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Original Article

Production of a Ric c3 hypo-allergen with no IgE binding or anaphylactogenic activity

Produção de uma proteína hipoalergênica mutante de Ric c3, não-ligante de IgE ou atividade anafilactogênica

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Abstract

Several studies have been carried out to expand the use of *Ricinus communis* L. castor bean (*Ricinus communis L castor bean.*). This oilseed finds appropriate conditions for its development in Brazil, with more than 700 applications. The main allergens of this plant are Ric c1 and Ric c3, that cross-react with various aeroallergens and food allergens such as peanuts, soybeans, corn, and wheat. This study aimed to determine the effect of mutations in Ric c3 amino acid residues known to affect IgE binding and allergy challenges. Based on the Ric c3 structure, B-cell epitopes, and amino acid involved in IgE binding, we produce recombinant mutant protein, mrRic c3, secreted from *E. coli*. Strategic glutamic acid residues in IgE-biding regions were changed by Leucine. The allergenicity of mrRic c3 was evaluated by determination of IgE, IgG1, and total IgG in immunized Balb/c mice and by degranulation assays of mast cells isolated from Wistar rats. The mrRic c3 presented a percentage of mast cell degranulation close to that seen in the negative control, and the immunization of mice with mrRic c3 presented lower levels of IgE and IgG1 than the group treated with the protein without mutations. The mutant mrRic c3 had an altered structure and reduced ability to stimulate pro-inflammatory responses and bind IgE but retained its ability to induce blocking antibodies. Thus, producing a hypoallergenic mutant allergen (mrRic c3) may be essential in developing new AIT strategies.

Keywords: 2S albumin, castor allergen, *Ricinus communis*, recombinant hypoallergenic, AIT.

Resumo

Vários estudos têm sido realizados para ampliar o uso da mamona *Ricinus communis* L. (*Ricinus communis* L), oleaginosa que encontra condições adequadas para seu desenvolvimento no Brasil com mais de 700 aplicações. Os principais alérgenos desta planta são Ric c1 e Ric c3 que apresentam reação cruzada com vários alérgenos dispersos no ar e alérgenos alimentares, como amendoim, soja, milho e trigo. Este estudo teve como objetivo determinar o efeito de mutações nos resíduos de aminoácidos, presentes, em epítopos ligantes de IgE, de Ric c3 envolvidos no desencadeamento de respostas alérgicas. Com base na estrutura de Ric c3, epítopos de células B, e aminoácidos envolvidos na ligação de IgE, produzimos, em *E. coli*. uma proteína mutante recombinante, denominada mrRic c3. Resíduos estratégicos de ácido glutâmico, presentes nestes epítopos, foram alterados por Leucina. A alergenicidade da proteína mrRic c3 foi avaliada pela determinação dos níveis séricos de IgE, IgG1 e IgG total em camundongos Balb/c imunizados com a proteína mutante e por ensaios de degranulação de mastócitos isolados de ratos Wistar. A proteína mrRic c3 promoveu um percentual de degranulação de mastócitos próximo ao observado no controle negativo e camundongos imunizados com mrRic c3 apresentou níveis de IgE e IgG1 menores que o grupo tratado com a proteína recombinante nativa. A mutante mrRic c3 apresentou estrutura alterada e capacidade reduzida de estimular respostas pró-inflamatórias e de se ligar a IgE, mas manteve sua capacidade de induzir anticorpos bloqueadores. Assim, a produção de um alérgeno mutante hipoalergênico (mrRic c3) pode ser uma etapa essencial no desenvolvimento de novas estratégias para a AIT.

Palavras-chave: albumina 2S, alérgenos de mamona, *Ricinus commnis*, recombinante hipoalergênica, Imunoterapia alérgeno específica.

