



Design of peptides with high affinity binding to a monoclonal antibody as a basis for immunotherapy

Surendra S. Negi^a, Randall M. Goldblum^{a,b}, Werner Braun^{a,*}, Terumi Midoro-Horiuti^{b,**}

^a Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, 77555-0304, United States

^b Department of Pediatrics, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, 77555-0372, United States

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ABSTRACT

About half of the US population is sensitized to one or more allergens, as found by a National Health and Nutrition Examination Survey (NHANES). The most common treatment for seasonal allergic responses is the daily use of oral antihistamines, which can control some of the symptoms, but are not effective for nasal congestion, and can be debilitating in many patients. Peptide immunotherapy is a promising new approach to treat allergic airway diseases. The small size of the immunogens cannot lead to an unwanted allergic reaction in sensitized patients, and the production of peptides with sufficient amounts for immunotherapy is time- and cost-effective. However, it is not known what peptides are the most effective for an immunotherapy of allergens. We previously produced a unique monoclonal antibody (mAb) E58, which can inhibit the binding of multiple groups of mAbs and human IgEs from patients affected by the major group 1 allergens of ragweed (Amb a 1) and conifer pollens (Jun a 1, Cup s 1, and Cry j 1). Here, we demonstrated that a combined approach, starting from two linear E58 epitopes of the tree pollen allergen Jun a 1 and the ragweed pollen allergen Amb a 1, and residue modifications suggested by molecular docking calculations and peptide design could identify a large number of high affinity binding peptides. We propose that this combined experimental and computational approach by structural analysis of linear IgE epitopes and peptide design, can lead to potential new candidates for peptide immunotherapy.

1. Introduction

Bronchial asthma (BA) and allergic rhinitis (AR) are common chronic respiratory diseases that have had dramatic increases in prevalence and severity during the late 20th century. This trend is still ongoing [1–5] and affect up to 50 % of certain populations [6,7]. Despite improvements in clinical diagnosis and management, allergic diseases remain a leading cause of work and school absences [8–11]. In the US population, AR is a leading cause of medical treatment (direct) and secondary societal (indirect) costs, estimated up to \$15 billion/year in 2008. BA caused another \$82 billion/year in 2013 [12]. In addition, most of the patients with BA also have AR with seasonal worsening, which often exacerbates their BA. A recent National Health and Nutrition Examination Survey (NHANES) study indicated that 45 % of the US population is sensitized to one or more allergens and that grasses, dust mites, and ragweed are the most common allergens [13]. The pollen of various

trees, weeds, and grasses are responsible for many of the allergic reactions that are triggered when people are outdoors during specific seasons.

Currently, the most common treatment for AR is the daily use of oral antihistamines, which can control some of the symptoms (itchy nose and eyes, runny nose, and sneezing), but are not effective for nasal congestion, which can be debilitating in many patients [14]. Intranasal or oral sprays containing glucocorticoids and anticholinergics are often taken to reduce the congestion, as well as the other symptoms of ongoing allergic inflammation. However, these are often used improperly, may take weeks to become fully effective, and can have severe side effects. Current immunotherapy for established allergic diseases is limited by the need for numerous injections or ingestion of the putative allergens, over a period of 3–5 years, and patients can also have severe anaphylactic reactions. This approach has a high direct and indirect cost, in terms of time and inconvenience.

* Corresponding author at: Department of Biochemistry and Molecular Biology, 301 University Blvd, Research Building 6, Rm 5.422, University of Texas Medical Branch, Galveston, TX, 77555-0304, United States.

** Corresponding author.

E-mail addresses: webraun@utmb.edu (W. Braun), tmidoro@utmb.edu (T. Midoro-Horiuti).

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Peptide immunotherapy is a promising new approach to treat allergic airways diseases [15–17]. The use of fragments of an allergen, instead of the whole allergenic protein as an immunogen for immunotherapy has several advantages. The small size of the immunogens prevents an unwanted allergic reaction of sensitized patients, while the production of peptides sufficient for immunotherapy, is time- and cost-effective. Previous studies with epitope peptide immunotherapy from grass or birch pollen allergens have shown that T- or B-cell epitope peptides of allergens can induce blocking IgG antibodies (Abs) in humans and thus, prevent an allergic reaction [18–20]. However, it is not known which peptides are most effective for an immunotherapy of allergens. We suggest here a combined experimental and state of the art computational approach to find novel peptide immunogens and assess their potential for immunotherapy by examining the binding affinity of the peptides to mAb E58 (Fig. 1).

