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Mapping IgE-binding epitopes of Ric c 1 and Ric c 3, allergens from *Ricinus communis*, by mast cell degranulation assay

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ABSTRACT

Ric c 1 and Ric c 3 are the major castor bean allergens. In order to identify continuous IgE-epitopes in Ric c 1 and Ric c 3, pools of sera from rats immunized with a pool of 2S albumin from these seeds, Ric c 1 and Ric c 3 overlapping synthetic peptides, were used to screen for IgE-binding epitopes. The allergenic properties were monitored by mast cell degranulation assays, histamine quantification and human-IgE binding. Large and small chains isolated from these proteins present allergenic properties. Four continuous epitopes were identified in Ric c 3 and two in Ric c 1. This knowledge may allow the induction of protective antibody responses to antagonize the IgE recognition.

1. Introduction

The castor bean plant (*Ricinus communis*) originated in Ethiopia and gradually dispersed towards South Africa, the Mediterranean region and warm areas of Asia, until finally establishing itself as a natural species in the majority of warm climate regions of the world [12]. The castor bean contains about 50% oil, which has special characteristics such as high viscosity, stability to heat and pressure, low freezing point, and ability to form waxy substances upon chemical treatments [7]. The modern industry uses the oil extracted from its seeds in the manufacture of explosives, varnishes, lubricants, dyes, plastics, fertilizers, leather, candles and cosmetics, as well as laxatives, antifungals and antiparasitic preparations [7]. As energy demands increase and fossil fuels are limited, researches are developed to obtain alternative renewable fuels. Biodiesel, consisting of methyl esters of fatty acids transesterified by vegetable oils with methanol, has become

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more attractive recently due to its environmental benefits and the fact that it comes from renewable resources [6,13]. Castor bean is an oleaginous candidate for such oil production [13], which will contribute to enhance the cultivation of this plant. Castor bean seeds, however, contain a strong toxin (ricin) and an allergenic protein fraction (CB-1A or 2S albumin isoforms), which severely limits the usefulness of the castor meal after oil extraction [5,12,27]. Medical problems such as conjunctivitis, rhinitis and urticaria have also been related with this kind of seed, as well as to castor bean leaves and pollen [12], described as a producer of atopic dermatitis. Recently, the atmospheric prevalence potency of castor bean pollen was demonstrated by Raju et al [21] in Chennai, India. The authors have recommended the inclusion of this pollen allergen in the diagnosis of allergy.

The mechanisms of the action of the toxin, ricin, have been established [5,22] and some treatments have been proposed to eliminate its toxicity [2]. In contrast, there is no available method for the inactivation of the allergenic fraction although the primary structure of major allergens present in the 2S albumin pool, Ric c 1 and Ric c 3 have been determined [8,15,17,24] since 1982 and 1992, respectively. A better understanding of the functional role of the castor bean allergens is fundamental to prevent allergy challenged by *R. communis*. The aim of this study was to determine the continuous IgE-epitope of the castor bean seed allergens, Ric c 1 and Ric c 3.

2. Methods

2.1. Plant material and 2S albumin purification

Castor bean (*R. communis* L., cultivar IAC-226) seeds were obtained from the Instituto Agronômico de Campinas, São Paulo/Brazil and 2S albumin fractions were isolated and identified by SDS-PAGE and immunoblotting experiments as described by Machado et al. [17,18].

2.2. Isolation of 2S albumin isoforms, Ric c 1 and Ric c 3

The pool of 2S albumin was subjected to reverse-phase liquid chromatography on a C18 column (Sephacryl peptide C18 5 μ ST 4.6/250 from Pharmacia Biotech) using a Shimadzu apparatus. The chromatography was developed at a flow rate of 0.7 mL min^{-1} using 0.1% TFA as solvent A and 80% of acetonitrile containing 0.1% TFA as solvent B. The gradient was 0–80% of solvent B for 55 min. The elution profile was monitored by on-line measurement of the absorbance at 220 nm. Fractions containing the major peaks were dried in a speed Vac system. The UV spectrum was obtained using a photo-diode detector.

2.3. Denaturation, reduction and pyridylethylation

Ric c 1 and Ric c 3 were dissolved at 10 mg/mL in a solution containing 6 M guanidinium chloride, 0.1 M Tris–HCl buffer, pH 8.4 and 1 M EDTA for 2 h at 37 °C. After denaturation, dithiothreitol was added to a concentration of 2 mM to reduce disulphide bonds and 4-vinylpyridine (560 μmol) to convert the cysteine residues to stable derivatives. The mixture was incubated for 6 h at 37 °C. [11]. The reaction mixture was submitted to C18 reverse-phase HPLC for *S*- and *L*-chain separation. The chromatography was developed at a flow rate of 0.7 mL min^{-1} using 0.1% TFA as solvent A and 80% of acetonitrile containing 0.1% TFA as solvent B. The gradient was 0–80% of solvent B for 55 min. Proteins were detected by monitoring the absorbance at 220 nm.

2.4. Circular dichroism (CD)

The native isoforms of 2S albumin, Ric c 1 and Ric c 3, and their isolated *S*-pyridyl-ethylated chains were dissolved in distilled water and CD spectra were obtained on a Jasco spectropolarimeter model J-715 (Jasco Corporation, Tokyo, Japan). The spectra were collected at 25 °C using a 0.1 cm path length quartz cell. Spectra were the average of three scans at a speed of 50 nm/min and the water spectrum was subtracted. Only the far UV region from 190 to 260 nm was analyzed.

2.5. N-terminal amino acid sequences

The N-terminal partial amino acid sequences of the 2S albumin isoforms were obtained on a Shimadzu PPSQ-10 automated protein sequencer using the Edman degradation principle. Sequences were determined from both purified proteins. PTH amino acids were detected at 269 nm after separation on a reverse-phase C18 column under isocratic conditions, according to the manufacturer's instructions. The polypeptide sequences obtained were submitted to automatic alignment using a BLAST search system [3].

2.6. Animals and antiserum

Isogenic female R/A Tor rats weighing 150 g were obtained from the animal house of the Universidade Federal Fluminense, Niteroi, RJ and all experimental procedures were approved by the animal research ethics of this University.

Rats were sub-cutaneously injected with 10 μg of 2S albumin pool in the presence of adjuvant mixture: 5 mg of Al_2OH_3 and 5 mg of nigrosin. After 15 days, rats were anesthetized and bled by cardiac puncture. The sera were pooled and tested by mast cell degranulation assay.

2.7. Mast cell degranulation assay

2.7.1. Rat peritoneal mast cells

Wistar rats were bred at the animal house of the Universidade Estadual do Norte Fluminense. All experimental procedures were approved by the animal research ethics board of the Universidade Estadual do Norte Fluminense.

Rats (~250 g) were euthanized with CO_2 and a peritoneal wash performed with the injection of 20 mL of DMEM (Gibco) containing 12 U/mL of heparin. The abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened and the fluid containing peritoneal cells was aspirated with a Pasteur pipette. Thereafter, the cells were transferred to Petri plates and incubated for 30 min at 37 °C. Two thirds of the superior layer were then aspirated and discarded. Supernatant