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1. Introduction

The leading group of plant allergenic proteins belongs to cupin and prolamin superfamily proteins. The prolamin superfamily includes glutelin, gliadin, tree nuts, cereals, and storage proteins such as 2S albumin (Breieneder and Radauer, 2014). In castor bean seeds, the major allergens are the 2S albumin isoforms Ric c1 and Ric c3. They are synthesized in the rough endoplasmic reticulum as a high molecular weight protein precursor (Irwin et al., 1990; Youle and Huang, 1978). These proteins cross-react with allergens from fungi, tobacco, gluten, wheat, soy, peanuts, and corn (Felix et al., 2008), which belong to the prolamin superfamily (Breieneder and Radauer, 2014; Irwin et al., 1990). Six epitopes are responsible for triggering allergy in these isoforms were identified in castor allergens, two of which were found in Ric c1 and four in Ric c3 (Youle and Huang, 1978; Deus-de-Oliveira et al., 2011). It was found in these epitopes that in order to bind to the IgEs present in the membrane of mast cells or basophils, there must be at least two glutamic acid residues (E) at a distance of at most 12 residues between them to induce the process of degranulation of these cells. It triggers allergy symptoms, supporting the hypothesis that the carboxylic side groups of these chains may be necessary for the interaction with IgE molecules (Youle and Huang, 1978; Aalberse, 2000).

According to the Brazilian Association of Allergy and Immunology (ASBAI), there are approximately 16 million allergic people in Brazil alone. Among the main types of allergy, the most prevalent are those of the respiratory tract, such as rhinitis, rhinoconjunctivitis, sinusitis, and asthma and skin allergies, the main ones being atopic dermatitis and contact dermatitis. Therefore, therapeutic strategies have been studied recently to develop more effective and safe methods to alleviate the symptoms triggered by allergic reactions. It has improved the understanding of the allergic process, enabling new treatment and diagnostic approaches (Moreno and Clemente, 2008; Jensen-Jarolim et al., 2017).

Several procedures are used to obtain hypoallergenic recombinant derivatives: the protein can be fragmented by changing its amino acid sequences; mutations in the protein structure can be made; creation of hybrid proteins from fragments of other different proteins; change in the spatial orientation of the protein molecule; allergen fragments can be reassembled in the form of a mosaic (Marth et al., 2014; Valenta et al., 2010). Genetically modified allergens with reduced immunoglobulin IgE-binding can reduce allergic side effects in immunotherapy. Their therapeutic potential has been demonstrated by their ability to induce IgG-blocking antibodies and to modify Th2-cell-mediated hypersensitivity (Marth et al., 2014; Petrova et al., 2021).

In silico study proposed the theoretical deactivation of the allergenic epitopes of Ric c1 and Ric c3 using mutations in the glutamic acid residues (E), present in IgE -binding. Glutamic residues were exchanged for leucine residues (L), making -them the least allergenic and without significantly altering the structures of these proteins (Nascimento et al., 2011; Pacheco-Soares and Carvalho, 2018). Based on this, Pacheco-Soares and Carvalho (2018) established conditions for expressing the Ric c1 isoform in a bacterial system using *Escherichia coli* (*E. coli*), containing mutations in specific fragments of allergenic epitopes.

Due to the cross-reaction between the 2S albumins of *Ricinus communis* with allergens from different sources, some of them with glutamic acid residues in IgE-binding or B-cell epitopes, we aim to produce a recombinant protein containing specific mutations in the Ric c3 isoform of castor bean 2S albumins and verify its hypoallergenicity.

2. Material and Methods

2.1. In silico studies – B cell epitope prediction

Bepipred 2.0 server, and Bcepred immunoinformatic approaches were used for predicting linear B cell epitopes of Ric c3. The combined parameters hydrophobicity, polarity, flexibility, and exposed surface were selected in this research.

2.2. Biological material and animals

R. communis L. (castor oil bean seeds), cultivar IAC-226, were obtained from the Instituto Agrônomico de Campinas, São Paulo, Brazil.

Cells of the *E. coli* strain Rosetta-gami2 (DE3) pLysS, genotype: D (ara–leu)7697DlacX74 DphoA PvuII phoR araD139 ahpC galE galK rpsL (DE3) F'[lac+lacIq pro] gor522::Tn10 trxBpLysSRARE23 (CamR, StrR, TetR)4)], were acquired from Novagen, and cells of the *E. coli* strain Nova Blue (genotype: *endA1 hsdR17* (rK12– mK12+) *supE44 thi-1 recA1 gyrA96 relA1 lac* F′[*proA+B+ lacIqZΔM15*::Tn*10*] (TetR)) were acquired from Stratagene.

Competent cells of these strains were chemically prepared, as previously reported (Pacheco-Soares and Carvalho, 2018).

Animals: Isogenic female R/A Tor rats, generally high producers of IgE, were obtained from the animal facility of the Universidade Federal Fluminense, Niteroi, RJ, Brazil.