We have tested our approach on data for two allergenic pectate lyases (PL) from cedar trees (Jun a 1) and ragweed (Amb a 1) pollens [21–25]. PL forms a family of major allergens [26] that are the main elicitors of respiratory allergies in global regions. Our research group has isolated, cloned, sequenced, and identified IgE epitopes of the two major allergens in mountain cedar pollen, Jun a 1 and Jun a 3 [21–23, 27,28]. We resolved the crystal structure of the dominant allergen Jun a 1 with a resolution of 1.8 Å [29–32]. We also produced a unique mAb E58, which can inhibit the binding of multiple groups of mAbs and human IgEs from patients affected by the major group 1 allergens of ragweed and conifer pollens, e.g. Amb a 1, Jun a 1, Cup s 1, and Cry j 1 [33]. We demonstrated here that the combined approach using two linear E58 epitopes on Jun a 1 and Amb a 1 as found by overlapping synthetic peptides for Jun a 1 and its sequence alignment with Amb a 1, and computational improvements by molecular docking and peptide design of these epitopes identified large number of high affinity binding peptides that exceed the binding affinities of these two linear E58 epitopes.

2. Material and methods

2.1. Synthesis of overlapping peptides of Jun a 1

In order to initiate the epitope analyses of E58 on Jun a 1 and Amb a 1, we synthesized an array of 167 overlapping peptides of Jun a 1 (15 mer peptides with 13 amino acid overlap, JPT, Berlin, Germany).

2.2. Anti-conformational monoclonal antibodies G1-4

Four Jun a 1 specific mAbs G1-4 have been purified and structurally characterized as we described previously [30]. Briefly, we produced a

panel of mouse IgG mAbs and selected those that (1) reacted with native but not denatured Jun a 1 and (2) were inhibited from binding to Jun a 1 by human sera, indicating that these mouse IgG antibodies recognize conformational structures that are also recognized by IgE antibodies in sera from patients highly sensitized to mountain cedar pollens. Further competition experiments with human IgE antibodies showed that the selected four mAbs G1-4 recognize different epitopes, which are also recognized by human IgE.

2.3. Production, screening, and characterization of E58

All of the mouse experiments were approved by the Institutional Animal Care and Use Committee at UTMB. Jun a 1, Cup s 1 and Cry j 1 were purified as we described previously [21]. Mouse IgE monoclonal antibody (mAb) E58 was induced in BALB/c mice by subcutaneous injections of Jun a 1 protein, as described [34]. Briefly, female BALB/c mice were immunized intraperitoneally with 10 µg of purified native Jun a 1 protein adsorbed on 2.25 mg alum (Imject Alum; Pierce, Rockford, IL) two times. One week after the last immunization, mice were euthanized, spleens were removed and mononuclear cells were fused with FO cells (SP2/0 myeloma cells, ATCC) according to a standard protocol [35]. Supernatant from individual wells, containing a single focus of growing hybridoma cells were tested for antibodies to Jun a 1 by ELISA, using 2 µg/mL of Biotinylated-anti mouse IgE as the detector (BD Pharmingen, San Jose, CA) and Streptavidin-horseradish peroxidase (HRP, Zymed) and 3,3',5,5'-Tetramethylbenzidine (KPL, Gaithersburg, MD) as substrates. Hybridoma cells in wells that tested positive were subcloned at least 3 times, by limiting dilution.

2.4. Epitope mapping of E58

The mAb E58 was purified from the culture supernatant of hybridoma in serum free AIM-V medium (ThermoFisher Scientific) using Jun a 1 affinity column [33]. The mAb E58 (0.01, 0.1, 1, and 10 µg/mL) was incubated for 1 h at 30 °C on multiwell microarray slides. Secondary biotinylated anti-mouse-IgE (BD Pharmingen) at 1 µg/mL was incubated for 1 h, subsequently with Cy5-Streptavidin conjugate (Jackson ImmunoResearch) at 0.1 µg/mL for 1 h. After washing and drying, the slide was scanned with a high-resolution laser scanner at 635 nm to obtain fluorescence intensity profiles. Resulting images were quantified to yield a mean pixel value for each peptide.

