



RESEARCH ARTICLE

REVISED Human Proteinase 3, an important autoantigen of c-ANCA associated vasculitis, shares cross-reactive epitopes with serine protease allergens from mites: an *in silico* analysis

[version 2; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background: In autoimmune vasculitis, autoantibodies to Human Proteinase 3 (PR3), a human serine protease, seems to have a role on the inception of c-ANCA associated vasculitis. The origin of this autoreactive response remains unclear. However, for several autoreactive responses, molecular mimicry between environmental antigens and human proteins is key to trigger autoantibodies and finally autoimmunity manifestations. Considering that PR3 is a serine protease and house dust mite (HDM) group 3 allergens share this biochemical activity, the aim of this study was to identify cross-reactive epitopes between serine proteases from human and mites using an *in silico* approach.

Methods: Multi alignment among amino acid sequences of PR3 and HDM group 3 allergens was performed to explore identity and structural homology. ElliPro and BepiPred *in silico* tools were used to predict B and T cell epitopes. Consurf tool was used to conduct identification of conserved regions in serine proteases family.

Results: PR3 and HDM group 3 allergens shared moderate identity and structural homology (root mean square deviation < 1). One B cell cross reactive epitope among serine proteases was identified (29I, 30V, 31G, 32G, 34E, 36K, 37A, 38L, 39A and 54C) and two T cell epitopes.

Conclusions: PR3 have structural homology and share cross reactive

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Approval Status ? ? ✓

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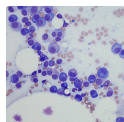
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epitopes with HDM group 3 allergens.

Keywords

Serine proteases, human proteinase 3, house dust mites group 3 allergens, ANCA associated vasculitis, sequence homology, T and B cell epitopes, cross-reactivity, epitope modelling



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This article is included in the **Bioinformatics** gateway.

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REVISED Amendments from Version 1

The manuscript was revised and refined by a language style corrector. Some sentences in the methods and results section were improved giving more detail and explanations in accord to revisor suggestions.

Any further responses from the reviewers can be found at the end of the article

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a life-threatening autoimmune disease affecting small vessels, compromising the respiratory mucosa, skin, lung, and the kidney¹. This group of small vessel vasculitis includes various diseases: granulomatosis with polyangiitis, microscopic polyangiitis, kidney-limited vasculitis and Eosinophilic granulomatosis with polyangiitis, all of them having in common some degree of autoimmune response to the Human Proteinase 3 protein (PR3). Previous studies have shown that autoantibody binding to PR3 expressed on the neutrophil surface may activate its degranulation, eliciting tissue damage in small vessels and their irrigated organs. Also, while proinflammatory effector T cells have been implicated in vasculitis pathogenesis², a specific PR3 T cell epitope has not been reported in AAV patients³. PR3 is a serine protease physiologically expressed in human neutrophils. Due to its enzymatic activity, it degrades various intercellular gap-junction proteins and collagen and may play a role in neutrophil transendothelial migration. In addition, this protein is an important autoantigen in AAV, and sera from patients with severe and relapsing forms of the disease can bind it in IgG ELISA assays^{4–6}. Further, although a cause-effect relationship between PR3-autoantibodies and vasculitis is not clearly defined, animal models support a pathogenic role^{7,8}, revealing that they may be involved in disease inception, progression and severity¹.

Environmental exposures, specially to microbial components mimicking self-antigens have been proposed as triggers of autoimmunity^{9,10}. Also, in AAV, it has been proposed that an endogenous immune response to a complementary protein to PR3 autoantigen could be implicated in disease inception, and this antisense protein harbors homology to various bacterial peptides¹¹. PR3 crystal structure has been elucidated, and various epitopes are recognized by patients suffering AAV; however, its cross-reactivity with environmental antigens is poorly studied^{12–14}.

Previous studies have shown that specific IgE to some self-proteins have been identified in autoimmune and allergic diseases like lupus, urticaria, dermatitis, allergic pulmonary aspergillosis and have a strong association with disease activity^{15–18}. Some allergens can cross-react with human proteins and participates in autoimmunity inception in pemphigus vulgaris by a “hit-and-run” mechanism, opening the theoretical possibility for a similar mechanism to occur in another autoimmune disease such as AAV^{19–22}.

In the tropics, house dust mites (HDM) are important ubiquitous allergen sources and exposure is perennial, increasing the possibilities of exposure in the general population²³, and IgE sensitization to their components^{24,25}. Sensitization to HDM group 3 allergens is common²⁶, as they harbor serine protease activity and conserved structural homology²⁷, making them potential PR3 cross reactive antigens; this has not been explored before. Here, we show *in silico* data suggesting cross-reactivity and epitope sharing between PR3 and HDM group 3 allergens.