Black mice-C57 (C57/BL6) and Wistar rats were obtained from the Universidade Federal do Paraná – UFPR under the Animal Use Ethics Committee (CEUA – UENF) approval, protocol nº 297.

Serum containing IgE: Isogenic female R/A Tor rats were immunized with the 2S albumin pool, and the sera were separated as described by Deus-de-Oliveira et al. (2011). Anti-2S albumin serum: An anti-2S albumin from castor seeds was generated in rabbits as described (Deus-de-Oliveira et al., 2011)

2.3. Natural Ric c3 (nRic c3) isolation and insertion in a cloning vector

nRic c3 isolation: The 2S albumin pool (Ric c1 and Ric c3) was isolated and characterized by SDS/PAGE and immunoblotting as previously described (Pacheco-Soares and Carvalho, 2018), and the isoforms Ric c1 and Ric c3 were isolated as described by Felix et al. (2008).

The insertion of the nRic c3 coding sequence into the cloning vector was performed as described by Pacheco-Soares and Carvalho (2018).

2.4. Production of mutant recombinant Ric c3 (mrRic c3)

The Ric c3 coding sequence was designed to contain mutations in allergenic epitopes exchange of glutamic acid residues (E) for leucine (L), as suggested by molecular modeling described by Nascimento et al. (2011). The gene was synthesized by the GeneArt tool (Invitrogen). The synthetic coding sequence was produced, sequenced, and cloned into the cloning vector pMA to produce the construct mRic c3-pMA. mRic c3-pMA was transformed into *E. coli* strain Nova Blue by heat shock to propagate and conserve this vector in a biological system. The transformed clones were analyzed by plating the cells on LB solid medium containing 100 µg/mL ampicillin. Colonies were collected and grown in LB medium with the same antibiotic, and the transformed Nova Blue cells were stored at −70 °C until needed.

mRic c3-pMA was used as a template for PCR to amplify mRic c3 containing the regions that bind to the pET-32 EK/LIC expression vector, producing the construct mRic c3-pET.

2.5. Cloning, expression, and purification of recombinant proteins (nrRic c3 e mrRic c3)

The host cell used to overexpress the natural and mutant Ric c3 sequences was *E. coli* strain Rosetta-gami2 (DE3) pLysS. The vector containing the nRic c3-pET and mRic c3-pET constructs was incubated in 100 µL of cells and taken to the electroporator (Bio-render gene Pulser X cell). The samples were transferred to a liquid LB medium and incubated for 1 hour at 37 °C under agitation at 200 rpm. After the incubation, the cells were resuspended and plated in a solid LB medium containing 100 µg/mL of ampicillin and 34 µg/mL of chloramphenicol and incubated at 37 °C for 16 hours.

Isolated colonies of *E. coli* strain Rosetta-gami2 (DE3) pLysS transformed with nRic c3-pET and mRic c3-pET were inoculated in liquid LB medium containing ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL). They were maintained at 37 °C under stirring at 200 rpm for 16 hours. The inoculums were placed in a new liquid LB medium with the same antibiotics and left under agitation for approximately 3.5 hours until an O.D.600 of 0.3-0.6.

After bacterial growth, 1 mM isopropyl-β-D-1-thiogalactopyranosideIPTG (isopropyl-β-D-1-thiogalactopyranosideIPTG) was added to activate the process of inducing the translation of recombinant Ric c3 (nrRic c3) and recombinant mutant Ric c3 (mrRic c3). Cultures with no addition of IPTG were evaluated as "non-induced" controls. All cultures were shaken at 200 rpm and kept at 37 °C for 16 hours. After the induction step, "induced" and "non-induced" bacterial extracts were centrifuged for 30 min at 5,000g. Subsequently, 8 M Urea Buffer was added, remaining in the orbital for at least 15 minutes until all the material was resuspended. Cell lysis was performed in a French® Press, and new centrifugation was performed for 10 minutes at 5000g.

The purification of recombinant proteins was done by nickel affinity chromatography and dialysis extensively against phosphorus buffer saline (PBS) 1x for 24 hours at 4 °C with 3 buffer changes. The material was collected and stored in a -20 °C freezer for the following steps.

2.6. Characterization of recombinant proteins

To confirm the expression of recombinant proteins, 15% Polyacrylamide Gel Electrophoresis (15% SDS-PAGE) was performed as described by Laemmli (1970) and Western Blotting (Towbin et al., 1979) using anti-6x-His Tag.