2.5. Design of E58 epitope variants

2.5.1. Molecular modeling of the E58 single chain antibody (ScAb)

In the absence of a E58 ScAb crystal structure, we used two different

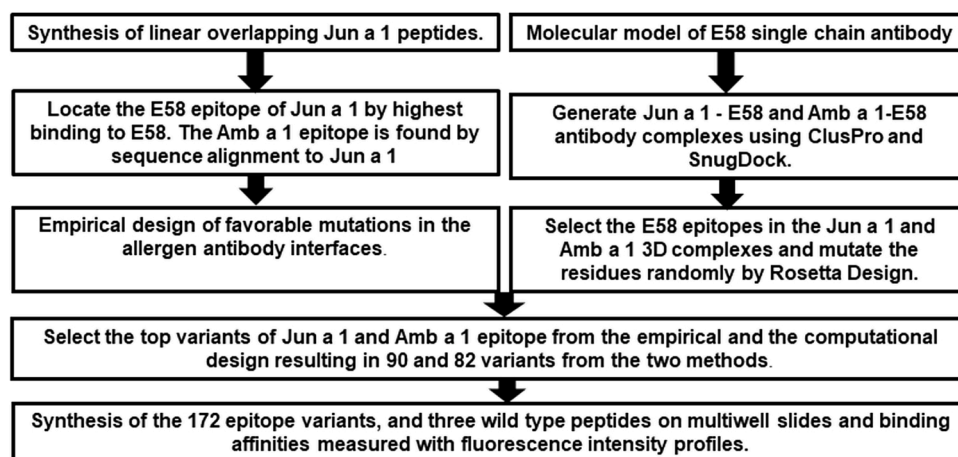


Fig. 1. Flow chart showing the design of high affinity variants of E58 epitopes.

molecular modeling programs: MPACK [36] and RosettaAntibody [37] to build a 3D model structure of E58 ScAb. In the MPACK modeling, both heavy (H) and light (L) chain sequences of E58 ScAb were first submitted to the fold recognition server HHPRED [38], and the best 3D templates selected for the H chain (PDB id 1I9R, 81 % sequence ID) and the L chain (PDB id 2AEQ, 86 % sequence ID) were used to build the model structures of E58 ScAb H and L chains. To build a complete E58 ScAb structure, the model and template structures were structurally aligned (RMSD: ~ 1 Å) and the coordinates for both H and L chains of E58 ScAb were saved. The final structure was energy minimized using NAMD [39]. In RosettaAntibody modeling, the sequence of E58 was submitted to a RosettaAntibody web-server (<https://rosie.graylab.jhu.edu/>) and a best model structure was selected. A structural alignment between the E58 ScAb model structures obtained using MPACK and RosettaAntibody revealed that the positions of amino acids in the complementarity determining regions (CDR) of E58 antibody are almost identical (RMSD 1.2 Å). We also checked the quality of both structures using the QMEAN program [40], and found that the RosettaAntibody model structure score (0.78 ± 0.06) was slightly better than MPACK (0.77 ± 0.06) compared to a perfect score of 1.0. Since, the RMSD value is very low, we selected the model structure obtained from RosettaAntibody for our rigid body docking simulation.

2.5.2. Molecular docking

In order to predict the molecular interactions between Jun a 1 and E58 ScAb, a rigid body docking simulation was performed using the ClusPro webserver [41–43]. During the docking process, the E58 ScAb structure was held in a fixed position while the residues in the CDR loop region of E58 ScAb were allowed to interact with freely rotating Jun a 1 structure. We obtained thirty docked structures of the Jun a 1-E58 ScAb complex. The amino acids at the complex interface were obtained by calculating a change in solvent accessible surface area of amino acids upon complex formation, and the frequency of amino acids at the interface. Using this strategy, we obtained ten docked structures that include the previously determined epitopes 2 and 3 of Jun a 1 [28] at the Jun a 1-E58 interface. We obtained two clusters of docked conformations: The first cluster contains amino acids present in the epitope site 2 and 3, and the second cluster contains amino acids present in the N-terminal region of epitope 2. Finally, one structure from each cluster that contains the maximum number of amino acids in the epitope region was selected for the Jun a 1 peptide design. The docked structure obtained from ClusPro was further docked using SnugDock [44,45] docking program to remove any clashes that may arise from rigid body docking. For each input structure, SnugDock generated several docked structures which were further clustered based on RMSD values. The process was repeated for top ten ClusPro docked structures to get twenty docked structures of Jun a 1-E58 complex (ten each cluster 1 and cluster 2).