Methods

Searching homologous with BLAST (Basic Local Alignment Search Tool)

The amino acid sequence from the human PR3 (Uniprot accession: [P24158](#)) was used as query to perform a search for serine protease homologous reported in allergenic sources: *Dermatophagoides pteronyssinus* (Der p 3: Accession number [P39675](#)), *Blomia tropicalis* (Blo t 3: [A1KX11](#)), *Glycyphagus domesticus* (Gly d 3: [Q1M2M8](#)), *Lepidoglyphus destructor* (Lep p 3: [Q1M2L7](#)) and *Tyrophagus putrescentiae* (Tyr p 3: [C6ZDB5](#)) with the [PSI-BLAST](#) tool. Parameters were set as default.

Multiple alignment

Identity among all allergenic sequences homologous to PR3 was analyzed using the [Jalview tool](#) 2.11.0²⁸. First, all allergens and human PR3 codes were used as inputs in the Jalview tool. Second, the [T coffee tool](#) was chosen to assess alignment. Third, alignment was displayed as an identity percentage.

Construction of 3D model

The 3D model of Der p 3, a serine protease of *Dermatophagoides pteronyssinus* was generated by homology in the [SWISS-MODEL](#) server using the zymogen catalytic region of human MASP-2 (PDB: 1zjk f) as a template. The 3D model of Der p 3 was loaded into the ProSA-web server²⁹, which was used to analyze its quality.

The model was refined in [DeepView](#) v4.1 (energy minimization and rotamer replacements). Its quality was evaluated by several tools, including Ramachandran graphs, WHATIF, QMEAN4 index, and energy values (GROMOS96 force field). For the validation of the Der p 3 structure we used the Minimize Structure option in the UCSF Chimera software, a procedure that adjust the energy and reduce the entropy of the model³⁰.

Three-dimensional structure (PDB: [1FUJ](#)) of the human PR3 serine protease was retrieved from the Protein Data Bank. A cartoon model was created using [Pymol software](#) v2.4. Root median square deviation (RMSD) value between Der p 3 and PR3 was calculated using [Chimera software](#) v1.0³⁰.

B and T cell epitope prediction

[ElliPro](#) v3.0 and [BepiPred](#) v2.0 tools were used to predict B and T cell epitopes on Der p 3^{31,32}. With ElliPro, the 3D structure of Der p 3 was used to predict epitopes. Minimum score and maximum distance (Angstrom) were set to 0.5

and 6, respectively. Epitopes with high conserved rates were visualized in the 3D model. For prediction using BepiPred, an amino acid sequence of Der p 3 was used as input.

Conservation analysis

The 3D structure of Der p 3 was submitted to the [ConSurf server](#) to generate evolutionarily related conservation scores to help to identify functional regions in the proteins. HMMER algorithm, 1 iteration, E-value cutoff (0.0001) and UNIREF-90 database was set as default to generate multiple alignment, prior to evolutive analysis. All amino acid sequences in FASTA format were used.

Results

Human PR3 and HDM group 3 allergens exhibited identity and features of the serine protease family

BLAST search identified various serine protease family members from HDM as homologous. The multiple sequence alignment analysis showed that Der p 3, Blo t 3, Gly d 3, Lep d 3 and Tyr p 3 allergens shared 45% of identity in their amino acid sequences with PR3. The most conserved region is located between residues 53 to 75, indicating the existence of molecular mimicry ([Figure 1](#)). Among the members of HDM group 3

allergens, an identity until 41% was reported ([Table 1](#)), and a highly conserved region between residues 40 to 90 was found. When identity between PR3 and each allergen used in study was analyzed, a moderate level of identity was found (30%) ([Table 1](#)).

A structural model of Der p 3 was obtained by homology modelling using the 3D structure of PR3 reported in the PDB database. According to modelling, the Der p 3 tertiary structure exhibited a typical fold of serine protease family, conformed by four α -helices and fifteen β -strands with structural homology with PR3 (RMSD = 0.8) ([Figure 2](#)).