The transfer was carried out in a semi-dry system on a "sandwich" of filter paper soaked in transfer buffer (20 mM Tris, 145 mM glycine, and 20% methanol) under a current of 400 mA/100 V constant for 1 hour. Afterward, the membrane was soaked in blocking buffer (PBS Tween 0.05% and 3% skimmed milk powder) for one h, diluted in blocking buffer containing the primary anti-His-Tag antibody, and incubated for 18 hours at 4 °C. Then, the membrane was washed thrice in PBS Tween 0.05% (PBST), with 5 minutes per wash.

The membrane was incubated for 1 hour with HPR (Horseradish Peroxidase)-conjugated secondary anti-mouse antibody diluted in a blocking buffer. Again, three washes of 5 minutes of PSBT were done. The Kit - ECL™ Western Blotting Detection Reagent was used for the development.

2.7. Biological assays

2.7.1. Immunization of animals

Black-C57 (C57/BL6) (n = 6/group) mice were immunized with nrRic c3 and mrRic c3 proteins emulsified in 100 µL of complete Freund's adjuvant (CFA), followed by two booster doses of protein with an interval of 21 days each. Seven days after the second immunization, blood from immunized animals was collected via retro-orbital plexus puncture. The collected blood was centrifuged for 10 minutes at 7,000 rpm to obtain the serum. These sera were used in the following steps.

2.7.2. Enzyme immunosorbent assay (ELISA)

Indindirect ELISA determined specific IgE, IgG, and IgG1 levels in immunized animals. Plates were coated with 500 ng of the recombinant proteins, nrRic c3 or mrRic c3, in 50 µL of carbonate buffer pH 9.6 and incubated for 1 hour at 37 °C. Then, 200 µL of blocking solution (PBS containing Fetal Bovine Serum -FBS), and incubated for 1one h. After the incubation period, a wash with PBS was performed.

50µLFifty microliters of immunized mouse sera (primary antibody) diluted 1:10 in PBS containing 10% FBS and 5% bacterial extract were added to the wells and incubated for one h at 4 °C, followed by 6 PBS-washing steps. The incubation with the secondary antibody was 1 h at 4 °C and followed the following proportions: IgE - 1: 2000; IgG - 1: 5000; IgG1 - 1: 5000. Another 6 washing steps with PBS followed this.

Detection was performed using TMB (Tetramethylbenzidine), and 50 µL of 1 M sulfuric acid (H_2SO_4) was added after the reaction turned blue. Optical density was analyzed in a 450 nm microplate reader. The test was performed in triplicate and repeated 3 times.

3. Results

3.1. Prediction of linear B cell

According to the sequence, properties using Bepipred 2.0 server, three continuous B-cell epitopes (residues 4-25, 31-60, and 81-111) were obtained (Figure 1, Table 1), and four epitopes (residues 1-21, 31-58, 69-79, 82-99) were predicted by Bcepred 2.0 server (Table 1). We observed overlapping regions in the predicted B-cell epitopes, and, as expected, these predicted peptides contain IgE-binding epitopes identified by Deus de Oliveira et al. (2011). In all predicted B-cell epitopes and identified IgE-binding epitopes, we observed at least two glutamic acid residues between 4 and 12 residues. The glutamic acid residues are essential for binding to IgE-prefixed on mast cells and subsequent triggering of the allergic response. Thus, based on the propositions of Nascimento et al. (2011), we performed mutations (Glu-Leu) to produce genetically modified allergens with reduced immunoglobulin IgE binding (Figure 2).

3.2. Induction of the expression of nrRic c3 and mrRic c3

Strains of *E. coli* strain Rosetta-gami2 (DE3) pLysS were transformed with nRic c3-pET and mRic c3-pET, and 1 mM of IPTG was added to induce protein expression.

Through electrophoretic analysis on 15% SDS-PAGE, we observed that *E. coli* - Rosetta-gami cells submitted to induction expressed a protein profile with a band of approximately 29 kDa, not visualized in cells that were not induced (Figure 3A).

The expressed proteins purified by nickel affinity chromatography were confirmed by Western Blotting using an anti-6x-His Tag primary antibody (Figure 3B).

