by reducing severe allergic reactions and improving immune response [15].

Conserve regions of the native allergens are essential for formulating the hypoallergenic vaccine design. Conserved regions enable the immune system to recognize its native allergens when exposed and act accordingly without overly reacting when the hypoallergenic vaccine is introduced to the body. T-regulatory cells will be activated when the hypoallergenic vaccine is introduced instead of the classic IgE-mediated allergic Th-2 response to the allergens [36]. The Th2:Th1 shift and the interplay of T regulatory cells will condition the immune system to treat the native allergens as harmless foreign material rather than a threat [37]. This principle was integrated into the hypoallergenic Sui p 2, resulting in a high structural and sequence-based conservation of the native regions, with a TM-score of 0.9492 and 76.19% identity, respectively.. This result insinuates that even though perturbation on the native sequence of Sui p 2 was done, its identity was conserved and potentially will enable the building of immune tolerance towards Sui p 2.

The alanine mutation in the hypoallergenic Sui p 2 reduced its molecular weight by about ~1kDa relative to its native counterpart (Native: 13.52862 kDa, Hypoallergen: 12.43210 kDa). Both of the proteins are stable and have low molecular weights. The isoelectric points of the native and hypoallergenic Sui p 2 are 6.63 and 5.59, respectively. And their GRAVY scores are -0.092 for the native and 0.443 for the hypoallergenic design. These physiochemical reduction results indicated that the alanine mutations made the hypoallergenic construct much simpler and less bulky, characteristics of an ideal vaccine and a reagent for specific immunotheraphy.

Modern analysis tools, AI-based predictions, and hypoallergenic validation analyses make rational vaccine design possible. Hypoallergenic Sui p 2 was successfully formulated using alanine mutagenesis. This research contributes to the foundational research on rational formulation of hypoallergenic design.

Conclusion

This study has formulated a hypoallergenic Sui p 2 derivative, which can potentially serve as a basis for the development of a prophylactic vaccine and a reagent for specific allergen immunotherapy for HDM allergies. With the alanine mutagenesis and artificial intelligence, the mutated sequence was less allergenic, had improved immunogenic properties, and non-toxic. This study is a great avenue for exploring rational modalities of vaccine formulation, especially in constructing allergen-specific vaccines. Further in vitro and in vivo studies would be required to validate the efficacy and effectivity of the hypoallergenic Sui p 2 design.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, methodology, data collection, data analysis and interpretation, original draft preparation, review and editing of the draft, C.F.L.A. Conceptualization, review, and editing of the draft, supervision, J.D.A.R. All authors have read and agreed to the final version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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