2.5.3. Peptide design

The Rosetta Design software [46] was used for the Jun a 1 peptide design by using the co-ordinates of the Jun a 1 wild-type peptide in the context of the Jun a 1/E58 complex. The Jun a 1 peptide-E58 docked structure was refined using the relax protocol implemented in Rosetta Design and then the fixed backbone (fixbb) protocol was used to mutate all amino acids in the peptide sequence to another residue type. The fixbb protocol uses side chain packaging algorithm to search for the rotamer space using the Dunbrack rotamer library. For each run, we generated thousand structures. The structures were sorted based on the Rosetta score, and the sequences of the top hundred structures with the lowest Rosetta scores were analyzed using WebLogo (<https://weblogo.berkeley.edu/logo.cgi>). The process was repeated for all twenty docked structures to generate a large set of Jun a 1 peptide sequences. We have also designed E58 epitope variants for Amb a 1, as described above. Using these strategies, we designed a total of 82 from the Rosetta analysis. We combined these peptides with 90 peptides from the

empirical design by single and double mutations of the linear WT epitopes for the multiwell microarray analysis (see below).

2.6. Validation of the E58 epitope variants

The binding of the mAb E58 to these variants and wild-type (WT) peptides were analyzed with synthesized peptides on multiwell microarray slides as described for 2.2 above. We considered the peptides which exceed the binding affinities of the WT peptides as examples of a successful design process.

3. Results

3.1. Linear epitope mapping of mAb E58 and production of Abs to E58 WT epitopes

We synthesized an array of 167 overlapping linear 15-mer peptides with 13 amino acid overlap of Jun a 1 and determined the linear peptide Q²²¹FGPNAGQRMPPR²³⁵, denoted as E58JunWT, as the peptide with the highest binding affinity to E58. The E58 epitope of Amb a 1, T²⁶⁵FTDNVDQRMPCRH²⁷⁹ (E58AmbWT), was found by sequence alignment between Jun a 1 and Amb a 1. The aligned sequences of Jun a 1 and the two isoforms of Amb a 1 were used to map the location of the epitope on Amb a 1 based on the equivalent position of the experimentally determined epitope in Jun a 1.

We synthesized these peptides, conjugated with keyhole limpet hemocyanin (KLH) and immunized rabbits to see if these peptides induce E58-like Abs. We obtained two polyclonal IgG Abs, anti-E58JunWT and anti-E58AmbWT. We first tested the effects of preincubating Jun a 1 or Amb a 1-coated wells with anti-E58JunWT and anti-E58AmbWT on the subsequent binding of each of four groups of mAbs (G1-4) each of which recognize unique conformational epitopes on Jun a 1 [30]. These IgG Abs bound to native Jun a 1 and Amb a 1 and also had the ability to inhibit the hIgE and anti-conformational mAb G1-4 binding to cedar and ragweed allergens (Fig. 2). The inhibitory effect of these IgGs is promising: the preincubation of anti-E58JunWT to Jun a 1 inhibited hIgE binding to Jun a 1 9–60 %, and preincubation of anti-E58AmbWT to Amb a 1 inhibited hIgE binding 0–32% but needs further optimization for peptide immunotherapy. We proceeded to optimize the linear E58 epitopes with the computational design to generate peptides with higher binding affinities than the WT peptides, E58JunWT and E58AmbWT, to E58.