T and B cell cross-reactive epitopes were predicted between HDM group 3 allergens and PR3

Using ElliPro and BepiPred servers, a cross reactive B cell epitope was predicted on all serine protease used in this study. This epitope is formed by ten residues and is on the N-terminal region, spanning amino acids 29 and 39 with a surface area of 470 Å², not forming part of any domain within the protein. Conservative analysis indicated that the antigenic region predicted was highly conserved in the serine proteases ([Figure 3](#)). According to ConSurf analysis, the region covering

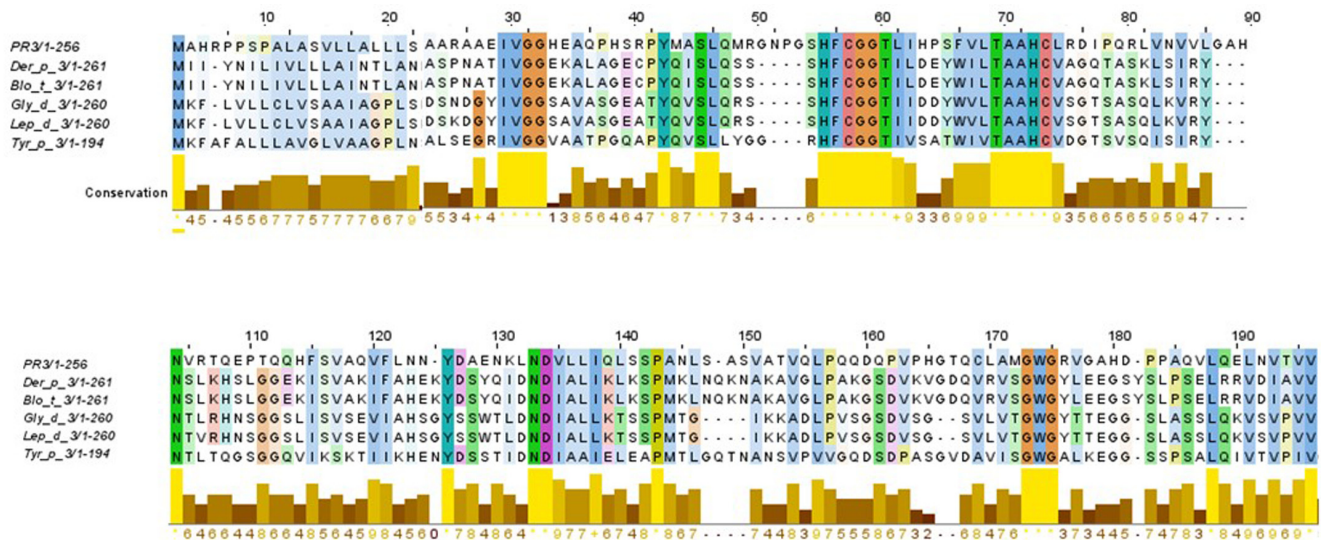


Figure 1. Multiple alignment among PR3 and the HDM allergens belonging to group 3. An identity of 45% in their amino acid sequences was found.

Table 1. Identity matrix among serine proteases used in study. All comparisons of PR3 with HDM group 3 allergens showed a moderate identity.

	PR3	Der p 3	Blo t 3	Gly d 3	Lep d 3	Tyr p 3
PR3	100	33	27	30	30	27
Der p 3	33	100	48	52	53	43
Blo t 3	27	48	100	58	58	47
Gly d 3	30	52	58	100	99	41
Lep d 3	30	53	58	99	100	41
Tyr p 3	27	43	47	41	41	100

