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Oral administration of banana lectin modulates cytokine profile and abundance of T-cell populations in mice

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ABSTRACT

Banana lectin (BanLec) is a dimeric protein occurring in fruit pulp that modulates immune cell functioning *in vitro*. In order to assess the immune response *in vivo*, BanLec from ripe banana (*Musa acuminata*) fruit was purified and orally given to mice for seven days. The analysis of cytokines in the mice peripheral blood revealed increased IL-10, IL-17 and TNFα, and a reduction of IFNγ and IL-6. In the thymus, an increase of CD4+ and a decrease of CD8+ T-cells were observed after oral administration of BanLec. The modulation of pro- and anti-inflammatory cytokines and T-cells in the peripheral blood and thymus of mice demonstrated the immunomodulatory properties of natural BanLec *in vivo*. This research brings new data on a protein from a fresh fruit consumed worldwide that may act as an immunomodulator, potentially affecting the host response to infections, immune diseases and cancer.

1. Introduction

Lectins are proteins of non-immune origin that are able to bind specifically to carbohydrates, and can recognize and interact with plant polysaccharides, glycoproteins and glycolipids [1]. Although these proteins are widely distributed in nature, plants are the most abundant source of lectins [2]. In this regard, various plant lectins have been described as biologically active proteins [3], including those present in plants usually consumed as food, such as peanuts, beans, soybean [2,4], amaranth [5] and so forth. Therefore, there is great interest in the consequences of the ingestion of lectins present in foods, as in the case of the modulation of the immune system, since these proteins could play a role in the response to infectious or immune diseases, and the prevention of cancer.

The lectin present in banana pulp, BanLec, specifically binds to glucose and mannose [6–8] and its ability to modulate immune cells has been demonstrated *in vitro* [8–10]. Koshte et al. [10] reported that BanLec could induce the production of IgG4 by human peripheral blood cell cultures. Furthermore, this protein stimulated nitric oxide production by mice macrophages [11–13]. The recombinant form of BanLec (rBanLec) was also able to stimulate the proliferation of T-lymphocytes from human peripheral blood when used in small doses, and the ability to bind T-cells was higher than the natural protein extracted from banana pulp [9].

In contrast to the abundant demonstrations of effects on cultured cells, *in vivo* studies on BanLec are very limited [12,14]. Nevertheless, Dimitrijevic et al. [15] showed that orally fed recombinant banana lectin was stable to gastric and intestinal fluids and was able to interact with cells in the mucosal surface of the digestive tract of mice, and trigger the production of antibodies after oral administration. More recently, Marinkovic et al. [16] showed that rBanLec administered through the rectum promoted balanced pro-inflammatory response in the colon of mice.

Although the stability of BanLec and its interaction with the intestinal mucosa had already been proven, the immunomodulatory effect *in vivo* by exposition through the oral route simulating the consumption of bananas is still an open question. Thus, we hypothesize that the interaction of orally administered BanLec with

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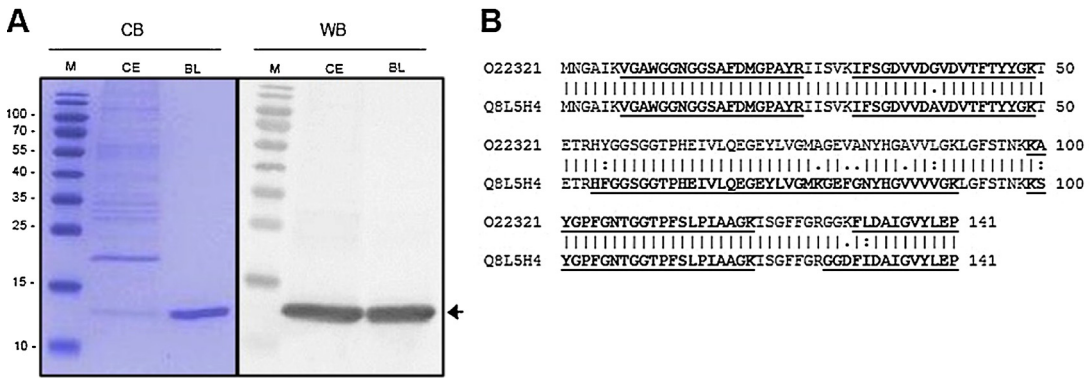

Fig. 1. Immunoprobing and sequencing of purified BanLec. (A) SDS-PAGE of banana crude extract (CE) and affinity purified BanLec (BL) stained for total protein with Coomassie Blue (CB) or revealed with anti-Banlec specific antibodies in western-bloting (WB). The numbers on the left correspond to the molecular weight of the bands of the protein marker (M) in kDa. The arrow on the right points the BanLec band. (B) Comparison of the two sequences of almost identical lectins (O22321 and Q8L5H4) present in the affinity-purified BanLec preparation using the program needle, showing the identical (|), conservative (:) or semi-conservative (.) amino acids. The sequences of the peptides identified by MS sequencing are in bold underlined letters, and the number of amino acids in each line are on the right.

immune cells at the gut mucosal barrier may promote systemic modulation *in vivo*. Therefore, the aim of this research was to evaluate the levels of cytokines and lymphocytes in both the peripheral blood and thymus of BALB/c male mice that received BanLec orally on a daily basis for seven days. Furthermore, since the study of Gavrovic-Jankulovic et al. [9] reported rBanLec as more stimulating than the natural one, we chose to assay the lectin purified from ripe bananas as a way to test the protein in the form in which it usually occurs in the human diet.