3.2. Conformational E58 Epitope of Jun a 1

In order to predict the molecular interaction between Jun a 1 and E58 Fab, we generated a complex structure of Jun a 1/E58 ScAb using two different docking programs ClusPro and SnugDock as described in Section 2.5.2. Subsequent analysis of the docked structure predicted two E58 ScAb potential binding sites on the Jun a 1 surface. In one of the binding modes E58JunWT is near the Jun a 1/E58 interface (Fig. 3a), which shows that docking calculations identified a promising binding area for the conformational E58 epitope (Fig. 3b). This Jun a 1/E58 complex was then used as a basis for modifying amino acid residues in the linear peptide E58JunWT. We did not use energy as a selection criterion, instead we used the consistency with the spatial location of the WT epitope of Jun a 1 with the previously identified linear epitope in the docked structures as a selection criterion. Residues in the Jun a 1/E58 interface were then used for modifying residues in E58JunWT and predict the effect on the binding affinity by an empirical approach using positive and negative design criteria (see Section 3.3) and an automated computational design of linear peptide/E58 ScAb complexes with Rosetta (see Section 3.4).

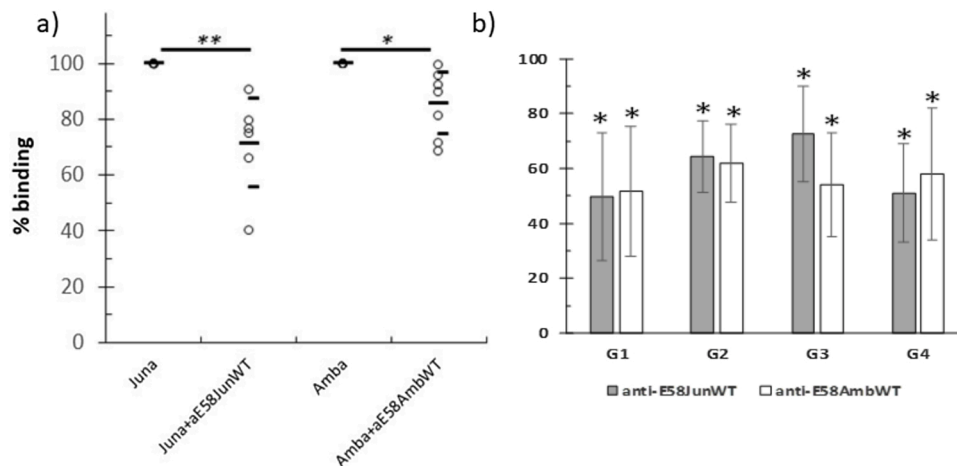


Fig. 2. Anti-E58JunWT and anti-E58AmbWT Abs inhibit hIgE binding to cedar and ragweed allergens (A) and mAb G1-4 binding to cedar (B). (A) Preincubation of anti-E58JunWT and anti-E58AmbWT Abs (20 µg/mL) partially inhibited the binding of hIgE Abs from subjects sensitized to cedar and ragweed, N = 7, mean ± SD, **: $p < 0.001$, *: $p < 0.01$. (B) Preincubation of anti-E58JunWT and anti-E58AmbWT Abs (20 µg/mL) inhibited the binding of mAb G1-4 mAbs to cedar extract. N=3, mean ± SD.