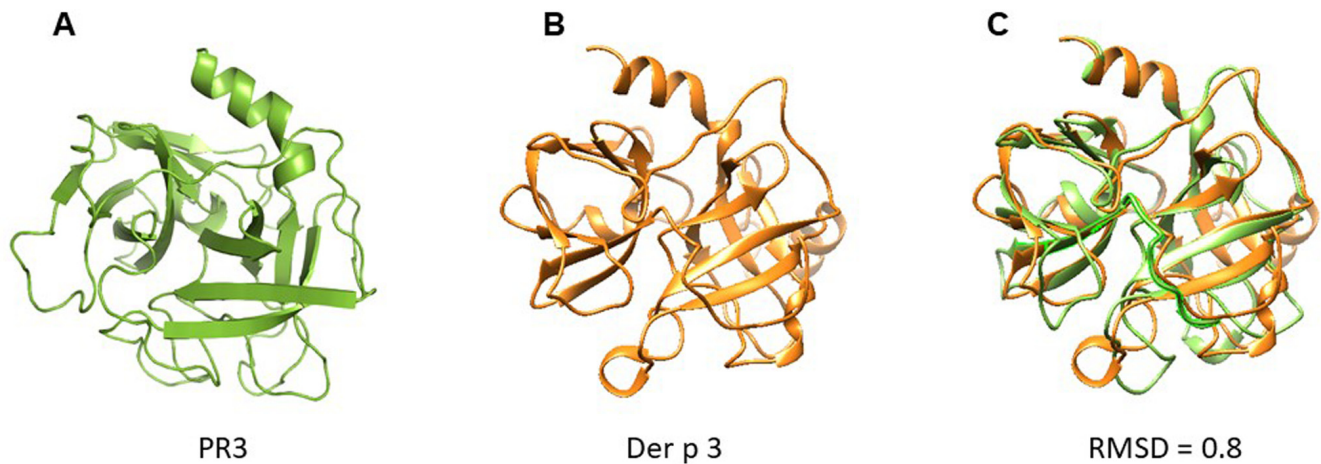


Figure 2. Cartoon models of PR3 and Der p 3. (A) PR3; (B) Der p 3. According to modelling by homology, Der p 3 exhibited a typical fold of serine protease family. (C) Overlapping of 3D model of PR3 and Der p 3. RMSD value of 0.8 is reported, suggesting structural homology.

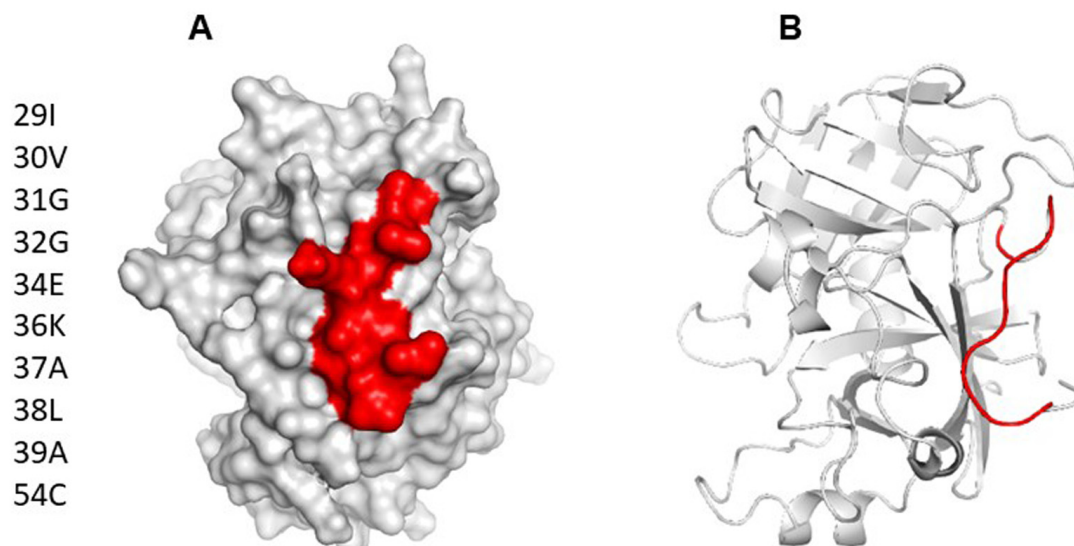


Figure 3. (A) Surface model of Der p 3, showing area occupied by B cell epitope predicted as cross reactive. **(B)** Cartoon model showing location of epitope on tridimensional structure. It can be appreciated that the predicted epitope is on a loop spanning residues 29 to 39.

the cross-reactive epitope is conserved among the serine protease family (Figure 4). T cell epitope prediction identified at least two epitopes with potential cross-reactivity among all sequences analyzed. Both epitopes are located on the first and second β strands: the first epitope spans the 45 to 59 region (ISLQSSSHFCGGTIL); and the second, the 63 to 77 region (WILTAAHCVAGQTAS) (Figure 5; Table 2).

Discussion

In this study we found that PR3 and HDM group 3 serine protease allergens have conserved identity and homology. Also, for the first time, we predicted various T and B cell cross

reactive epitopes between them through an *in silico* approach. PR3 is an important autoantigen in small vessel vasculitis and it seems to participate in disease inception, progression, and severity¹. Our results have potential implications for the understanding of autoreactive response in AAV and open the possibility for a new environmental trigger of the autoreactive response in AAV.

