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Recombinantly produced banana lectin isoform promotes balanced pro-inflammatory response in the colon

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ABSTRACT

Recombinant banana lectin isoform (rBanLec) attaches specifically to the mucosal surface, crosses the epithelial barrier and then directly affects the immune response in mouse colon. Structural characteristics, specificity and physiological impacts of rBanLec reported until now highly resemble those of its natural counterpart. Here, we demonstrated that a dose-dependent stimulation of the colon with rBanLec skewed the immune response towards Th1/Th17 direction and this effect was counterbalanced by the rise in IL-10 production. Qualitative and quantitative characteristics of the established cytokine network were dependent on the applied rBanLec concentration. In addition, rBanLec enhanced local NO production and myeloperoxidase activity and promoted an increase in local IgA and IgG production. Stimulation with rBanLec can be beneficial in prevention of pathologies raised due to inappropriate cell-mediated immune response as well as in prevention of the pathogen invasion via the colon.

1. Introduction

Nutrition affects the whole organism, including the modulation of immune functions, in multiple ways. Numerous studies have demonstrated a beneficial role of essential nutrients and functional food ingredients within the immune system (Calder & Kew, 2002). Functional food components, beneficial components found naturally in foods or added as functional ingredients, include probiotics and a variety of molecules such as poly- and oligosaccharides, polyols, plant proteins, plant stanols and sterols, carotenoids, fatty acids, flavonoids, isothiocyanates, phenolic acids, phytoestrogens, vitamins and minerals.

Functioning of the gastrointestinal tract (GIT), a food entry place, and juxtaposed gut-associated lymphoid tissue (GALT) is mostly affected by food constituents. Gut epithelium serves as a dynamic barrier, which regulates the uptake of

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nutrients, minerals and water. The small intestine is mainly responsible for the absorption of nutrients, while resorption of minerals and water is dominant in the colon (Sandle, 1998). The other important function of the gut epithelium is exclusion of potential pathogen entry (Peterson & Artis, 2014). Proper functioning of an immune system in the gut is very important, especially in the colon, which is a portal of entry for many infectious agents but also susceptible to the infections. Therefore, bioactive substances that could properly stimulate/modulate immune response in the colon and adjacent mucosa would be beneficial.

Functioning of local immune response in the colon is highly influenced by microbiota as well as bioactive food constituents. Lectins represent a broad group of bioactive molecules that can bind the gut epithelium and influence processes within the gut (Lavelle, Grant, Pusztai, Pfuller, & O'Hagan, 2001; Majee & Biswass, 2013; Souza, Caravalho, Ruas, Ricci-Azevedo, & Roque-Barreira, 2013). They are present in plant-based foods in appreciable amount. Lectins are defined as (glyco)proteins which can specifically bind to carbohydrate structures. Due to their unique specificity they discriminate sugars based on the subtle structural differences. Consequently, lectins exert very specific mode of action, including distinctive immunostimulatory/immunomodulatory effects. As they are highly abundant in everyday nutrition, it would be important to clarify in more details which events are triggered upon their ingestion.

Banana lectin (BanLec) was isolated for the first time from a natural source *Musa paradisiac* by Koshte, van Dijk, van der Stelt, and Aalberse (1990). It is reported to be a glucose–mannose-specific lectin, which is a potent mitogen of both human and mouse T cells (Gavrovic-Jankulovic et al., 2008; Peumans et al., 2000), a promoter of pro-inflammatory cytokines expression within splenocytes (Cheng, Wong, & Ng, 2009), and enhancer of NO production in macrophages (Wong & Ng, 2006).

Our research is focused on the evaluation of immunomodulatory and immunostimulatory potential of recombinantly produced banana lectin isoform (rBanLec). The pI value calculated for rBanLec is in the range of those recorded for naturally occurring BanLec (Gavrovic-Jankulovic et al., 2008). The alignment of rBanLec 141 amino acids long sequence to the one of BanLec isolated from natural sources shows a high degree of similarity (~95%). The rBanLec at C-terminus possess GSRSHHHHHH sequence that originates from the expression vector used, Q to N exchange at position 2 due to cloning strategy, and K to N exchange at position 98 as unique for rBanLec. However, the sequences coding its ligand binding loops are highly preserved. The sequence of the first binding loop of rBanLec differed from corresponding sequence in naturally occurring counterparts only at positions 130 (D replaced by K) and 132 (L replaced by I), while the second ligand binding loop (GDVVD) is completely preserved in rBanLec (Gavrovic-Jankulovic et al., 2008).