A

Sequence	^{1}ESKGEREGSSSQQCRQEVQRKDLSSCERYLRQSSSRRSPGEEVLRMPGDENQQQESQQLQQCCNQV KQVRDECQCEAIKYIAEDQQQGQLHGEESERVAQRAGEIVSSCGVRCMR^{115}
Hydrophilicity	^{1}ESKGEREGSSSQQCRQEVQRKDLSSCERYLRQSSSRRSPGEEVLRMPGDENQQQESQQLQQCCNQV KQVRDECQCEAIKYIAEDQQQGQLHGEESERVAQRAGEIVSSCGVRCMR^{115}

B

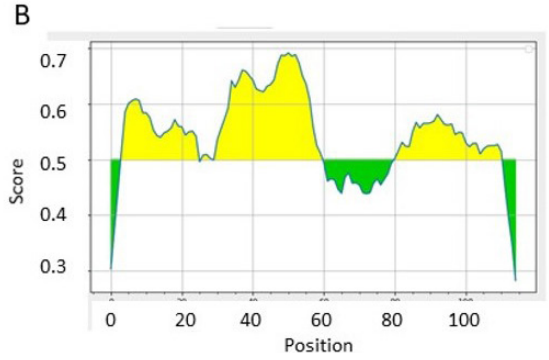

Figure 1. Ric c3, B-cell predicted epitopes (A) BcePred Method (PHI, 2023). In this research, the combined parameters were selected: hydrophobicity, polarity, flexibility, and exposed surface: The predicted B-cell epitopes are shown in blue color and underlined; (B) The graph represents the propensity scale for B cell epitopes by the Bepipred Linear Epitope Prediction 2.0. The horizontal axis indicates the number of amino acids of the complete sequence of the protein of Ric c3. The vertical axis indicates the predicted regions for the epitopes, highlighting the regions in yellow, the amino acids that form the linear epitopes of the allergen, and in the green color, the amino acids that do not form epitopes considering the values: (Minimum 0.282; Maximum 0.692; Threshold 0.542).

Table 1. B-cell epitopes predicted using Bepipred or PcePred. IgE binding epitopes were identified by Deus de Oliveira et al. (2011).

Start/End	Predicted Peptide – B-Cell epitopes Bepipred	Predicted Peptide – B-Cell epitopes BcePred	Start/End	IgE- binding peptide (Deus de Oliveira et al., 2011)
4/25	G**ER**EGSSSQQCRQ**E**VQRKDLSS	**E**SKG**ER**EGSSSQQCRQ**E**VQRKD	1/15	**E**SKG**ER**EGSSSQQCR
			16/29	Q**E**VQRKDLSSC**ER**YLR
31/60	RQSSSRRSPG**EE**VLRMPGD**E**NQQQ**E**SQQLQ	RQSSSRRSPG**EE**VLRMPGD**E**NQQQ**E**SQQ		Linked peptide
69/79		KQVRD**E**CQC**E**A	73/85	D**E**CQC**E**AIKYIA**E**DQ
81/111	IA**E**DQQQGQLHG**EESE**RVAQRAG**E**IVSSCGV	A**E**DQQQGQLHG**EESE**RVA	76/104	**E**GLRQIA**E**QQQSQGQ
			105/119	LHG**EESE**RVAQRAG**E**