In AAV, it has been proposed that autoantibodies directed to a complementary protein to PR3 autoantigen could be implicated in disease inception, and this antisense protein harbors homology to various bacterial peptides¹¹ – a theory named

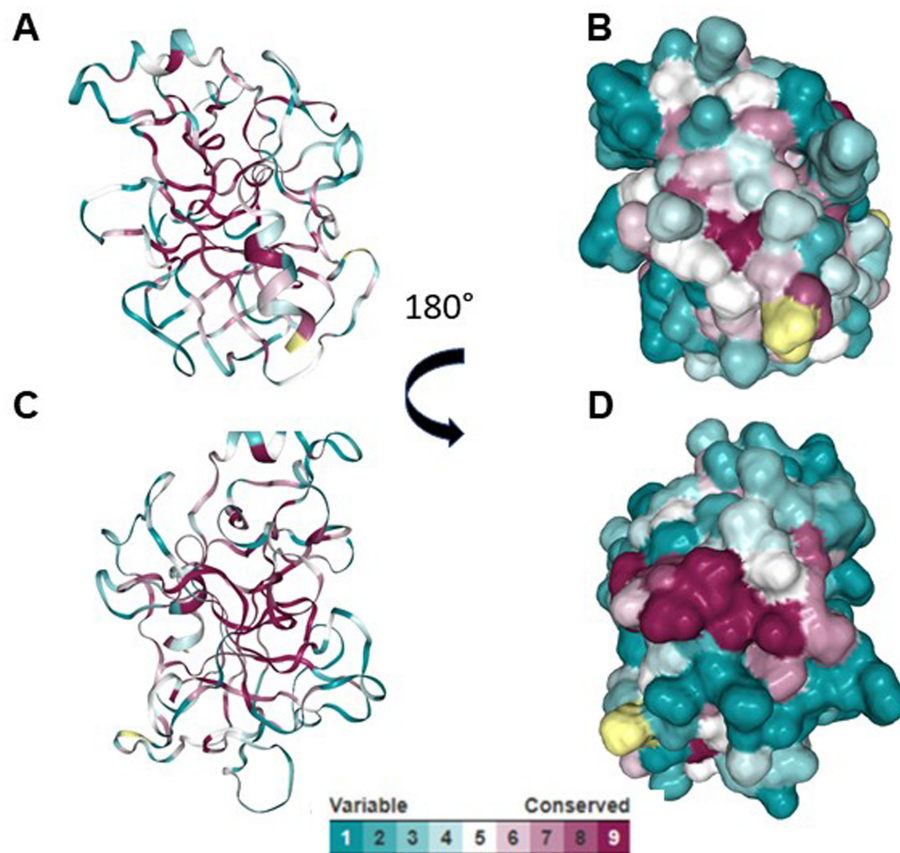


Figure 4. Phylogenetic analysis of the serine proteases using Consurf. (A and C) Cartoon models showing the conserved region among serine proteases. (B and D) Surface models showing the conserved region among serine proteases.

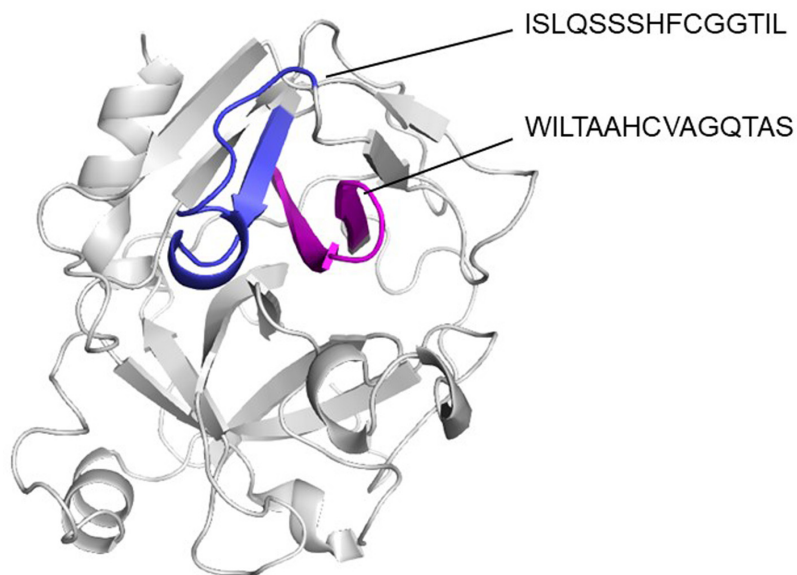


Figure 5. Cartoon model showing the location of T cell epitopes in their tridimensional structure. It can be appreciated that predicted epitopes are in a continuous β strands (blue and magenta).

Table 2. T cell epitopes predicted *in silico*. Both are conserved among PR3 and HDM group 3 allergens.

Allele	Start	End	Sequence
HLA-DRB1*01:01	45	59	ISLQSSSHFCGGTIL
HLA-DRB1*01:01	63	77	WILTAHCVAGQTAS

autoantigen complementarity³³. However, in epidemiological studies, autoantigen complementarity hypothesis testing has showed conflicting results, since sera from some patients suffering from AAV do not recognize complementary PR3, while others do^{34–36}. Also, molecular mimicry of PR3 protein by infectious microorganism components have been proposed as a possible environmental trigger of the disease based on the reports of infections preceding the manifestations of vasculitis^{10,37–39}, although a cross reactive antigen have not been reported yet.