The data from the literature show that rBanLec specificity and certain physiological impacts (T cells mitogen, recognition by human IgG4) are the same as those of its natural counterpart (Gavrovic-Jankulovic et al., 2008; Stojanovic et al., 2010). Moreover, rBanLec (i) is resistant to proteolysis and denaturation within the GIT, (ii) binds to the luminal surface of small intestine and passes across the epithelium, and (iii) is significantly less immunogenic when applied orally than parenterally (Dimitrijevic, Stojanovic, Micic, Dimitrijevic, & Gavrovic-Jankulovic, 2012).

Previous studies have demonstrated that the consumption of bananas is helpful in treatments of diarrhoea, gastric ulcers and preventions of some cancer types (review in Sampath Kumar, Bhowmik, Duraivel, & Umadevi, 2012). These beneficial effects could be due to the large amount of non-digestible fructooligosaccharides, substances with antioxidant and antimicrobial activity, but also due to the BanLec influence. Thus, the aim of this study was to characterize a local immune response in the colon of healthy mice after a single rectal application of rBanLec in various concentrations. To evaluate local immunostimulatory/immunomodulatory impact of rBanLec, we analysed the production of major effector and regulatory cytokines, antimicrobial substances and antibodies.

2. Materials and methods

2.1. *Production of rBanLec*

rBanLec used in this study is 6His-tagged protein (Gavrovic-Jankulovic et al., 2008). It was produced in *Escherichia coli* SG13009 [pREP4] transformed with expression vector pQE70 (Qiagen, Hilden, Germany) which contained BanLec-encoding insert (GenBank accession number EU055641; Gavrovic-Jankulovic et al., 2008). The protocol for isolation of total RNA from banana pulp (*Musa acuminata*, Cavendish) and amplification of the *BanLec* gene is given in Gavrovic-Jankulovic et al. (2008). Production of rBanLec was performed according to already described procedures (Dimitrijevic et al., 2012; Gavrovic-Jankulovic et al., 2008). Briefly, rBanLec production proceeded through three main stages:

(i) Cultivation of transformed *E. coli* SG13009 and induction of rBanLec synthesis – Transformed *E. coli* SG13009 were grown in 2 × YT-medium (Sigma, St. Louis, MO, USA), supplemented with ampicillin (50 μg/ml; Sigma) and kanamycin (25 μg/ml; Sigma) at 37 °C until OD_{600} 0.7. Then rBanLec synthesis was induced by addition of isopropyl-d-thio-galactopyranoside (1 mM; Sigma), bacterial suspension was incubated at 37 °C for additional 5 h and stored at 4 °C overnight (Gavrovic-Jankulovic et al., 2008).

(ii) Disruption of rBanLec-producing bacterial cells – After pelleting (5000 × *g* for 15 min, 4 °C), rBanLec-producing *E. coli* SG13009 were resuspended in TBS (100 mM NaCl, 50 mM Tris pH 7.4). Disruption of rBanLec-producing bacterial cells proceeded trough lysozyme treatment (1 mg/ml lysozyme (Sigma) in TBS, 1 h at room temperature (RT) plus centrifugation at 5000 × *g*, 15 min at 4 °C), treatment with Na-deoxycholate containing solution (0.1% Na-deoxycholate (Sigma) in TBS, 1 h at 4 °C, plus centrifugation at 5000 × *g*, 15 min at 4 °C) and 3 freezing/thawing cycles. rBanLec-containing solution which is used for its purification is obtained upon centrifugation at 5000 × *g* for 15 min at 4 °C (Gavrovic-Jankulovic et al., 2008).

(iii) Purification of rBanLec from the obtained supernatant by combination of chromatographic methods – immobilized metal-affinity chromatography (IMAC)