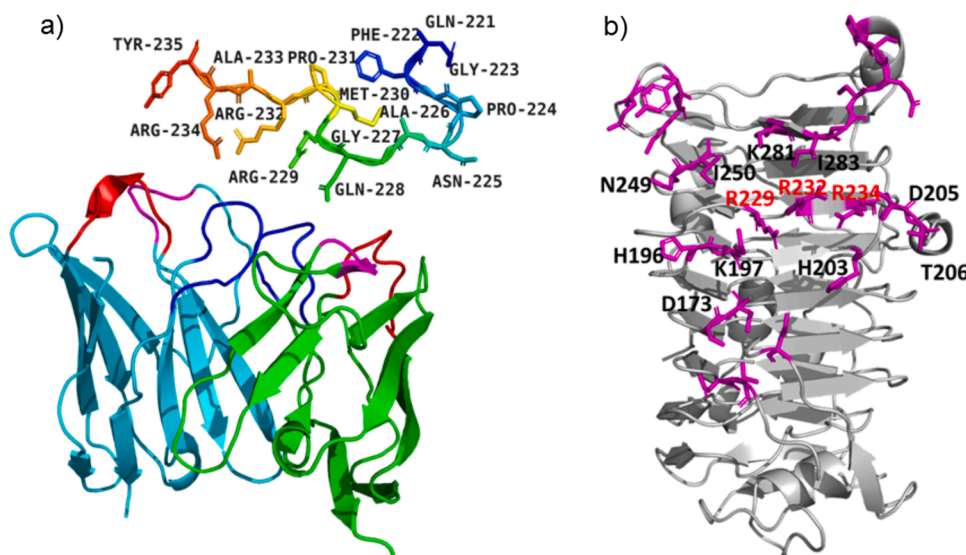


Fig. 3. Interface of the Jun a 1/E58 Fab complex obtained by rigid body docking. a) Residues of the linear epitope E58JunWT in the context of the 3D complex of the Jun a 1/E58 Fab, colored blue to red from the N-terminal to the C-terminal the peptide. The two chains of the E58 Fab structure are shown in green and light blue, and the CDR loop regions on the E58 Fab structure are shown in red, magenta and blue color. The interaction between E58JunWT is mostly dominated by the charged and polar residues (Gln228, Arg229, Arg232, and Arg234). b) 3D structure of Jun a 1 with marked residues that are in contact with E58 (magenta). The residues in red color are part of the E58JunWT peptide.

3.3. Empirical design of the WT peptides identifying the residues in the Jun a 1 and Amb a 1 E58 interface

To understand the importance of individual residues in the peptides for the E58 interaction, we made single and double mutants of E58JunWT and E58 AmbWT and predict their potential effect on binding based on physico-chemical properties such as charge, hydrophobicity or hydrogen bonding. As a positive design we changed the residues in the linear epitopes to increase potentially the binding affinity in the complex. Examples for a positive design are to mutate residues to amino acids if they can form new hydrogen bonds or increase hydrophobic interactions in the complex. Another basis for a positive design are the modified peptides of Jun a 1 that were measured in a previous study how well they are recognized by sera from Jun a 1 allergic patients [47]. For example, the peptide E58JunWT overlaps with the two linear epitopes 2 and 3 (Tables 1 and 2) identified in that study. Mutations in epitopes 2 and 3 with higher binding affinities relative to the WT in that study were then also included in E58JunWT. As a negative design we wanted to reduce binding of mutated E58JunWT or E58AmbWT peptides to E58. To test those residues we mutated polar and charged amino acids to the nonpolar residue A (both single and double mutants) to reduce binding. We also used our previously established physico-chemical property distance PD [47] as a criterion for a positive or negative design. The PD distance measures the similarity of two

Table 1

Variants of E58JunWT with normalized binding affinities greater than 2.0 to E58 are listed. The WT sequence is also included as a reference. Amino acids shown in bold are mutated from wild type, where Score is the normalized binding affinity defined as the ratio of the signal intensities of the variant peptides to the wild-type peptide.

Peptide No.	Sequence	Score	Description
66	QFGPNAGQRM ~~PR~~ FRY	4.20	From epitope 3 peptide 9
93	QFGPNIGQRM ~~PR~~ IY	3.20	Jun a 1 peptides based on PD (E1) vector distance
55	QFGPNAGQRY ~~PH~~ PRY	2.80	Rosetta Design Peptide 2
53	QFGPNAG ~~RD~~ YPRY	2.70	AMB A 1 based on PD (E1) distance vector
101	QFIPNAIQRM ~~PR~~ ARY	2.30	Jun a 1 peptides based on PD (E1) vector distance
63	QFGPNAGQRM ~~PR~~ ARF	2.30	From epitope 3 peptide 6
102	QFGPNAGQRL ~~PR~~ ARY	2.10	Jun a 1 peptides based on PD (E1) vector distance
12	QFGPNSAQRM ~~PR~~ ARY	2.10	Double A/S mutations
24	QFGPQAGQRM ~~PR~~ ARY	2.10	Test of interacting contacts in Jun a1/Ab
1	QFGPNAGQRM ~~PR~~ ARY	1.00	Jun a 1 WT

Table 2
High affinity binding variants of E58AmbWT.