In their seminal publication, Pendergraft *et al.* run a BLAST query to find homologues of PR3 protein in microbial or fungal microorganisms, and do not find matching sequences at that time¹¹. However, they do not include Arachnida or other environmental sources of cross-reactivity. In our analysis we find matching PR3 protein sequences with various HDM group 3 serine protease allergens, and at least theoretically this finding could have many implications for the understanding of inception and even diagnosis of autoreactive response in AAV. Recently, Qian *et al.* have shown that some allergens can cross-react with human proteins¹⁹ and participate in autoimmunity inception in pemphigus vulgaris by a “hit-and-run” mechanism²², opening the theoretical possibility for a similar mechanism to occur in another autoimmune disease such as AAV. Similarly, in atopic dermatitis, Valenta and collaborators observe that some patients with severe complications from the disease, had IgE directed to the profilin of the *Betula verrucosa*, but also to the human homologue⁴⁰.

In the tropics, HDM are important ubiquitous sources of protease allergens. Exposure is perennial, increasing the possibilities of exposure and IgE sensitization to their components in the

general population^{23–25}. Sensitization to HDM group 3 allergens is common²⁶, and they harbor serine protease activity²⁷, a characteristic that make them highly allergenic. Moreover, their conserved structural homology makes them highly immunogenic^{41,42} and suitable for epitope spreading⁴³. In this context, “hit-and-run” and epitope spreading establish framework mechanisms for environmental allergens with homology to autoantigens to potentially participate in the development of autoimmunity. We speculate that HDM group 3 allergens harbor two characteristics that make them suitable candidates for environmental triggering of AAV: their proteolytic activity that, as other protease allergens, set a tissue damaging microenvironment during antigen recognition⁴¹; and molecular homology-epitope sharing with human PR3, that would elicit B cell autoantibody production and autoreactive T cell receptor generation. In conclusion, we observe that PR3 and HDM group 3 serine protease allergens have conserved identity, and for the first time we predict cross-reactive epitopes between them through an *in silico* approach.

Data availability

UniProtKB: PRTN3_HUMAN, Accession number P24158: <https://www.uniprot.org/uniprot/P24158>

Protein Data Bank: PR3 (MYELOBLASTIN), Accession number 1FUJ: <https://www.rcsb.org/structure/1FUJ>

UniProtKB: Mite allergen Der p 3, Accession number P39675: <https://www.uniprot.org/uniprot/P39675>

UniProtKB: Trypsin Blo t 3, Accession number A1KXI1: <https://www.uniprot.org/uniprot/A1KXI1>

UniProtKB: Gly d 3, Accession number Q1M2M8: <https://www.uniprot.org/uniprot/Q1M2M8>

UniProtKB: Allergen Lep d 3, Accession number Q1M2L7: <https://www.uniprot.org/uniprot/Q1M2L7>

UniProtKB: Trypsin Tyr p 3.0101, Accession number C6ZDB5: <https://www.uniprot.org/uniprot/C6ZDB5>

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Version 2

Reviewer Report 12 May 2022

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Hiroshi Tanaka 

Department of School Health Science, Hirosaki University Hospital, Hirosaki, Japan

To elucidate the origin of the autoreactive response of human proteinase 3 (PR3), the authors examined molecular similarity between environmental antigens and PR3. They found that some house dust mites showed structural homology to PR3. Some environmental antigens, such as house dust mites, may be attributable to the production of PR3-ANCA.

This is an interesting paper. The results obtained is informative enough for readers. It is nice to add some comments or speculation about how the results obtained are relevant in real-world clinical practice, particularly the etiology and treatment of patients with C-ANCA-related vasculitis. This issue is very relevant for physicians who treat patients with ANCA-related vasculitis.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nephrology, Rheumatology, Clinical immunology, Pediatrics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 29 March 2022

<https://doi.org/10.5256/f1000research.81402.r124319>

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Tomas Erban 

Crop Research Institute, Prague, Czech Republic

Buendia et al. investigated in their manuscript possible link between human proteinase 3 (PR3) and house dust mite allergen protease that is denoted as allergen group 3. Authors suggest that similarity between PR3 and the group 3 mite allergens may be link to autoimmune vasculitis. Methodology and conclusions are based on structural homology based on in silico analysis. I believe that recommendations will improve the manuscript, which is of interest.