2. Material and methods

2.1. Bananas

Slightly overripe bananas (*Musa acuminata* cv. Nanicão) at a ripening stage of 6–7 on the peel colour index (PCI) were peeled, and the pulp was cut and frozen in liquid N_2, and stored at −80 °C for extraction of BanLec.

2.2. Mice

Male BALB/c mice aged 6–8 weeks were obtained from the animal house of the School of Pharmaceutical Sciences at the University of Sao Paulo. Only males were used to avoid any variability caused by the hormonal cycles from female mice. The animals were allowed at least seven days for a period of conditioning to the accommodation at the animal house. The mice were housed in ventilated cages and given a chow diet and tap water *ad libitum* in the pathogen-free part of the local animal house under environmental conditions of relative humidity of 55 ± 10%, a temperature of 22 °C ± 2, and a 12/12 h light/dark cycle. Autoclaved hardwood-derived bedding was provided in the cage, as well as in-cage shelters, which were changed three times a week, as were the water bottles. All procedures were according to the guidelines of the Brazilian Society of Science of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (Protocols CEUA/FCF/416 and CEUA/FCF/448).

2.3. Affinity purification of BanLec and sequencing

The extraction of proteins from banana fruit was done according to Marinkovic et al. [16]. Twenty grams of frozen banana pulp were ground in a mortar and pestle in the presence of liquid N_2, and homogenized in 120 mL of a 20 mM 1,3-diaminopropane (Sigma-Aldrich, USA) solution. The homogenate was transferred to 30 mL tubes and centrifuged (6000*g* for 30 min at 4 °C), and the supernatant was the banana crude extracts. The proteins from the crude extract were precipitated with 80% ammonium sulphate (Merck, Germany) in an ice bath. The suspension was centrifuged at 7500*g* for 20 min at 4 °C and the pellet was dialyzed (Spectra/Por—MWCO 6000–8000 Da, Spectrum, USA) against phosphate-buffered saline solution (PBS—pH 7.4) for a minimum of 36 h at 4 °C, with several changes of PBS. The soluble proteins from the dialysate were applied on a Sephadex G-75 (GE Healthcare Life Sciences, Sweden) XK 26 column (26 cm × 20 mm– Pharmacia Biotech, USA) previously equilibrated with PBS solution, and washed with three column volumes of PBS. The BanLec bound to the resin was eluted with 0.5 M methyl α-D-mannopyranoside (Sigma-Aldrich, USA) in PBS. The eluted fractions were screened for the presence of BanLec by SDS-PAGE, and the fractions positive for lectin were pooled and dialyzed against PBS to get rid of the methyl α-D-mannopyranoside. The eluted BanLec was rechromatographed on the Sephadex G-75 column as described above to provide BanLec at higher purity. Protein was assayed with a 2-D Quant Kit (GE Healthcare, USA) according to the manufacturer's instructions. In order to ensure BanLec identity, the protein was sequenced at the mass spectrometry core facility (BioMass/CEFAP) of the Biomedical Sciences Institute at the University of Sao Paulo on an LTQ-Orbitrap Velos ETD (Thermo Scientific, USA) spectrometer coupled with Easy nano LC II (Thermo Scientific, USA). The samples were trypsin digested by adding 15 μL of a 20 ng/μL trypsin solution (Promega, USA) in a microtube containing pieces of the gel for 15 min at 4 °C. After that, 40 μL of 50 mM ammonium bicarbonate (Sigma-Aldrich, USA) was added and the incubation proceeded for 14 h at 37 °C. The peptides were separated on a C18RP column at a 60 min gradient. The samples were analysed in duplicate and results were searched against the Musaceae UniProt database. The protein sequences of the two lectins identified (Accession O22321 and Q8L5H4) were aligned using the program Needle version 6.6.0 from the European Molecular Biology Open Software Suite available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/.

2.4. SDS-PAGE and western blotting

Proteins were separated in SDS-PAGE slab gel at 15% (w/v) acrylamide/bisacrylamide (both from Amersham, Sweden), according to Green and Sambrook [17]. Protein markers were from 10 to 200 kDa (Page Ruler Unstained Protein Ladder—Fermentas, USA). The gel was run at constant voltage (200 V) for 45 min at 4 °C, and the separated proteins were revealed by Coomassie Blue R-250 (Bio-Rad, USA) staining. Alternatively, replicate gel was transferred onto nitrocellulose membranes (Hybond ECL 0.45 μm—Amersham