Peptide No.	Sequence	Score	Description
152	TFTINVIQRMPCRHR	2.90	Amb a 1 based on PD (E1) distance vector
69	TFRDGVQRMPCRHR	2.80	Peptide group 1
89	TFTDNVQSRMPCRHR	2.70	Peptide group 2
153	TFTDIVDQRMPCRHR	2.70	Amb a 1 based on PD (E1) distance vector
85	TFTGNVEQRMPCRHR	2.50	Peptide group 2
86	TFTGNVQRMPCRHR	2.50	Peptide group 2
158	TFTDNVDIRMPCRHR	2.40	Amb a 1 based on PD (E1) distance vector
150	TFTDNVDQRMPCRHR	2.00	Amb a 1 based on PD (E1) distance vector
68	TFTDNVDQRMPCRHR	1.00	Amb a 1 WT

peptides by the physical-chemical properties of the 20 amino residues. The PD value between two identical peptides is 0, and peptides with conservative substitutions have a low value typically less than 4. We correlated PD values of peptides with their experimentally determined binding affinities to patient sera, and found that peptides with PD values to the WT epitopes of Jun a 1 of less than 6 have a high probability to bind with a similar affinity as the wild type epitope [47]. For the peptide design we chose one component of the PD, the differences of the E1 quantitative descriptors [48–50] as a criterion for the design. We mutated residues in E58JunWT and E58AmbWT to residues with a difference in their E1 less than 5 in the positive design, and to residues with a difference greater than 15 in the negative design. Examples are mutations M to L, A to T, Q to N and P to G as a positive design and A to I or G to I as a negative design. These suggested mutated linear sequences of the WT epitopes resulted in 90 peptides for the assessment by the experimental binding assay with E58.

3.4. Linear E58JunWT and E58AmbWT docking to ScAb and computational design with Rosetta

We selected only the linear WT peptide sequence in the two complex structures of Jun a 1 and Amb a 1 with the mAb E58 that overlaps with the previously identified epitope sites 2 and 3 [28], and refined it further using SnugDock to optimize side chains and hydrogen bonds. The sequences of the peptides were then further modified by introducing random mutations in the E58JunWT peptide using the ROSETTA software. Based on the ROSETTA score, we obtained 27 peptides that are predicted to bind strongly to mAb E58. A similar approach was used for the E58AmbWT peptide, resulting in 55 predicted peptide sequences.

3.5. Analysis of the binding affinity of the predicted E58JunWT and E58AmbWT variants

We selected the top predicted E58 binding peptides for Jun a 1 and Amb a 1 peptides, found by the empirical and the computational design approach for the experimental assessment, and identified 43 variants of E58JunWT for Jun a 1 and 35 for Amb a 1 with a higher binding affinity than the WT peptides (Fig. 4). The top high affinity binders with a 2-fold higher affinity than the WT peptides are given in Tables 1 and 2 with an indication of mutated residues (**in bold font**) and an explanation of the applied approach for that peptide.

Comparing the top high affinity binding peptides suggested the importance of three conserved R residues. The importance of these R residues were also confirmed by the negative designs. Mutation of the R to an A reduced binding, as expected. Interestingly, also an R to K substitution resulted in reduced binding. This might be due to the unique property of the guanidinium group of R in comparison with K for making several hydrogen bonds, which is well established as a preference of R compared to K for protein stability [51]. Exchanging the polar residues Q and N, typically increased binding affinity. A dramatic increase of the binding affinity was observed for a substitution of the A residue next the two Rs in RAR to RFR which could be explained by a potential cation- π interaction that stabilizes the conformation of the peptide. In general, the data set identified the sequence RMPRAR as an important binding motif.

4. Discussion

Although peptide immunotherapy for allergens has been previously proposed [19,20], the computational design of novel peptides for the treatment of allergies has not previously been done. The design process consists of three challenging steps, which includes the precise docking of the allergenic protein and flexible peptides to a known Ab structure, the computational modification of the peptide sequence for increased binding to the Ab, and the choice of criteria to select optimal binding peptides and the necessary facilities for the experimental validation. The individual steps have been explored by others in drug and protein designs [52–54], the identification of epitopes and antigen-Ab interactions of influenza, human immunodeficiency and dengue viruses [55–57], and the design of anti-microbial [58], anti-cancer [59] peptides and the design of recombinant allergens for immunotherapy [60]. Particular challenging problems in the design process are the flexibility of the peptide backbone and the CDR loops of the Abs in the docking calculation [61–64], and the sampling of the sequence space in the design process [65–67]. Our study showed that computational design approaches are promising in the design of peptides for allergen specific immunotherapy.