Because methodology of the study is based on in silico tools, it is necessary to consider that the function is “possible” and needs to be confirmed in future.

Please note, that group 3 mite allergens become subject of debate in the domestic mites, that include both house dust (HDMs) and stored product mite (SPMs) species. Immortally, authors should clarify that allergens of HDMs and SPMs were included in the study. In addition, these allergens are present in mite feces and, therefore, they are abundant in the environment. Thus, introduction should be improved.

It was found that occurrence of trypsin allergens in mites differs in species and strains. The expression of group 3 mite allergens was observed to be affected by presence of intracellular symbionts in mites. There is suggested link to PAR2 (PAR-2) in mites (– for instance, see: <https://doi.org/10.1016/j.jprot.2021.104356>¹). The affected pathway in mites can be affected also in humans. Could be a suggested link to PR3 in this regard? e.g. “immune functions of PR3 with respect to PR3 modulation of cell activation via cleavage of protease-activated receptor-2 (PAR-2)” - Ann N Y Acad Sci. 2007 Aug;1109:84-92. doi: 10.1196/annals.1398.010². Authors can also consider the suggested “Orchestration of an uncommon maturation cascade of the house dust mite protease allergen quartet” (<https://doi.org/10.3389/fimmu.2014.00138>)³

Comment to approach

- Authors used different sequences of group 3 mite allergens to predict similarity. It should be clear whether “pro” or mature form was used in analyses. It is recommended that authors will consider change “identity” to similarity. Usually, high identity is “only” to partial

sequence. It is important to consider overall similarity.

- In initial stage, authors used multiple Grp3 sequences, i.e.: Der p 3, Blo t 3, Gly d 3, Led p 3 and Tyr p 3. Is it possible to report similarity?
- In further analysis, authors used Der p 3 sequence. Incidentally, research showed that *D. pteronyssinus* is depleted of this allergen in mite feces, although it is high-abundance in *D. farinae* – for instance, see *J Proteomics*. 2017 Jun 6;162:11-19 doi: 10.1016/j.jprot.2017.04.021⁴

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Invertebrate physiology, proteomics, acarology, allergens, multiomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 09 August 2021

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**Chandrabose Selvaraj**

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Reviewer Recommendation: Major Revision

1. Primary check on this manuscript for language refinement is mandatory as I see that it is not satisfactory, and moreover, there are more typographical errors. I suggest the authors go with language and grammar checks.
2. The following sentence seems incomplete and difficult to understand, reframe it "Here, allergens such as: Der p 3, Blo t 3, Gly d 3, Led p 3 and Tyr p 3 shared an identity of 45% among them according to multiple sequence alignment"
3. Why have authors selected PR-3 for the homologous evaluation study? Since the 3-D structure is available in the PDB database?
4. What basis authors have selected allergenic sources such as Dermatophagoides pteronyssinus (Der p 3: Accession number P39675), Blomia tropicalis (Blo t 3: A1KXI1), Glycyphagus domesticus (Gly d 3: Q1M2M8), Lepidoglyphus destructor (Led p 3: Q1M2L7) and Tyrophagus putrescentiae (Tyr p 3: C6ZDB5) in this study? In BLAST it did not show the above mentioned allergens.
5. Did authors find the sequence similarity analysis of target sequence Der p 3 with its template; if so provide that information more in detail.
6. Swiss-Modeler does not show the 1FUJ as template in template searching, and then what basis authors have checked RMSD calculation between the modeled structure and IFUJ as template?

7. In methodology part authors have mentioned that the modeled structure was subjected to validation, but lack results and interpretation. Provide more in detail.
8. Provide abbreviation for AAV
9. Is the residues present in the epitope region responsible for the antigenic effect?
10. In epitope prediction, the author only provides the sequence and length of the epitopes, if the author provides the information on score value, identity and whether it is present in any domain or motif it could be more informative.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunoinformatics, Computational Biology, Molecular Modeling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Dec 2021

Emiro Buendía

Emiro Buendía, Division of Health Sciences, Universidad del Norte, Barranquilla, Colombia.

Dear reviewer, thanks for the reading of the paper, we have taken note of all your comments, questions and suggestions. Our responses are bellow:

1. Reviewer: Primary check on this manuscript for language refinement is mandatory as I

see that it is not satisfactory, and moreover, there are more typographical errors. I suggest the authors go with language and grammar checks.

Answer: We appreciate your suggestion. The manuscript was revised and refined by a language style corrector.