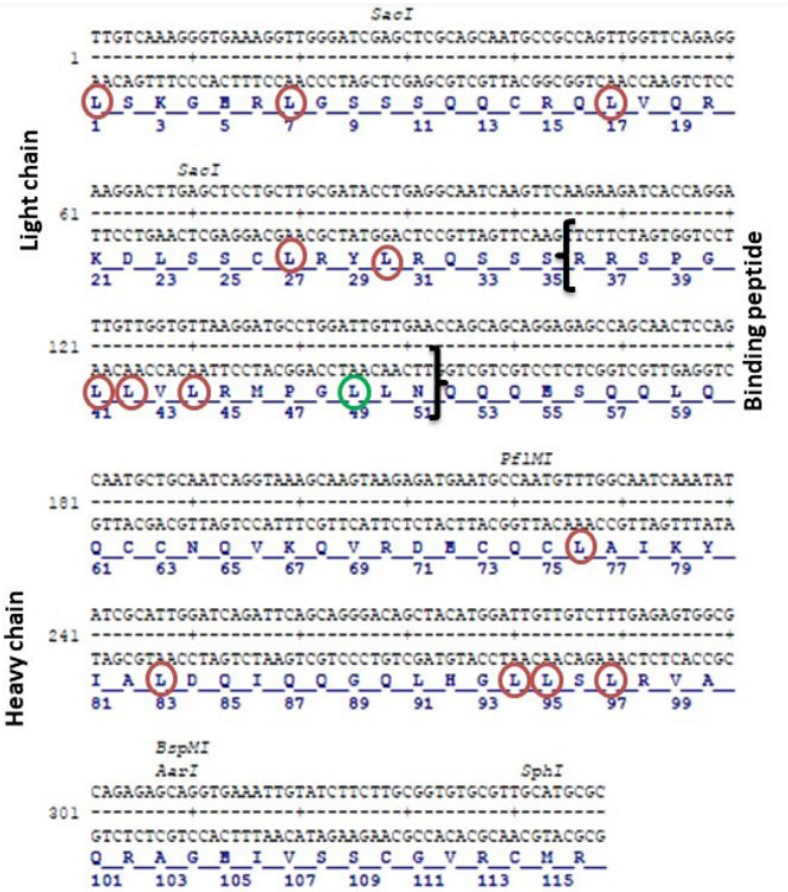

Figure 2. Gene and protein sequence of mRic c3. Gene name: Ric c3_mari_2018; Constructed 18AC5XNC 348bp. The red circles indicate the leucine residues (L) that replaced the glutamic acid residues (E), as proposed by Nascimento et al. (2011) and the green circle indicates an exchange in the aspartic acid residue (D) for a leucine residue (L) within the linker peptide between the light and heavy chains. Source: Invitrogen (with author's markings).

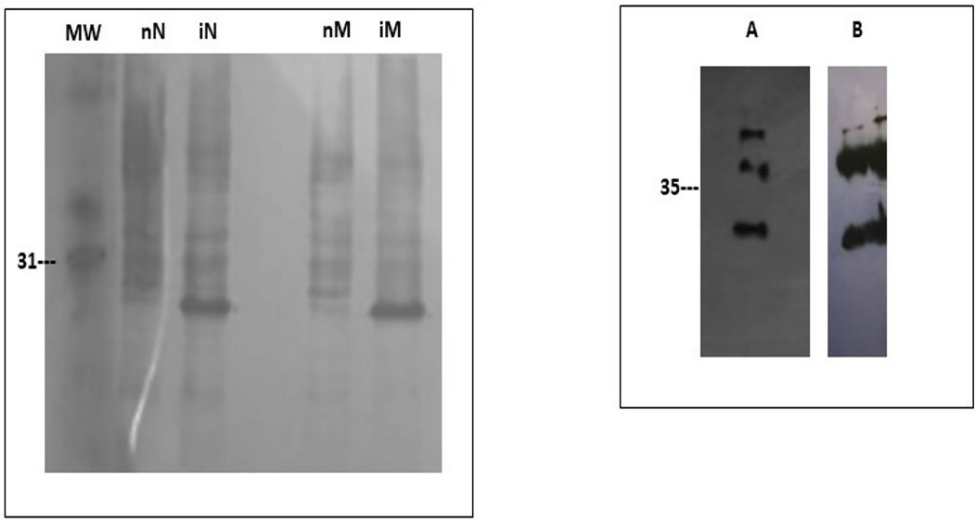

Figure 3. (A) Electrophoretic visualization of nrRic c3 and mrRic c3 expression induction with 1 mM IPTG by SDS-PAGE. MW Molecular weight; nN and nM: represent the "Not Induced" control extracts of nrRic c3 and mrRic c3, respectively; iN and iM: represent the Induced extracts of nrRic c3 and mrRic c3, respectively; (B) Analysis of expression induction by Western Blotting after purification step on Ni-NTA column using an anti-His primary antibody (1:4000). (A) expression of mrRic c3; (B) nrRic expression c 3. Source: Research data.