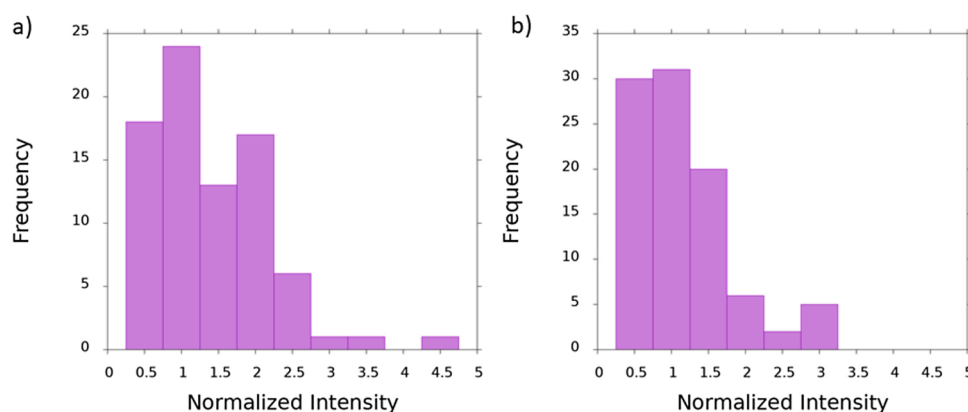


Fig. 4. Number of peptides as a function of binding affinities of all peptide variants for Jun a 1 (A) and Amb a 1 (B). The binding affinities were normalized to the WT values. Thus, peptides with normalized intensities >1 bind better than E58JunWT or E58AmbWT. The number of the peptides are plotted using a bin size of 0.5.

As in the case of infectious diseases, specific immunotherapy (SIT) is the only potentially curative treatment approach in allergic diseases. However, traditional SITs are limited by the need for weekly and then monthly injections of the crude extract of whole allergens over a period of 3–5 years, and also have the potential for life-threatening anaphylactic reactions, due to cross-linking pathogenic IgE by the allergen. New approaches have been attempted to use T-cell peptide vaccines to modulate T-cell response, while avoiding the pathogenic IgE stimulation [68]. However, in a phase 3 study of cat allergy and a phase 2b study in house dust mite allergy, this approach failed to demonstrate any significant benefits [16,69]. The importance of blocking IgG responses to reduce allergenicity was also demonstrated for the cat allergen Fel d 1 by pre-selected monoclonal antibodies with subnanomolar affinities in mechanistic and clinical studies [70].

The peptides we reported here have the potential to be peptide vaccines, which can be injected or taken sublingually. These peptides will induce the patient's own antibodies, which prevent these allergens from binding to their own pathogenic IgE antibodies. To test this hypothesis we will in future work use the designed high affinity binding peptides in conjugation with KLH to induce E58-like Abs in immunized rabbits. The sera from the rabbits can then be tested for binding to native Jun a 1 and Amb a 1 and for inhibiting human IgE binding to cedar and ragweed allergens. Previous research work by others have shown that this is a feasible approach. For example, peptides of the cat allergen Fel d 1 fused to a hepatitis B virus derived PreS domain exhibited more than 1,000 fold reduction in allergenic activity in a basophil activation test [71]. A recombinant peptide-based vaccine for grass pollen allergens was also successfully developed by a similar approach [19,72].

5. Conclusion

We identified many variants of the linear WT E58 epitopes of Jun a 1 and Amb a 1, which have a higher binding affinity than the originally selected linear peptides from overlapping WT segments of the allergens. The best binders and the empirical design approach also suggested the partial sequence RMPRAR as an important binding motif. We suggest that these peptides have promising potential as therapeutic agents for seasonal pollen allergies.

CRediT authorship contribution statement

Surendra S. Negi: Conceptualization, Investigation, Writing-First draft and Editing. **Randall M. Goldblum:** Conceptualization. **Werner Braun:** Project Administration, Methodology, Writing Reviewing and Editing. **Terumi Midoro-Horiuti:** Investigation, Data Curation, Writing- First draft and Editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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