2. Reviewer: The following sentence seems incomplete and difficult to understand, reframe it "Here, allergens such as: Der p 3, Blo t 3, Gly d 3, Led p 3 and Tyr p 3 shared an identity of 45% among them according to multiple sequence alignment"

Answer: We appreciate your suggestion. A new sentence was added: "Multiple sequence alignment showed that Der p 3, Blo t 3, Gly d 3, Led p 3 and Tyr p 3 allergens shared 45% of identity in their aminoacid sequences with PR3. The most conserved region is located between residues 53 to 75, indicating the existence of molecular mimicry."

3. Reviewer: Why have authors selected PR-3 for the homologous evaluation study? Since the 3-D structure is available in the PDB database?

Answer: We appreciate your question. We selected PR3 for the study because it is the most important autoantigen in ANCA + vasculitis. Since, this human protein is a serine protease, in the sequence alignment analysis we seek for other serine proteases in mites, because it is a previously described environmentally ubiquitous serine protease source. In our study we selected the 3D model of PR3 from the Protein Data Bank as mentioned in your observation because its structure was already deciphered, the structural homology analysis was done keeping in mind that its serine protease folding could make more possible for us to model homologous serine in mites.

4. Reviewer: What basis authors have selected allergenic sources such as Dermatophagoides pteronyssinus (Der p 3: Accession number P39675), Blomia tropicalis (Blo t 3: A1KXI1), Glycyphagus domesticus (Gly d 3: Q1M2M8), Lepidoglyphus destructor (Led p 3: Q1M2L7) and Tyrophagus putrescentiae (Tyr p 3:C6ZDB5) in this study? In BLAST it did not show the above-mentioned allergens.

Answer: We appreciate your question. These allergens were chosen on the basis that they belong to the serine protease family, and our hypothesis was that the sensitization to these allergens is cross-reactive with the PR3 autoantigen. So, search was limited using terms: American house dust mite (taxid:6954). In this way, results were only about mites, an important allergenic source.

5. Reviewer: Did authors find the sequence similarity analysis of target sequence Der p 3 with its template; if so provide that information more in detail.

Answer: We appreciate your question. Yes, the binary alignment showed 33% identity between the template amino acid sequences, and Der p 3. An image of the alignment was uploaded in the new manuscript version.

6. Reviewer: Swiss-Modeler does not show the 1FUJ as template in template searching, and

then what basis authors have checked RMSD calculation between the modeled structure and IFUJ as template?

Answer: We appreciate your question. For modelling the mite allergen Der p 3 structure, we used the zymogen catalytic region of MASP-2, a lectin binding mannose from human (PDB: 1zjk f), as a template because 1FUJ code was not listed in Swiss-Modeler query results for this protein. Next we calculated the RMSD based on the predicted structure of the mite Der p3 protease and the previously resolved structure of the human PR3. The following sentence was improved to answer your question in the method section of the paper (**Construction of 3D model**): "The 3D model of Der p 3, a serine protease of *Dermatophagoides pteronyssinus* was generated by homology in the **SWISS-MODEL** server using the zymogen catalytic region of human MASP-2 (PDB: 1zjk f) as a template. The 3D model of Der p 3 was loaded into the ProSA-web server ²⁹, which was used to analyze its quality".

7. Reviewer: In methodology part authors have mentioned that the modeled structure was subjected to validation, but lack results and interpretation. Provide more in detail.

Answer: We appreciate your suggestion. We added the next sentence in the method section (**Construction of 3D model**): For the validation of the Der p 3 structure we used the Minimize Structure option in the UCSF Chimera software, a procedure that adjust the energy (entropy) of the model.

8. Reviewer: Provide abbreviation for AAV.

Answer: We appreciate your suggestion. ANCA Associated Vasculitis (AAV) was included in the corrected paper text.

9. Reviewer: Is the residues present in the epitope region responsible for the antigenic effect?

Answer: We appreciate your question. We think that the residues found in the epitope region are involved in the cross reactivity between the studied serine protease and hypothesized have a role in autoimmunity inception. But this are preliminary results awaiting experimental validation.

10. Reviewer: In epitope prediction, the author only provides the sequence and length of the epitopes, if the author provides the information on score value, identity and whether it is present in any domain or motif it could be more informative.

Answer: We appreciate your suggestion. The B epitope is part of the N-terminal region of PR3 and is not within any domain of the protein. The T epitope spanned between residues 45-59 is located on the first and second beta lamina. While the second epitope T (63-77) is located in the third beta sheet, and first alpha helix in the protein structure. In the result section (**T and B cell cross-reactive epitopes were predicted between HDM group 3 allergens and PR3**), two sentences were improved to consider your suggestion.

Competing Interests: No competing interests were disclosed.

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