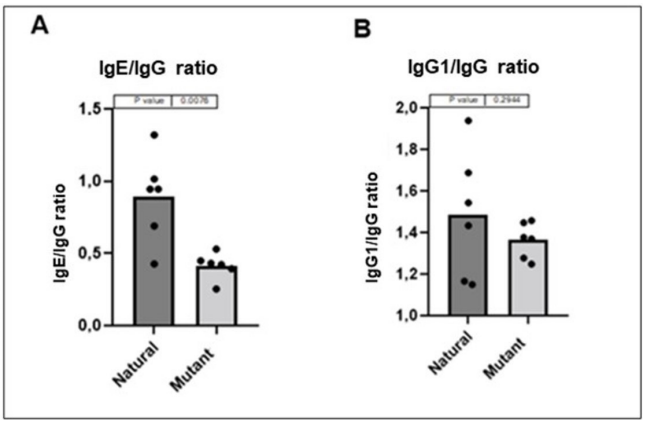

Figure 4. Antibodies produced after immunization with nrRic c3 and mrRic c3. (A) Total IgE/IgG ratio; and (B) IgG1/IgG ratio of groups treated with natural and mutant proteins, respectively. The assay was performed in duplicate – GraphPad Prism 8. Source: Research data.

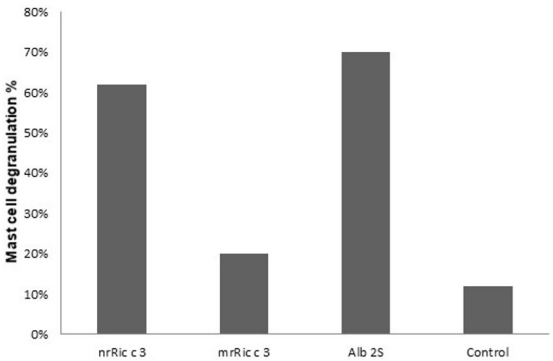

Figure 5. Mast cell degranulation assay. nrRic c3 (mast cells + 2S anti-albumin serum + recombinant Ric c3); mrRic c3 (mast cells + 2S anti-albumin serum + recombinant mutant Ric c3). 2S albumins/ positive control (mast cells + 2S anti-albumin serum + 2S Albumin Pool - Ric c1 and Ric c3); Negative control (mast cells incubated with 2S anti-albumin serum only). Source: Research data.

3.3. Evaluation of the profile of antibodies produced in the immunization process in Black-C57 mice

The ELISA assay was performed from the serum obtained from each immunized animal (6 animals per group) following the protocol of 21 days between immunizations.

We observed, through the evaluation of the production profile of IgE, total IgG, and IgG1 antibodies, that in the total IgE/IgG ratio of the group treated with the natural recombinant protein (nrRic c3), the production of IgE was higher than in the group treated with the mutant protein (mrRic c3).

In addition, when looking at the IgG1/total IgG ratio, the group treated with nrRic c3 also had high levels of IgG1 concerning total IgG, unlike the group treated with the mutant, which had lower values (Figure 4).

3.4. Analysis of allergenic activity

The allergenic activity of the recombinant proteins was analyzed using the mast cell degranulation assay. We observed that mrRic c3 (mutant recombinant) induced 20% degranulation, a value very close to the negative control, 12%. The recombinant protein without mutations (nrRic c3) induced 62% of mast cell degranulation, and the extract containing Ric c1 and Ric c3 (pool of 2S castor bean albumin) – positive control induced 70% (Figure 5).

4. Discussion

Immunotherapy began more than 100 years ago, and since then, the search for improvements in this form of treatment has continued. Clinical applications with allergen-extract based are restricted due to potential adverse reactions. Concern about the increased prevalence of IgE-mediated allergies, especially in Brazil, increases when we observe the growth of castor bean (*Ricinus communis*), as it has a tremendous toxic and allergenic potential given by the proteins present in its seeds and pollen and by the cross-responses between allergens from castor beans with allergens from food sources and aeroallergens.

The elucidation of the structures, knowledge of cross-responses between allergens, and the identification of epitopes involved in the allergic response have contributed to personalized and comprehensive allergy treatment. The B cell epitopes and IgE binding epitopes are generally located in exposed regions in the allergen structure; some may overlap. Based on these characteristics, we used bioinformatics strategies to determine IgE binding epitopes using synthetic peptides (Deus-de-Oliveira et al., 2011) and B cell epitopes. Integrated computational approaches assist in developing epitope-based vaccines and are essential for identifying regions susceptible to mutations to obtain hypoallergenic mutants. This strategy was used pr xxx to propose a multiepitope vaccine against multidrug-resistant Streptococcus pseudopneumoniae (Attar, 2024). Considering these properties, we produced a hypoallergenic mutant derived from the significant castor seed allergen, Ric c 3. To constructed mr Ric c3, ten specific glutamic acid residues (E) and one aspartic acid residue (D) were replaced by leucine residues (L).

It is essential to highlight that the allergenic isoforms of 2S albumins, in their natural form, in the plant, are formed by two polypeptide chains joined by sulfur disulfide bridges. These isoforms are produced from a single precursor that undergoes post-translational processing by endopeptidases and carboxypeptidases, releasing the two isoforms, Ric c1, and Ric c3, both consisting of two polypeptide chains. However, this processing could not occur in a bacterial recombinant protein production system. The production of recombinant mutant Ric c3 as a single polypeptide chain was also adopted by Pantoja-Uceda et al. (2003), who produced recombinant Ric c3 for structural studies of Nuclear Magnetic Resonance (NMR). The researchers elucidated the three-dimensional structure of Ric c3 but did not assess its biological properties.

We started an immunization protocol in Black-C57 (C57/BL6) mice to observe the profile of antibodies produced during immunization with nrRic c3, similar to that produced by (Pantoja-Uceda et al., 2003) and with mrRic c3. Our results showed that in animals immunized with nrRic c3, IgE and IgG1 were higher than in the group treated with the mutant protein (mrRic c3). It is known that both IgE and IgG1 are antibodies produced in hypersensitivity reactions and are directly related to the Th2 allergenic profile (Focke-Tejkl, et al., 2015; Lupinek et al., 2014).

With the data from the mast cell degranulation assay, we verified that the allergenic potential of the recombinant protein without mutation (nrRic c3) remained close to the positive control (2S Albumin pool). Nevertheless, this ability was reduced when pre-sensitized cells were incubated with mrRic c3, showing that the alteration of amino acid residues prevented the binding between the IgE in the cell membrane and the mutated protein. So we concluded that the major castor seeds allergen Ric c3 was converted into a hypoallergenic compound, mrRic c3-IgE - B cell epitope-based.

Recent works corroborate our proposal for producing hypoallergens from mutations in point amino acids. Kulwanich et al. (2019) proposed a point mutation of residue 47 of Der p 2 involved in immunoglobulin (Ig) E binding. It produced a mutant with reduced-IgE bind but retained its ability to induce blocking antibodies. Raith et al. (2019) produced a hypoallergenic variant of grass pollen (Phl p 7). The ability of Phl p 7 IgE-bind depends on the presence of calcium. The researchers performed specific point mutations in the calcium-binding regions of the allergen, and they observed, by Dot Blot assays, that sera from patients sensitized with Phl p 7 showed a dramatically reduced IgE reactivity of the mutant compared to the wild-type protein. A similar alternative to abolishing IgE binding and retaining anaphylactogenic potency was proposed by Tscheppe et al. (2020). Yu et al. (2022) recently converted the major house dust mite allergen Der f 34 into a B cell epitope-based hypoallergenic vaccine based on immunoinformatics and peptide-carrier fusion approaches. Three different fragments of major allergen Der f 34 that contained candidate B cell epitope and without T cell epitopes were linked at the N terminal and C terminal of the PreS carrier, and the interaction with immune receptors (toll-like receptor-3) by ligand-receptor docking were evaluated.

5. Conclusion

Our results may contribute to studies on the effectiveness of producing hypoallergenic recombinant proteins from point mutations in amino acids to allergen-specific immunotherapy. It is important to emphasize that every allergy study is based on the importance of the allergen for a given population. Creating a sera bank of sensitized patients is essential to provide a panel of the predominant and integrated networks to control and monitor the air and population allergy cases.

We constructed a hypoallergenic mutant, mr Ric c3. There is a cross-response between *Ricinus allergens* and food allergens such as peanuts, corn, and soy and with airborne allergens. The glutamic acid residues are essential for triggering the allergic response in these allergens' IgE binding and B cell epitopes. Investigating whether mrRic c3 would also protect responses triggered by these allergens is an investigation in development for our research group.

Projects such as these can significantly collaborate with Brazilian research, as they aim to understand the seasonality of aeroallergens and, through knowledge of the profile of the allergenic population, it would be easier to direct the development of immunomodulatory vaccines for the treatment and prevention of allergic disease.

In summary, given the problem of allergic diseases in Brazil, this work contributed to new therapeutic approaches based on producing a hypoallergenic protein.

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